Green Cone Opsin and Rhodopsin Regulation by CNTF and Staurosporine in Cultured Chick Photoreceptors

Han-Qing Xie and Ruben Adler

Purpose. To investigate the regulation of visual pigment expression in chick embryo photoreceptor cells by ciliary neurotrophic factor (CNTF), and by the protein kinase inhibitor staurosporine.

Methods. Embryonic day (ED) 8 chick embryo retinal cells were dissociated and cultured at low densities for 3 days, either in control medium or in medium supplemented with CNTF or staurosporine. The cultures were analyzed by immunocytochemistry with the monoclonal antibody Rho4D2, which recognizes chicken rhodopsin and green cone pigment, and by reverse transcription–polymerase chain reaction (RT-PCR) and Northern blot analysis to investigate visual pigment expression at the mRNA level.

Results. CNTF increased the number of Rho4D2-immunoreactive photoreceptors in retinal cell cultures, in agreement with previous reports. RT-PCR and Northern blot analysis, however, showed that rhodopsin mRNA was undetectable in both control and CNTF-treated cultures but that CNTF induced significant increases in mRNA levels for the green cone pigment. Staurosporine-treated cultures also had more Rho4D2-immunoreactive cells than control cultures, but this increase was accompanied by induction of rhodopsin expression, with concomitant decreases in levels of green cone pigment mRNA. No significant differences were found between CNTF- or staurosporine-treated cultures and the corresponding control cultures regarding the red cone pigment, which was expressed in all cases, and the blue and violet pigments, which were not detected in any of the samples.

Conclusions. The results suggest that multiple regulatory systems control visual pigment expression during differentiation of chick embryo photoreceptor cells. CNTF appears to stimulate specifically the differentiation of green cones, without the previously suggested effects on the differentiation of rod photoreceptors in ED 8 chick retinal cultures. (*Invest Ophthalmol Vis Sci.* 2000;41: 4317–4323)

Vertebrate vision requires multiple visual pigments, which are expressed in different types of photoreceptor cells. Although most vertebrates have a single-rod pigment (rhodopsin), the number of cone pigment genes is more variable among species (e.g., two in rodents, three in humans and other primates, four in birds). The mechanisms that determine the expression of particular visual pigment genes in each type of photoreceptor cell are not understood (for reviews see Morrow et al.,[1] and Adler[2]). Several molecular agents have been implicated as regulators of the type and/or level of visual pigment expression, including retinoic acid and other vitamin A derivatives,[3–6] taurine,[6,7] and growth factors such as fibroblast growth factor (FGF),[8] activin,[9] and ciliary neurotrophic factor (CNTF).[10–14]

CNTF is generally regarded as a regulator of rod photoreceptor development, with opposite effects in rodents and chickens. In rat retina explant cultures, CNTF treatment results in dramatic decreases in the number of differentiating rods, with concomitant increases in the number of cells expressing bipolar cell markers.[12] Decreases in opsin immunoreactive cells were also observed in dissociated rat retinal cultures treated with CNTF.[11,14] In chick retinal cultures, in contrast, Fuhrmann et al.[10] and Kirsch et al.[11] observed CNTF-induced increases in the number of photoreceptors immunoreactive with the monoclonal antibody Rho4D2. These immunoreactive cells were identified as rods based on morphologic characteristics, such as the absence of lipid droplets. However, the identity of the Rho4D2(+) cells remains uncertain, because the Rho4D2 antibody, although rhodopsin-specific in bovine retinas,[15] recognizes both rhodopsin and the green cone pigment in the chick,[10] in which these two genes are highly homologous.[16,17]

We have reevaluated the effects of CNTF on cultured chick photoreceptors, complementing Rho4D2 immunocytochemistry with RT-PCR and Northern blot analysis to verify the identity of the visual pigments regulated by this factor. The experiments showed that rhodopsin was not expressed in either CNTF-treated or control cultures, and that CNTF treatment markedly increased mRNA levels for the green cone

From The Johns Hopkins University School of Medicine, The Wilmer Eye Institute, Baltimore, Maryland.

Supported by National Institutes of Health Grant EYO4859; the Knights Templar Eye Foundation, Chicago, Illinois; National Institutes of Health Core Grant EY1765; a center grant from the Foundation Fighting Blindness; a grant from Mrs. Harry J. Duffey; and an unrestricted departmental grant from Research to Prevent Blindness. RA is the Arnall Patz Distinguished Professor of Ophthalmology, and a senior investigator of Research to Prevent Blindness.

Submitted for publication June 1, 2000; revised August 29, 2000; accepted September 8, 2000.

Commercial relationships policy: N.

Corresponding author: Ruben Adler, The Johns Hopkins School of Medicine, 519 Maumenee, 600 N. Wolfe Street, Baltimore, MD 21287-9257. radler@jhmi.edu

pigment. In experiments originally designed to examine the involvement of second-messenger systems in the effects of CNTF, we found that staurosporine, a microbial alkaloid widely used as a protein kinase inhibitor,[18] causes by itself an increase in the number of Rho4D2-immunoreactive photoreceptors, contrary to the situation with CNTF; however, this increase correlates with the induction of rhodopsin expression and a decrease in green cone mRNA levels, as demonstrated by RT-PCR and Northern blot analysis.

Materials and Methods

Materials

Monoclonal antibody Rho4D2 was a generous gift from David Hicks. Reagents and their sources were as follows: molecular biology reagents Trizol, DNase I, Superscript reverse transcriptase, *Taq* polymerase, random primers, oligo(dT) primer, a random primer labeling kit, and restriction enzymes were obtained from Life Technologies (Gaithersburg, MD); White Leghorn chick embryos from Truslow Farm (Chestertown, MD); horse anti-mouse antibody, the ABC Elite kit, and goat serum from Vector (Burlingame, CA); Hybond-N nylon membrane and α-^{32}P dcTP (3000 Ci/mmol) from Amersham (Buckinghamshire, UK); Ultrahyb hybridization solutions from Ambion (Austin, TX); fetal bovine serum from Hyclone (Logan, UT); CNTF from R&D (Minneapolis, MN); and staurosporine and other chemicals from Sigma (St. Louis, MO). Polymerase chain reaction (PCR) oligonucleotide primers for visual pigments were synthesized by Integrated DNA Technologies (Coralville, IA).

Retinal Cell Dissociation, Culture, and Treatment

White Leghorn chick embryos were used throughout the experiments. Retinal cells were dissociated from embryonic day (ED) 8 embryos, when most retinal cells are already postmitotic,[19] but no visual pigments are detectable.[20] The cells were cultured for 3 days at 8.0×10^5 cells per 35-mm dish, or 6.5×10^6 cells per 100-mm dish, as described.[21] Cultures were maintained in M199 medium supplemented with 1% heat-inactivated fetal bovine serum, penicillin-glutamine, and linoleic acid. Rat recombinant CNTF, diluted in phosphate-buffered saline (PBS) with 0.1% bovine serum albumin (BSA), or staurosporine, dissolved in dimethyl sulfoxide (DMSO), were added to the cultures 3 hours after seeding, when most cells had already attached to the substratum. The corresponding vehicles served as control treatments.

Immunocytochemistry

Immunocytochemistry was performed as described.[9] Cultured cells were fixed in 4% paraformaldehyde in PBS for 30 minutes, blocked in PBS containing 0.25% Triton X-100 and 10% goat serum, and incubated overnight with the primary antibody Rho4D2 (1:100 dilution). After appropriate washes, the cultures were incubated with biotinylated secondary antibodies and processed with the ABC kit (Elite; Vector) according to the manufacturer's protocol.

Quantitative Microscopic Analysis

Morphologic categorization of cultured retinal cells as photoreceptors, nonphotoreceptor neurons, and morphologically undifferentiated cells was performed according to established criteria.[9] Total number of cells and the number of Rho4D2(+) cells under various treatments were determined under phase-contrast and bright-field illumination, respectively, at $\times$400 magnification. At least 50 randomly selected fields per dish were counted for the assessment of Rho4D2(+) cells by an observer who was unaware of the identity of the samples. Each experiment was repeated at least three times, using triplicate dishes for each condition. A two-tailed Student's *t*-test was used to evaluate the significance of differences between treatments, and the dose dependency of staurosporine effects was determined by analysis of variance (ANOVA). $P \leq 0.05$ was considered significant.

Reverse Transcription–Polymerase Chain Reaction

For reverse transcription–polymerase chain reaction (RT-PCR), oligonucleotide primers for chicken rhodopsin and the red, green, blue, and violet cone pigments were designed using the Oligo 5.0 program (Molecular Biology Insights, Inc., Cascade, CO), based on reported gene sequences,[16,17,22,23] and are listed in Table 1. Total RNA was extracted from cultured retinal cells or freshly dissected ED 18 retina,[24] using the reagent according to the manufacturer's instructions (Trizol; Life Technologies), and incubated with DNase I to digest any residual genomic DNA. Reverse transcription reactions were performed with random hexamers using a commercially available reverse transcriptase (Superscript; Life Technologies). PCR amplification was conducted with a commercial system (DNA Engine; MJ Research, Waltham, MA) with denaturing at 94°C for 5 seconds, annealing at 64°C for 30 seconds, and extension at 72°C for 45 seconds (30 cycles), followed by final extension at 72°C for 5 minutes. Identity of PCR products was verified by sequencing, performed at the Johns Hopkins Genetics Core Facility.

Northern Hybridization

Northern hybridization was performed as described.[25] Total RNA was extracted as described, and 10 μg RNA from each sample were size fractionated in 1.5% formaldehyde-agarose gels and transferred to nylon membranes (Hybond-N; Hyclone). After UV cross-linking, the membranes were prehybridized (UltrAhyb; Ambion) and hybridized overnight at 42°C with the same solution containing 10^6 cpm/ml ^{32}P-labeled probes, followed by two washes with 2$\times$ SSC and 0.1% sodium dodecyl sulfate (SDS), and two washes with 0.1$\times$ SSC and 0.1% SDS for at least 15 minutes each. Red, green, rhodopsin, visi-

Table 1. Primer Sequences for Chick Visual Pigments and Visinin in RT-PCR Study

	Primer Sequence
Rhodopsin	aga aac tcc gga cgc ctc taa act gta ccg tga cca gcc gaa ca
Red	cgt ggc ggc tca gca gaa gga gtc tta ggc ggg cga gac gga gga gtt
Green	caa gag cat ggg aag ggg gaa aga ccg agg aga ggg gga cgc taa ca
Blue	gct atc gcg gcc ttt atg ttc ctc tgc cac ttg ttg tcc gtc gtg ta
Violet	gga cga cga ctt cta cct ctt cac cca act cgc aga ccc tct tc
Visinin	gcg cat tta cgg caa ctt ctt cc gca taa tgg cgt cgt tct tca tca

nin, and β-actin probes were labeled with α-P^{32} dCTP (3000 Ci/mmol) by random priming using a random primer labeling kit (Gibco, Grand Island, NY). Probes spanned nucleotides 76 to 494 of the red pigment, 52 to 519 of the green pigment, 160 to 766 of rhodopsin, and the full-length β-actin and visinin sequences. Blots were sequentially hybridized with at least three different probes (see the Results section) after stripping with boiling 0.1% SDS solution. The intensity of the bands was analyzed using image analysis software (NIH Image; National Institutes of Health, Bethesda, MD). In the case of CNTF experiments, the values were normalized to the actin signals, and data from three separate experiments are expressed as average ± SEM. Bands in the staurosporine Northern blot analysis were normalized in relation to visinin.

Results

Effects of CNTF Treatment on the Frequency of Rho4D2(+) Cells

Dissociated ED 8 chick embryo retinal cells differentiate in culture into distinct populations of photoreceptors and multipolar neurons, whereas approximately one third of the cells remain morphologically undifferentiated.[9,26] The frequencies of these three cell categories were not significantly different in CNTF-treated and control cultures after 3 days in vitro (Fig. 1A), suggesting that CNTF did not affect the overall differentiation of progenitor cells into photoreceptors or nonphotoreceptor neurons. Consistent with previous reports,[10,11] analysis with the monoclonal antibody Rho4D2 showed that some photoreceptors were strongly immunoreactive (Fig. 1C), and revealed statistically significant difference in the number of Rho4D2(+) cells between control and CNTF-treated cultures. As shown in Figure 1B, the frequency of Rho4D2(+) photoreceptors was approximately three times higher in CNTF-treated than in control cultures.

Effects of CNTF Treatment on mRNA Levels for Green Cone Pigment and Rhodopsin

RT-PCR and Northern blot analysis were used to determine whether CNTF affected mRNAs for rhodopsin and/or the green cone pigment, both of which are recognized by Rho4D2. ED 18 retinas, in which mRNAs for all visual pigments are readily detectable, were used as positive control (Fig. 2A). Transcripts for the blue and violet cone pigments and for rhodopsin were undetectable by RT-PCR in CNTF-treated and control cultures. Red and green mRNAs could be observed in both cases, but the PCR product for the green cone pigment appeared stronger in CNTF-treated than in control cultures (Fig. 2A). This apparent CNTF-induced increase in mRNA levels for the green cone pigment was verified by Northern hybridization (Fig. 2B). After normalization with respect to actin, the 2.8-kb band recognized by the green probe in CNTF-treated samples had an optical density 215% ± 27% higher than that in control cultures, with the difference being statistically significant. Although the 1.6-kb band recognized by the red visual pigment probe appears darker in CNTF-treated cultures by visual inspection of Figure 2B, its optical density normalized to the actin band in three separate experiments was only 123% ± 29% of control values, with the difference not statistically significant.

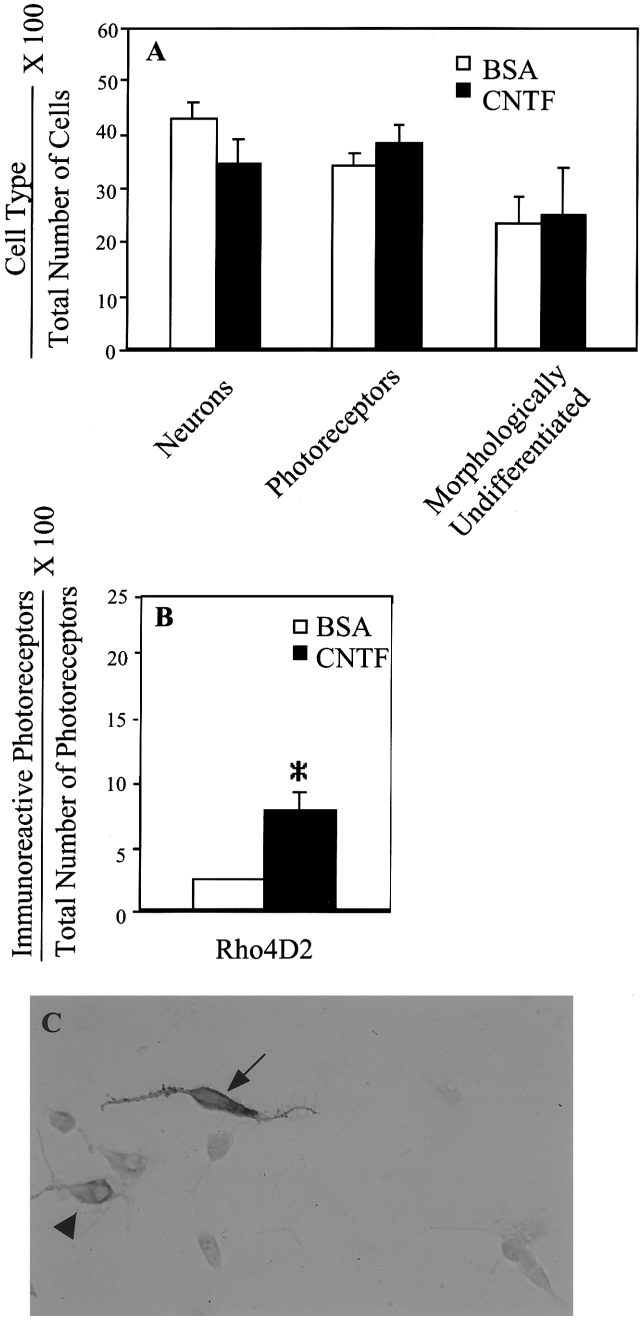

Figure 1. CNTF treatment significantly increased the number of Rho4D2(+) cells without any effects on the overall differentiation of retinal cells. ED 8 retinal cells were cultured for 3 days in the presence of 10 ng/ml CNTF or in the presence of vehicle. Immunocytochemical staining with Rho4D2 was then performed. (**A**) The numbers of morphologically identified neurons and photoreceptors, or of the morphologically undifferentiated cells, did not differ significantly between control and treated groups, suggesting that CNTF had no apparent effects on the overall differentiation of various cell types in retinal cell culture. Similar to the reported observation,[10] no significant difference in the total number of cells was found between control (2.35 ± 0.11 × 10^5 cells/dish) and CNTF-treated culture (2.61 ± 0.20 × 10^5 cells/dish), suggesting that CNTF did not affect the overall survival of retinal cells. (**B**) CNTF significantly increased the number of Rho4D2(+) cells, consistent with previously reported results.[10] (**C**) Bright-field photomicrograph of an ED 8 culture grown for 3 days in CNTF-containing medium and immunoreacted with the Rho4D2 monoclonal antibody. A strongly immunoreactive photoreceptor is indicated by the *arrow*, and a lightly stained cell by an *arrowhead*.

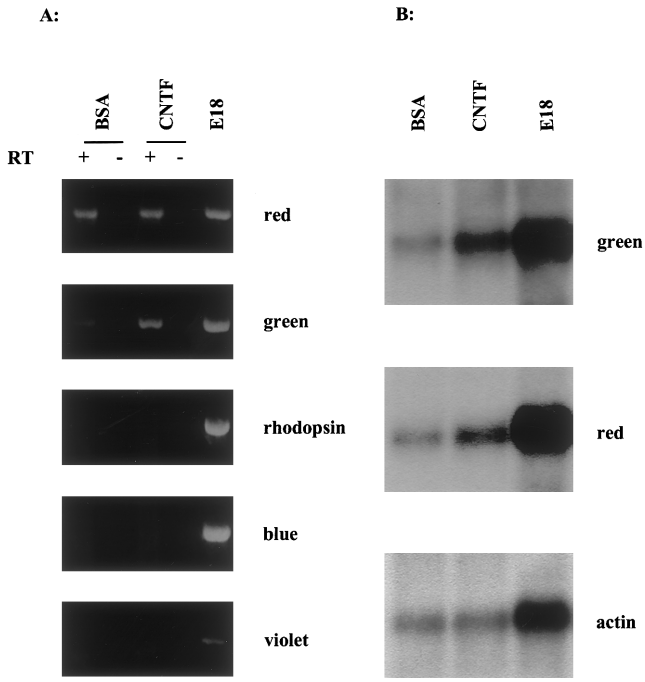

FIGURE 2. CNTF treatment promoted the expression of green cone visual pigment, without any detectable effects on rhodopsin. ED 8 retinal cells were cultured in the presence of CNTF or vehicle for 3 days. (**A**) RT-PCR was performed in samples prepared from control and CNTF-treated retinal cells. Red and green visual pigments were expressed in both control and CNTF samples, whereas rhodopsin and blue and violet visual pigments were not detectable in any case. These results demonstrate that CNTF did not induce the expression of rhodopsin. (**B**) Northern blot analysis showed stronger signal intensity with green visual pigment probe in CNTF-treated samples, indicating that CNTF promoted the expression of green visual pigment. Samples from freshly dissected ED 18 chick retina served as positive controls in both RT-PCR and Northern hybridization. Reverse transcriptase reaction (RT) is shown with (+) or without (−) reverse transcriptase.

Effects of Staurosporine Treatment on the Frequency of Rho4D2 Immunoreactive Photoreceptors in Retinal Cell Cultures

In pilot experiments designed to investigate whether the protein kinase inhibitor staurosporine blocks the effects of CNTF treatment, we observed that cultures treated with staurosporine, in the absence of CNTF, appeared to have more Rho4D2(+) photoreceptors than the untreated control cultures. Quantitative analysis corroborated this observation and showed concentration-dependent increases in Rho4D2 immunoreactive cells in staurosporine-treated cultures, with the effects reaching statistical significance at 100 nM (Fig. 3B). The total number of cells present in the cultures decreased slightly at this staurosporine concentration (Fig. 3A), but the decreases were not statistically significant and did not appear to be selective for any of the morphologic subpopulations present in the retinal cultures (Fig. 3C). The finding that staurosporine also increased Rho4D2-immunoreactive cells in the cultures precluded its use for studies of CNTF action but, as shown later, disclosed a separate regulatory mechanism for visual pigment expression.

Effects of Staurosporine Treatment on Rhodopsin Expression and mRNA Levels for the Green Cone Pigment

Similar to the results from the CNTF study (Fig. 2), RT-PCR detected mRNA for the red and green cone pigments but not for rhodopsin, blue, and violet in control ED 8 retinal cell cultures. As shown in Figure 4A, however, PCR products for rhodopsin were detectable in samples from cultures treated for

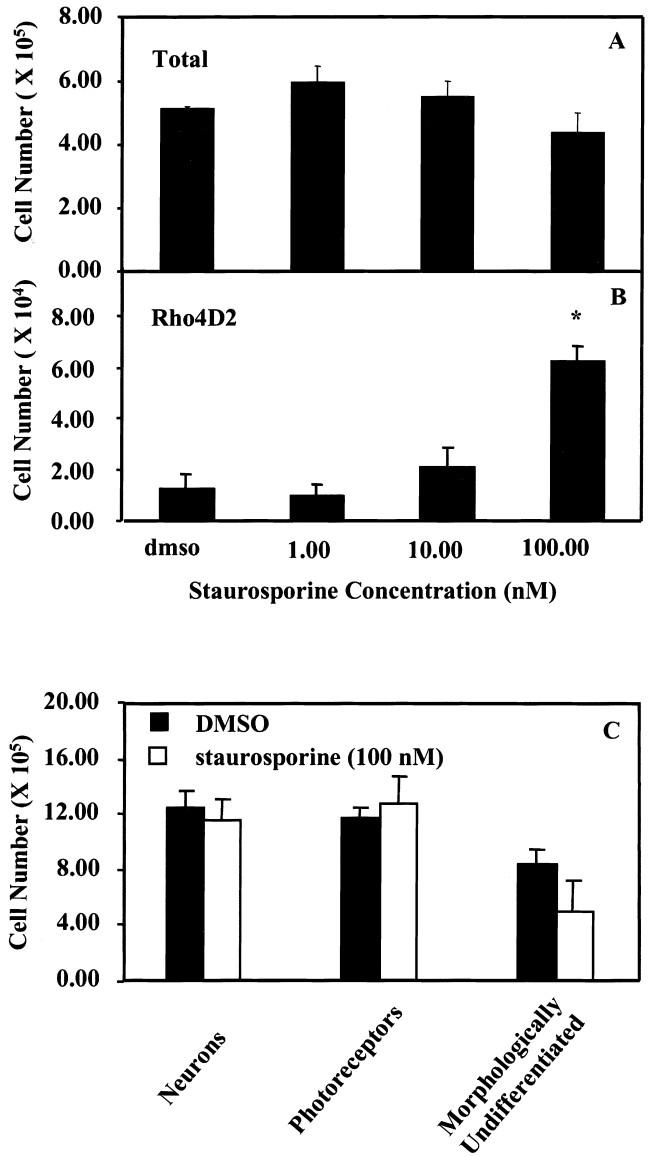

FIGURE 3. Staurosporine treatment increased the number of Rho4D2(+) cells. ED 8 retinal cells were cultured for 3 days in the presence of staurosporine at different concentrations or in the presence of vehicle and processed for immunocytochemistry with the Rho4D2 antibody. (**A**) Staurosporine treatment increased the number of Rho4D2(+) cells in a dose-dependent manner. ANOVA showed that there were significant differences between DMSO-treated samples and samples treated with 100 nM staurosporine. (**B**) Staurosporine treatment of ED 8 retinal cell cultures did not change the total number of cells. Decreases observed after 100 nM staurosporine treatment were not statistically significant. (**C**) The numbers of neurons, photoreceptors, and morphologically undifferentiated cells did not differ significantly between control and treated groups.

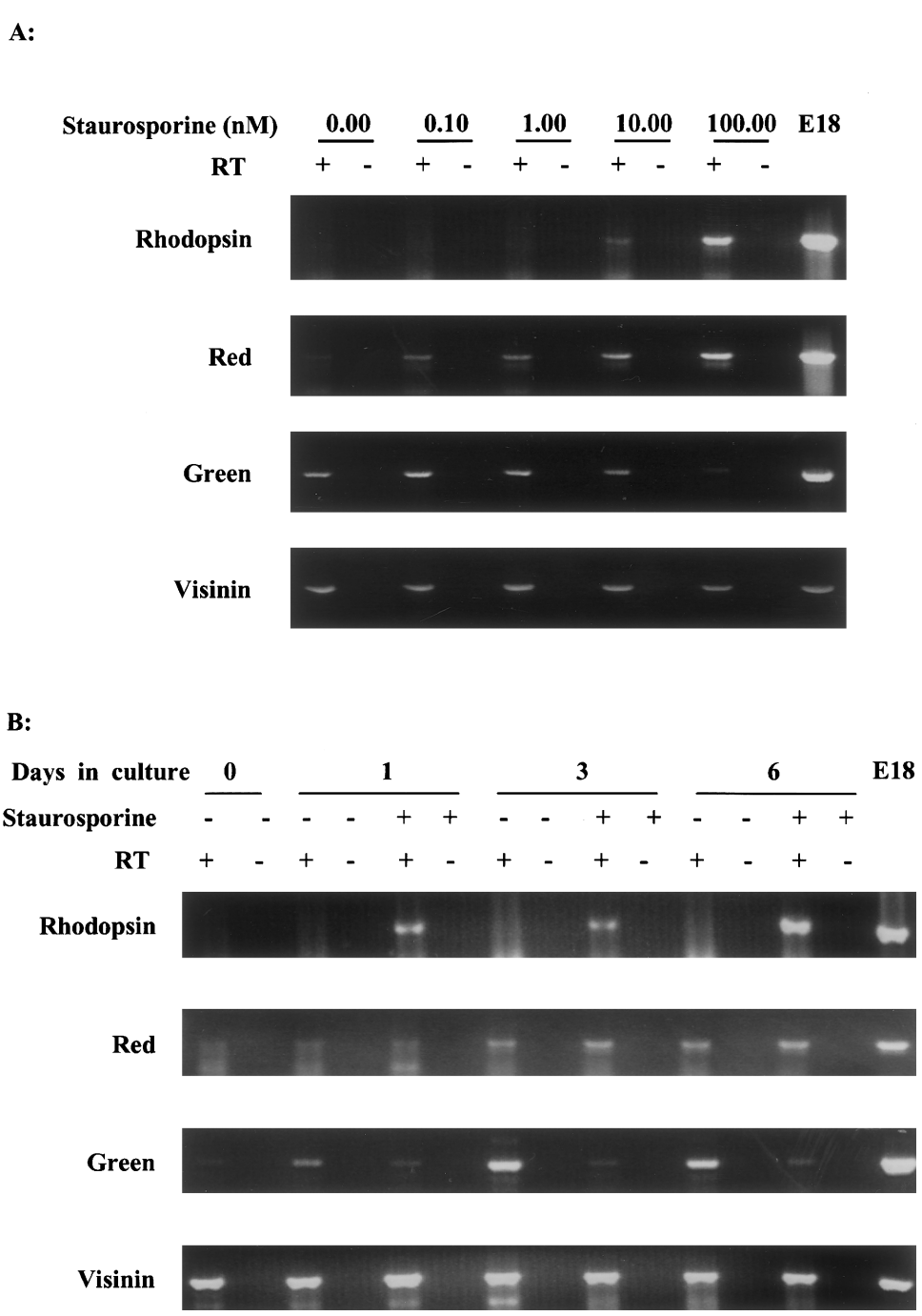

FIGURE 4. Staurosporine treatment induced rhodopsin expression. ED 8 retinal cells were treated with different concentrations of staurosporine for 3 days (**A**) or treated with 100 nM staurosporine for 1, 3, or 6 days (**B**). RT-PCR was performed to evaluate the kinetics of visual pigment expression after staurosporine treatment. (**A**) Rhodopsin PCR products were not detectable in samples treated with DMSO, or with 0.1 or 1.0 nM staurosporine but were apparent in samples treated with 10 and 100 nM staurosporine after 3 days in culture. (**B**) Rhodopsin expression was undetectable 3 hours after seeding (day 0). The induction of rhodopsin by staurosporine treatment was apparent after 1 day's treatment and persisted for at least 6 days. Blue and violet were below detection limits in both control and staurosporine-treated samples. These results suggest that staurosporine specifically induced rhodopsin expression. Samples from freshly dissected ED 18 chick retina served as positive controls. Reverse transcriptase reaction (RT) is shown with (+) or without (−) reverse transcriptase.

3 days with 10 or 100 nM staurosporine; rhodopsin mRNA was detectable in as early as 24 hours in culture, and its expression persisted for at least 6 days in vitro (Fig. 4B). PCR products for red and green cone pigments were present in both staurosporine-treated and control cultures, but at 100 nM staurosporine, the green product was barely detectable; blue and violet expression was never detected in these experiments. As shown in Figure 5, Northern blot analysis verified the induction of rhodopsin by staurosporine, with two transcripts (1.6 and 2.5 kb) being present in ED 18 retinas (positive controls), as well as in cultures treated with 100 nM staurosporine. Rhodopsin signals were very faint in samples treated with 10 nM staurosporine, which required longer exposures to be visualized (not shown) but were never detectable in DMSO-treated control cultures. The normalized density of the green cone pigment was reduced approximately 50% in samples treated with 10 nM staurosporine compared with control cultures. Green was practically undetectable in cultures treated with 100 nM staurosporine.

DISCUSSION

The experiments reported confirm previous reports,[10,11] showing that CNTF causes an increase in the number of photoreceptors present in chick embryo retinal cultures that are immunoreactive with the Rho4D2 monoclonal antibody. However, whereas those reports concluded that CNTF-responsive

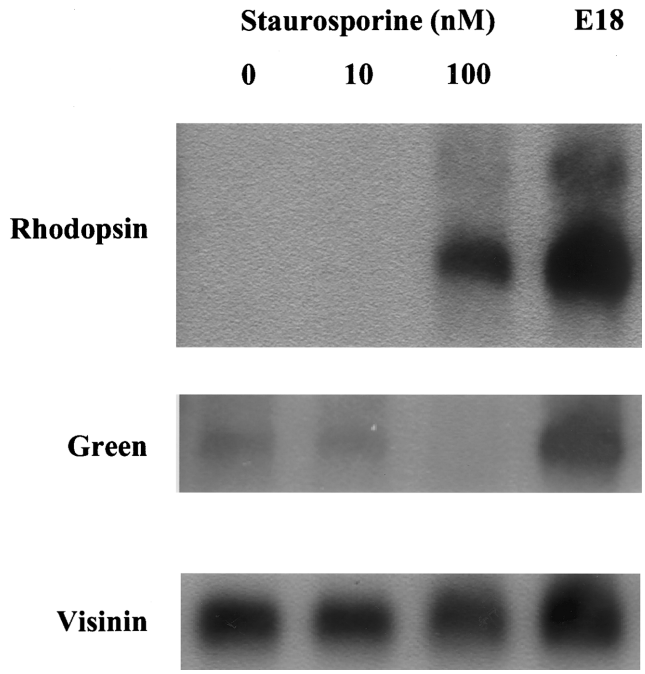

FIGURE 5. Staurosporine treatment inhibited the expression of green visual pigment. ED 8 retinal cells were treated with 0, 10, and 100 nM staurosporine for 3 days, and Northern blot analysis was performed. Northern hybridization analyses indicate that staurosporine treatment resulted in decreases in the intensity of green visual pigment signals. In addition, both transcripts for rhodopsin (1.6 and 2.5 kb) were detected in the staurosporine-treated samples by Northern blot analysis, suggesting that both forms of rhodopsin mRNA were induced by staurosporine treatment. Samples from freshly dissected ED 18 chick retina served as positive control.

photoreceptors were rods, our PCR and Northern hybridization data indicate that they are more likely to be green cones, because CNTF-treated cultures showed higher mRNA levels for the green cone pigment, without any detectable presence of rhodopsin mRNA. A likely explanation for this apparent discrepancy is the high sequence homology of rhodopsin and the green cone pigment,[16,17] which suggests that the Rho4D2 antibody may recognize both of these pigments.[10] It is also noteworthy that the identification of CNTF-responsive Rho4D2(+) cells as rods in previous studies was based on morphologic features of the immunoreactive cells, such as absence of oil droplets,[10,11] without direct verification of the identity of the visual pigments expressed in CNTF-treated and control cultures. Given that the culture conditions used in our experiments and those of Fuhrmann et al.[10] and Kirsch et al.[11] are essentially identical, it appears reasonable to conclude that CNTF regulates the expression of the green cone pigment in chick embryo retinal cultures without any effects on the expression of the rhodopsin gene. It remains an open possibility, however, that CNTF may affect rods under different conditions.

CNTF was discovered in the chick embryo eye based on its effects on the survival of ciliary ganglion neurons,[27] on which it has been found to exert a variety of effects.[28] CNTF was subsequently shown to have diverse functions both within and outside the nervous system (for review, see Ip and Yancopoulos[29]) Its relevance to the visual system includes the capacity to delay photoreceptor death in experimental animal models of human retinitis pigmentosa.[30] Our studies, extending the observations from the Hoffman laboratory,[10,11] suggest that CNTF is specifically involved in the regulation of green cone opsin, one of the five visual pigments found in chick photoreceptors. The selectivity of these effects, at least in vitro, is suggested by the presence of similar levels of mRNA for the red cone pigment, iodopsin, and the absence of rhodopsin and the blue and violet cone pigments in both CNTF-treated and control cultures.

Although both CNTF and staurosporine increase the number of Rho4D2 immunoreactive cells in chick retinal cultures, they seem to act through very different mechanisms, because staurosporine was found to induce the expression of rhodopsin while decreasing the expression of the green cone opsin. These two agents, however, resemble each other in that they have no effect on the expression of the blue and violet cone pigments. The biochemical pathways through which staurosporine acts on chick visual pigments remain unknown. One of its major effects is to inhibit protein kinase C (PKC) activity,[18] but it has also been shown to affect other kinases involved in intracellular cell signaling.[18,31] Given its multiple sites of action, it is not surprising that staurosporine has been reported to have a variety of effects on neuronal primary cultures and cell lines. They include induction of neurite outgrowth, effects on the cell cycle, and apoptotic cell death.[32–36] We did not investigate in detail the patterns of neurite development in staurosporine-treated cultures, but qualitative observations suggested that they were different from those in vehicle-treated control cultures (not shown). Although the relative frequencies of photoreceptors, nonphotoreceptor neurons, and morphologically undifferentiated cells were not significantly different between staurosporine-treated and control cultures, we observed modest cell losses in cultures treated with 100 nM staurosporine. It remains to be determined whether changes in cell proliferation and/or cell death contribute to the decrease in mRNA levels for the green cone pigment observed in staurosporine-treated samples.

Although the generation of photoreceptor cells is completed by the end of the first week of embryonic development, at least in the fundal region of the eye,[19] visual pigment expression cannot be detected by in situ hybridization in ovo until ED 14 or 15.[20] However, expression of the red cone pigment iodopsin is markedly accelerated when photoreceptors are grown in dissociated cultures, either in serum-containing or in serum-free cultures.[2,9,37] This accelerated expression, which was also seen in progenitor cells born in vitro, was suppressed almost completely by treatment of the cultures with the growth factor activin.[9] Taken together, these observations led to the hypothesis that retinal progenitor cells acquire the capacity to express the red cone pigment very early in their development,[2] and that additional inductive signals are necessary for other visual pigments to be expressed. The finding that rhodopsin and green cone pigment expression can be selectively induced by pharmacologic manipulation of the cultures with CNTF and staurosporine is consistent with this possibility. Further experimentation is needed to determine whether similar mechanisms operate during retinal development in vivo as well.

Acknowledgments

The authors thank Teri Belecky-Adams for providing plasmids, Andrea Tamres and Rebecca Bradford for technical support, and Karl Wahlin for assistance with densitometric analysis.

References

1. Morrow EM, Furukawa T, Cepko CL. Vertebrate photoreceptor cell development and disease. *Trends Cell Biol.* 1998;8:353–358.
2. Adler R. A model of retinal cell differentiation in the chick embryo. *Prog Retinal Eye Res.* 2000;19:529–557.
3. Stenkamp DL, Gregory JK, Adler R. Retinoid effects in purified cultures of chick embryo retina neurons and photoreceptors. *Invest Ophthalmol Vis Sci.* 1993;34:2425–2436.
4. Kelley MW, Turner JK, Reh TA. Ligands of steroid/thyroid receptors induce cone photoreceptors in vertebrate retina. *Development.* 1995;121:3777–3785.
5. Hyatt GA, Dowling JE. Retinoic acid: a key molecule for eye and photoreceptor development. *Invest Ophthalmol Vis Sci.* 1997;38: 1471–1475.
6. Wallace VA, Jensen AM. IBMX, taurine and 9-cis retinoic acid all act to accelerate rhodopsin expression in postmitotic cells. *Exp Eye Res.* 1999;69:617–627.
7. Altshuler D, Lo Turco JJ, Rush J, Cepko C. Taurine promotes the differentiation of a vertebrate retinal cell type in vitro. *Development.* 1993;119:1317–1328.
8. Hicks D, Courtois Y. Fibroblast growth factor stimulates photoreceptor differentiation in vitro. *J Neurosci.* 1992;12:2022–2033.
9. Belecky–Adams TL, Scheurer D, Adler R. Activin family members in the developing chick retina: expression patterns, protein distribution, and in vitro effects. *Dev Biol.* 1999;210:107–123.
10. Fuhrmann S, Kirsch M, Hofmann HD. Ciliary neurotrophic factor promotes chick photoreceptor development in vitro. *Development.* 1995;121:2695–2706.
11. Kirsch M, Fuhrmann S, Wiese A, Hofmann HD. CNTF exerts opposite effects on in vitro development of rat and chick photoreceptors. *Neuroreport.* 1996;7:697–700.
12. Ezzeddine ZD, Yang X, DeChiara T, Yancopoulos G, Cepko CL. Postmitotic cells fated to become rod photoreceptors can be respecified by CNTF treatment of the retina. *Development.* 1997; 124:1055–1067.
13. Fuhrmann S, Heller S, Rohrer H, Hofmann HD. A transient role for ciliary neurotrophic factor in chick photoreceptor development. *J Neurobiol.* 1998;37:672–683.
14. Kirsch M, Schulz–Key S, Wiese A, Fuhrmann S, Hofmann H. Ciliary neurotrophic factor blocks rod photoreceptor differentiation from postmitotic precursor cells in vitro. *Cell Tissue Res.* 1998;291:207–216.
15. Hicks D, Molday RS. Differential immunogold-dextran labeling of bovine and frog rod and cone cells using monoclonal antibodies against bovine rhodopsin. *Exp Eye Res.* 1986;42:55–71.
16. Okano T, Kojima D, Fukada Y, Shichida Y, Yoshizawa T. Primary structures of chicken cone visual pigments: vertebrate rhodopsins have evolved out of cone visual pigments. *Proc Natl Acad Sci USA.* 1992;89:5932–5936.
17. Wang SZ, Adler R, Nathans J. A visual pigment from chicken that resembles rhodopsin: amino acid sequence, gene structure, and functional expression. *Biochemistry.* 1992;31:3309–3315.
18. Tamaoki T, Nomoto H, Takahashi I, Kato Y, Morimoto M, Tomita F. Staurosporine, a potent inhibitor of phospholipid/Ca++ dependent protein kinase. *Biochem Biophys Res Commun.* 1986;135: 397–402.
19. Kahn AJ. An autoradiographic analysis of the time of appearance of neurons in the developing chick neural retina. *Dev Biol.* 1974;38: 30–40.
20. Bruhn SL, Cepko CL. Development of the pattern of photoreceptors in the chick retina. *J Neurosci.* 1996;16:1430–1439.
21. Adler R. Preparation, enrichment and growth of purified cultures of neurons and photoreceptors from chick embryos and from normal and mutant mice. *Methods Neurosci.* 1990;2:134–150.
22. Takao M, Yasui A, Tokunaga F. Isolation and sequence determination of the chicken rhodopsin gene. *Vision Res.* 1988;28:471–480.
23. Kuwata O, Imamoto Y, Okano T, et al. The primary structure of iodopsin, a chicken red-sensitive cone pigment. *FEBS Lett.* 1990; 272:128–132.
24. Saga T, Scheurer D, Adler R. Development and maintenance of outer segments by isolated chick embryo photoreceptor cells in culture. *Invest Ophthalmol Vis Sci.* 1996;37:561–573.
25. Xie HQ, Hu VW. Modulation of gap junctions in senescent endothelial cells. *Exp Cell Res.* 1994;214:172–176.
26. Adler R. Plasticity and differentiation of retinal precursor cells. *Int Rev Cytol.* 1993;146:145–190.
27. Adler R, Landa KB, Manthorpe M, Varon S. Cholinergic neuronotrophic factors: intraocular distribution of trophic activity for ciliary neurons. *Science.* 1979;204:1434–1436.
28. Nishi R. Target-derived molecules that influence the development of neurons in the avian ciliary ganglion. *J Neurobiol.* 1994;25:612–619.
29. Ip NY, Yancopoulos GD. The neurotrophins and CNTF: two families of collaborative neurotrophic factors. *Annu Rev Neurosci.* 1996;19:491–515.
30. LaVail MM, Yasumura D, Matthes MT, et al. Protection of mouse photoreceptors by survival factors in retinal degenerations. *Invest Ophthalmol Vis Sci.* 1998;39:592–602.
31. Groundwater PW, Solomons KR, Drewe JA, Munawar MA. Protein tyrosine kinase inhibitors. *Prog Med Chem.* 1996;33:233–329.
32. Hashimoto S, Hagino A. Staurosporine-induced neurite outgrowth in PC12h cells. *Exp Cell Res.* 1989;184:351–359.
33. Weil M, Jacobson MD, Coles HS, et al. Constitutive expression of the machinery for programmed cell death. *J Cell Biol.* 1996;133: 1053–1059.
34. Wiesner DA, Dawson G. Staurosporine induces programmed cell death in embryonic neurons and activation of the ceramide pathway. *J Neurochem.* 1996;66:1418–1425.
35. Yao R, Yoshihara M, Osada H. Specific activation of a c-Jun NH2-terminal kinase isoform and induction of neurite outgrowth in PC-12 cells by staurosporine. *J Biol Chem.* 1997;272:18261–18266.
36. Stokke T, Smedshammer L, Jonassen TS, Blomhoff HK, Skarstad K, Steen HB. Uncoupling of the order of the S and M phases: effects of staurosporine on human cell cycle kinases. *Cell Prolif.* 1997;30: 197–218.
37. Tamres AN, Belecky–Adams TL, Adler R. Microenvironmental regulation of visual pigment expression in the chick retina [ARVO Abstract]. *Invest Ophthalmol Vis Sci.* 1999;40(4):**S**457. Abstract nr 2411.