Prevention of Photoreceptor Apoptosis by Activation of the Glucocorticoid Receptor

Andreas Wenzel,[1] *Christian Grimm,*[1] *Mathias W. Seeliger,*[2] *Gesine Jaissle,*[2] *Farhad Hafezi,*[1] *Robert Kretschmer,*[3] *Eberhart Zrenner,*[2] *and Charlotte E. Remé*[1]

Purpose. Evidence has accumulated that excessive light exposure may promote age-related and inherited retinal degeneration, in which photoreceptor death by apoptosis leads to loss of vision. In the current study, the effect of elevated corticosteroid levels on light-induced apoptosis of photoreceptors was determined.

Methods. Photoreceptor apoptosis was induced in retinas of BALB/c mice by exposure to diffuse white light. High levels of corticosteroids were induced, either endogenously (fasting-mediated stress) or by a single intraperitoneal injection of dexamethasone (DEX). Photoreceptor damage was assessed morphologically and by electroretinography. Glucocorticoid receptor (GR) and activator protein (AP)-1 activities were shown by Western blot analysis and electrophoretic mobility shift assay (EMSA) of retinal nuclear extracts.

Results. Fasting and injection of DEX led to an activation of GR in the retina, as judged by its translocation to the nucleus of retinal cells. On induction of GR activity before light exposure, AP-1 activity, normally induced by damaging doses of light, remained at basal levels. Both treatments completely prevented photoreceptor apoptosis and preserved retinal function.

Conclusions. Activity of the transcription factor AP-1 is associated with light-induced apoptosis. In the current study, pharmacologic suppression of AP-1 activity protected against light damage. Inhibition of AP-1 activity may have occurred by the protein–protein interaction of GR and AP-1. (*Invest Ophthalmol Vis Sci.* 2001;42:1653–1659)

Evidence from epidemiologic studies and from animal models indicates that excessive light exposure may enhance many forms of retinal dystrophies in humans.[1–9] A common feature of these retinal dystrophies and of light-induced retinal degeneration is photoreceptor death by apoptosis.[10] Short-term exposure to high levels of white light is therefore used to synchronously induce and analyze this particular mode of cell death in the retina of mice.

Steroids can exert potent antiapoptotic effects,[11–14] and one mechanism proposed to underlie this protection, is GR-mediated inhibition of AP-1 activity.[15] The inhibitory crosstalk between AP-1 and GR may involve a protein–protein interaction between both transcription factors,[16–18] and transrepression of AP-1 target genes by activated GR has been well documented in in vitro systems.[19,20] Indeed, transrepression by interference with other transcription factors, rather than by transactivation of GR target genes, appears to be the essential mode of action for GR.[21]

c-Fos is a constituent of the transcription factor AP-1[22] and plays a significant role in light-induced apoptosis of photoreceptors.[23,24] In normal mice, light induces AP-1 activity and photoreceptors die by apoptosis. In mice without c-Fos, light does not induce AP-1 activity and light damage does not occur.[23,25] However, due to impaired retinal function and morphology in c-*fos* knockout mice,[26] developmental deficits that reduce light damage susceptibility cannot be excluded. To test for an involvement of AP-1 in light-induced photoreceptor apoptosis in genetically normal mice, we sought to inhibit AP-1 by activation of GR. Expression of both, AP-1 members and GR has been detected in a variety of mammalian retinal cell types including photoreceptors, thus GR/AP-1 interaction may occur in retinal cells.[27–29]

Activation of GR either by elevation of endogenous corticosterone (CS) levels through metabolic stress or by administration of the synthetic GR agonist dexamethasone (DEX) resulted in a complete protection of retinal function and morphology after exposure to excessive light. Our results point to inhibitory crosstalk between the activated glucocorticoid receptor and AP-1 that prevents the induction of photoreceptor apoptosis by light. These results may direct further research into the clinical application of glucocorticoids in the prevention of light-related retinal degeneration.

Methods

Mice

All procedures concerning animals were in accordance with the regulations of the Veterinary Authority of Zurich and with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Male BALB/c mice (21 days old) were obtained from Wiga (Sulzfeld, Germany). All animals were reared in a 12–12-hour (6 AM–6 PM) dark–light cycle with 60 to 100 lux within the cages. Experiments were performed at the age of 9 to 12 weeks. Dexamethasone sodium phosphate (Dexadresone; Intervet International BV, The Netherlands) was applied in a single intraperitoneal injection at the times indicated. Control animals received an equal volume of physiological saline solution.

Food Deprivation

Food was withdrawn for 16 hours overnight before light exposure. During exposure, food-deprived (FD) mice obtained food but were deprived again for 4 hours thereafter. Normally fed (NF) mice had access to food at any time. Water was available for all mice throughout the experiments.

From the [1]Laboratory of Retinal Cell Biology, University Hospital Zurich, Zürich, Switzerland; the [2]Retinal Electrodiagnostics Research Group, Department of Ophthalmology, University of Tübingen, Germany; and the [3]Central Laboratory of Chemistry, Inselspital Bern, Switzerland.

Supported by the Swiss National Science Foundation; the Velux Foundation, Glarus, Switzerland; Grants SFB430 C2, Se 837/1-1 and RE318/2-1 from the German Research Council; and Grant 517 from the University of Tübingen Fortüne.

Submitted for publication January 16, 2001; accepted February 28, 2001.

Commercial relationships policy: N.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Andreas Wenzel, Laboratory of Retinal Cell Biology, University Hospital Zurich, Sternwartstrasse 14, 8091 Zürich, Switzerland. awenzel@opht.unizh.ch

Light Damage

Light damage was induced in dark-adapted mice placed in cages with reflective interior by exposure to 5000 lux of diffuse white fluorescent light for up to 1 hour (lights on at 10 AM).[23] After light exposure, all mice were kept in darkness for 24 hours.

Morphology

Retinal morphology was analyzed 24 hours or 10 days after light exposure. Tissue preparation was performed as described previously.[26]

Electroretinogram Recordings

Ganzfeld electroretinograms (ERGs) were recorded with an International Society for Clinical Electrophysiology of Vision (ISCEV)-standard–compatible setup (Multiliner Vision; Jaeger/Toennies, Höchberg, Germany). ERGs were obtained from anesthetized mice 10 days after light exposure, after a 16-hour period of dark adaptation, according to described procedures.[30]

Determination of Serum CS

CS levels were determined by a commercial assay (DPC CAC Rat Corticosterone assay; Bühlmann Laboratories AG, Basel, Switzerland), before and after light exposure in serum from trunk blood collected within 60 seconds after handling and decapitation of the animal.

Electrophoretic Mobility Shift Assay

Nuclear extracts were prepared from dark-adapted mice and from mice immediately or 6 hours after light exposure. Electrophoretic mobility shift assays (EMSAs) were performed as described previously.[23] Briefly, 2.5 μg (5 μl) protein of nuclear extract was incubated on ice for 20 minutes with 19 μl of 5 mM $MgCl_2$, 0.1 mM EDTA, 0.75 mM dithiothreitol (DTT), 7.5% glycerol, 0.05% Nonidet P-40 containing 24 μg BSA, and 2 μg poly d(I-C) (Boehringer-Mannheim, Mannheim, Germany). ^{32}P end-labeled oligonucleotides (1 μl) coding for an activator protein (AP)-1–specific (5′-AAG CAT GAG TCA GAC AC-3′) DNA binding sequence (tetradecanoylphorbol acetate [TPA] response element, TRE) were added and incubation was continued for another 20 minutes. Protein–DNA complexes were resolved on a 6% polyacrylamide gel using 0.25× Tris borate electrophoresis (TBE) running buffer and were visualized on x-ray film.

Western Blot Analysis

Retinal nuclear extracts were separated by SDS-PAGE (10%), and Western blot analysis was performed according to standard protocols. For immunodetection, a polyclonal rabbit antiserum directed against GR (cat-no. sc 1004; Santa Cruz Biotechnology, Santa Cruz, CA) was applied. Horseradish peroxidase (HRP)–conjugated secondary antibody was applied (cat-no. sc 2004; Santa Cruz Biotechnology) and immunoreactivity visualized using a kit (Renaissance Western Blot Detection; Dupont NEN Life Science Products, Inc., Boston, MA).

Results

Elevated Serum CS and Resistance against Light-Induced Retinal Degeneration after Food Deprivation

Metabolic stress increases the release of CS.[31,32] To induce metabolic stress, mice were deprived of food for 16 hours. This treatment resulted in a more than sevenfold increase of serum CS levels as measured immediately before light exposure (Fig. 1). NF and FD mice were exposed for 1 hour to light of 5000 lux, an exposure duration exceeding the threshold for inducing light damage in NF mice at least threefold (Fig. 5 in Ref. 33). ERGs 10 days after light exposure showed a distinct loss of retinal function in NF mice (Figs. 2A, 2C). The morphologic analysis revealed a dramatic reduction of outer nuclear layer (ONL) thickness (Fig. 2E). In marked contrast, neither retinal function (Figs. 2B, 2D) nor retinal morphology (Fig. 2F) was affected by light exposure in FD mice.

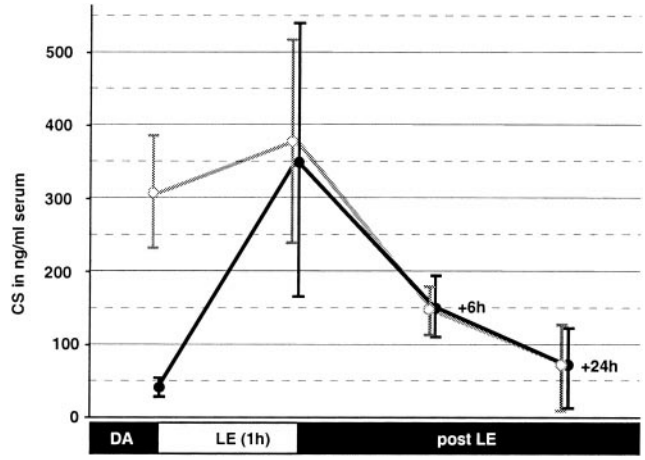

Figure 1. CS levels in serum of FD and NF mice before, during, and after light exposure. In NF mice (●) corticosterone (CS) levels were low after dark adaptation (DA) and increased approximately eightfold during light exposure (LE). In FD animals (○) CS levels were already elevated more than sevenfold before LE and were only marginally increased by the subsequent LE. After reaching comparable levels in both types of animals during LE, CS levels declined in parallel after LE (post LE), regardless of the prior feeding procedure. CS was measured in trunk blood; mean ± SD, $n = 3$.

An interesting observation was that light exposure per se induced a strong increase in serum CS levels in NF mice, whereas it did not further increase serum CS in FD mice (Fig. 1). Thus, immediately after light exposure, no difference in CS levels was detected between NF and FD mice. Similarly, serum CS in both types of mice declined in parallel, as determined 6 and 24 hours after the end of light exposure (Fig. 1).

Effect of Food Deprivation on the GR and AP-1

In FD mice, increased GR levels were detected in retinal nuclei preparations before light exposure (Fig. 3A; Nucleus: lane FD, d), whereas GR levels in the cytoplasm decreased (Fig. 3A; Cytoplasm: lane FD, d). Six hours after exposure, nuclear GR levels were still elevated (Fig. 3A; lane FD +6h). In NF mice, nuclear GR levels increased only after light exposure (Fig. 3A, compare NF, d with NF +6h), reaching levels similar to those in FD mice 6 hours after light exposure (Fig. 3A, compare NF +6h with FD +6h). This observation may reflect the delayed increase in serum CS during light exposure as a result of stress during the experimental procedure (Fig. 1). AP-1 DNA–binding activity, which normally increases during light exposure,[23,25] remained near basal levels in FD mice (Fig. 3B, FD: lanes d, i, and +6h) but increased in NF mice (Fig. 3B, NF: lanes d, i, +6h).

Several parameters, apart from GR-mediated inhibition of AP-1, may have the potential to modulate light damage susceptibility of retinal cells. Stress may upregulate, for example, Hsp70, which may inhibit apoptosis by interference with AP-1 activation[34,35] and which can protect against retinal light damage.[36] However, comparable levels of constitutive Hsp70 and inducible Hsp70 were found in both NF and FD mice before and after light exposure (Fig. 4), suggesting a protective mechanism independent of Hsp70.

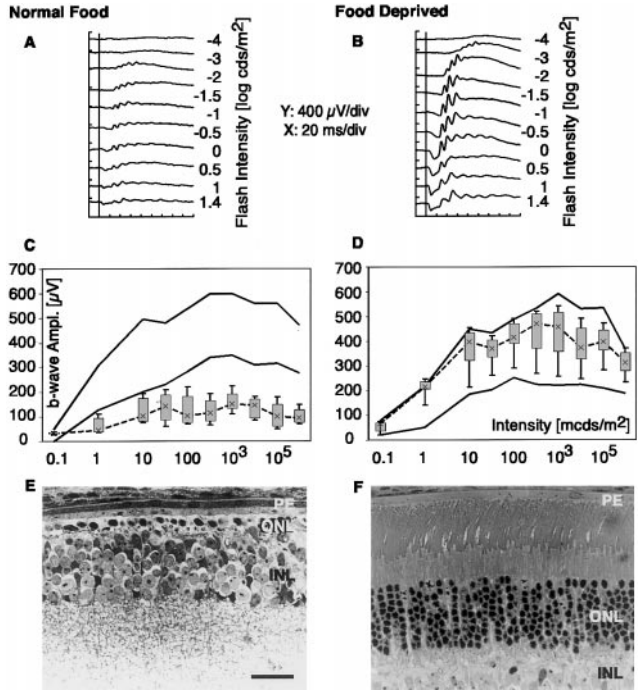

FIGURE 2. Food deprivation–induced protection against light-induced retinal degeneration. Exemplary retinal scotopic ERG intensity series in an NF mouse (**A**) and an FD mouse (**B**) after light exposure. All components of the ERG were strongly reduced in the NF animal but were unaffected in the FD mouse. A marked reduction of the average scotopic b-wave after light exposure was observed in NF mice (**C**), whereas no reduction resulted from light exposure in FD mice (**D**). *Dashed lines*: mark the median of the scotopic b-wave in three mice after light exposure, the *box* indicates 25% and 75% quantiles and the *whiskers* the 5% and 95% quantiles. *Solid lines*: the 5% and 95% quantiles (normal range) in unexposed mice, respectively. There is no significant difference between the normal range in unexposed NF and FD mice. Almost complete loss of photoreceptors was observed in NF mice 10 days after light exposure (**E**). The outer nuclear layer in large central areas was reduced to one row of photoreceptor nuclei and the inner nuclear layer (INL) was in proximity to the apparently unaffected pigment epithelium (PE). The same retinal area looked unaffected in an FD mouse 10 days after light exposure (**F**). Photoreceptors showed normal density and morphology, including well-conserved rod outer and inner segments. Histology was prepared from those eyes used for ERG recordings. Representative of three independent experiments. Scale bar, 25 μm.

Furthermore, levels of rhodopsin, the primary chromophore for light damage,[37] as well as the rate of rhodopsin regeneration after bleaching, which determines the light damage susceptibility of photoreceptors,[33] was comparable in FD and NF mice (not shown), excluding an influence of fasting-induced stress on rhodopsin metabolism. Altered signal flow in phototransduction may affect the vulnerability of photoreceptors for light damage.[3,4] However, from ERG recordings, no evidence for fasting-mediated effects on phototransduction were observed (Figs. 2C, 2D). Moreover, mice reared on a defined artificial diet were as susceptible to light damage as mice reared on the conventional diet (not shown), thus excluding contamination of the conventional diet with photosensitizing agents. Food deprivation was associated with a steep reduction (15%) in body weight (FD: 17 ± 1 g, $n = 15$; NF: 20 ± 1 g, $n = 11$) and may have caused a metabolic state that did not allow apoptosis of photoreceptors to occur. However, in both NF and FD mice photoreceptor apoptosis induced by *N*-methyl-*N*-nitrosourea[23] was comparable, excluding that the execution of apoptosis was inhibited in general (not shown).

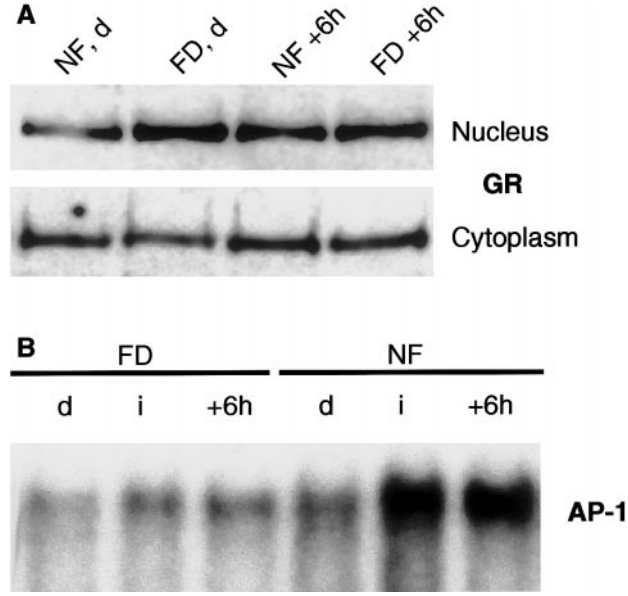

FIGURE 3. Effect of food deprivation and light on AP-1 and GR. (**A**) Nuclear translocation of GR after food deprivation. In retinal nuclear extracts from FD mice (10 μg protein loaded), levels of GR immunoreactivity were highest before (d) and were elevated 6 hours (6h) after light exposure. In extracts from NF mice, GR levels were low before light exposure but were increased after light exposure. The high levels of GR in nuclei of FD mice before light exposure were accompanied by lowered GR levels in the corresponding cytoplasmic fraction (20 μg protein loaded), indicating translocation of GR from the cytoplasm to the nucleus. (**B**) AP-1 DNA binding activity before (d), immediately (i), and 6 hours (6h) after light exposure (+6h) in the same extracts (2.5 μg protein). Light-induced activation of AP-1 was almost completely prevented by food-deprivation (FD), whereas in NF mice, the typical increase in AP-1 activity was observed immediately and 6 hours after 1 hour of light exposure. Representative of three independent experiments.

Stress-Mediated Protection against Light Damage Mimicked by DEX

To specifically test the hypothesis that elevation of GR activity can protect the retina from the deleterious effects of excessive light, we applied the synthetic GR agonist DEX. By using this selective pharmacologic compound, we not only excluded any undetected and nonspecific effects of the fasting-induced metabolic stress but simultaneously tested a compound with a long-standing record for application in patients.

Dark-adapted mice received a single intraperitoneal injection of DEX or saline solution immediately before light exposure or immediately thereafter. After 24 hours in darkness, the morphologic examination revealed substantial photoreceptor damage in the inferior central retina of saline-injected mice exposed for 20 minutes (Fig. 5B) and for 1 hour (Fig. 5C).

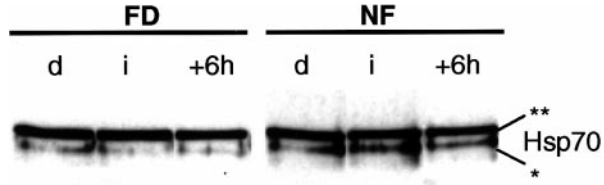

FIGURE 4. Effect of food deprivation and light on Hsp70. Neither light exposure nor food deprivation increased the amount of inducible (*) or constitutive (**) Hsp70 in retinal total homogenates (30 μg protein loaded). Representative of three independent experiments.

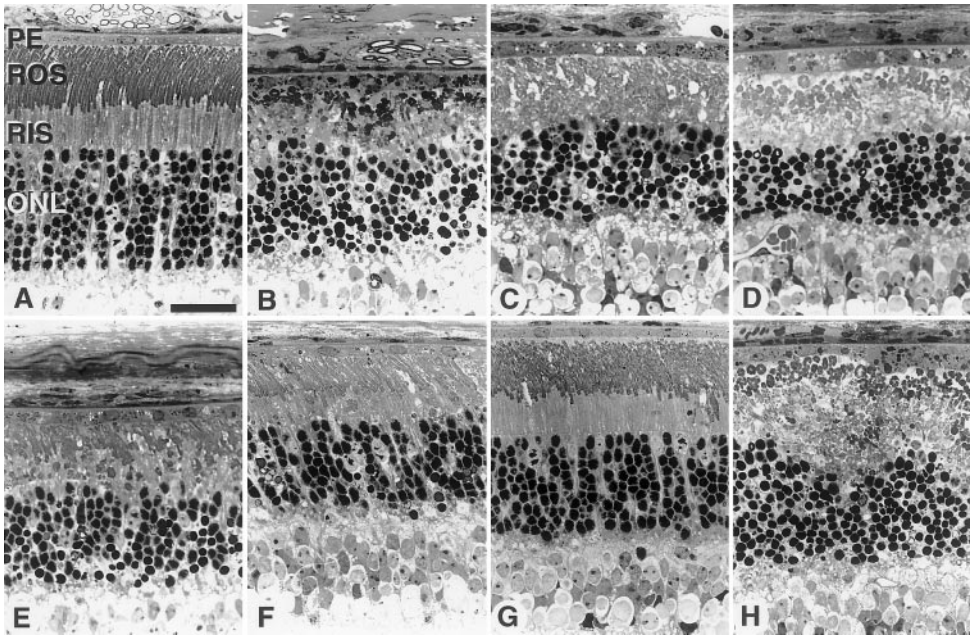

FIGURE 5. Protection of retinal morphology against light damage by DEX. (**A**) Retinal morphology of a BALB/c mouse injected with 52 mg/kg DEX and prepared after 24 hours in darkness. No signs of morphologic alterations due to the DEX treatment were found. (**B**) Saline-injected animal exposed for 20 minutes to fluorescent white light and analyzed 24 hours after the end of light exposure. The majority of photoreceptor nuclei in the inferior central retina were condensed, indicating ongoing apoptosis. The pigment epithelium (PE) was swollen, and rod outer segments (ROS) and rod inner segments (RIS) were largely disrupted. (**C**) Saline-injected animal exposed for 60 minutes and analyzed 24 hours after the end of light exposure. The morphologic damage appeared similar to that in a retina after a 20-minute exposure but was present in larger areas (not shown). (**D**) Pretreatment with 7 mg/kg DEX did not confer protection against light damage (60 minute). (**E**) In mice pretreated with 22 mg/kg DEX, retinal morphology was partially conserved after exposure for 60 minutes. Less photoreceptor nuclei in the outer nuclear layer (ONL) appeared pyknotic, and disintegration of ROS was less pronounced than in saline-injected mice. (**F**) A dose of 37 mg/kg DEX further facilitated this protective effect. (**G**) Pretreatment with 52 mg/kg DEX suppressed light damage completely on a morphologic level. (**H**) Posttreatment with the same dose of DEX immediately after light exposure had no beneficial effect. (**B-H**) Light intensity, 5000 lux. Representative of three independent experiments. Scale bar, 25 μm.

Application of DEX immediately before light exposure prevented photoreceptor damage in a dose-dependent manner (Figs. 5D–G). Whereas DEX at 7 mg/kg body weight had no effect, 22 and 37 mg/kg partially conserved retinal morphology after light exposure, and with a DEX dose of 52 mg/kg (Fig. 5G), retinal morphology was indistinguishable from that of DEX-injected mice that were not exposed to damaging light (Fig. 5A).

The DEX-mediated protection of the retina against light damage was complete. Retinal morphology was unaffected 10 days after light exposure (Fig. 6C). In contrast, the outer nuclear layer (ONL) in saline-treated mice was reduced from 10 to 12 rows of photoreceptor nuclei to 1 to 2 rows at that time (Fig. 6B). Similarly, data from ERG analysis reflected the morphologic findings: ERGs recorded from light-exposed mice pretreated with 52 mg/kg DEX (Fig. 6G) were indistinguishable from those of saline-injected nonexposed control mice (Fig. 6E), demonstrating that not only morphology, but also retinal function was fully protected. In contrast, light exposure sharply reduced a- and b-waves in mice injected with saline (Fig. 6F). DEX at 52 mg/kg, applied immediately after light exposure, had no beneficial effect (Figs. 5H, 6D, 6H).

DEX per se did not influence functional properties of the retina, which in turn might influence light damage susceptibility of photoreceptors. Phototransduction, judged from ERG recordings (not shown), rhodopsin levels, and rhodopsin regeneration kinetics were comparable to saline-injected or untreated control animals (not shown).

DEX-Induced Activation of GR and Inhibition of AP-1 in the Retina

In retinal nuclear extracts of saline-injected light-exposed mice, AP-1 DNA binding activity peaked approximately 6 hours after the end of exposure to damaging doses of light[23] (Fig. 7). This increase in AP-1 activity was largely abolished in nuclear extracts of mice pretreated with DEX (Fig. 7). Nuclear GR levels in saline-injected mice were unchanged immediately after light exposure and were moderately elevated after 6 hours (Fig. 7). In contrast, mice pretreated with DEX showed strongly increased levels of nuclear GR immediately and 6 hours after light exposure (Fig. 7). Thus, similar to the stress-induced elevation of serum corticosteroid levels, the systemic DEX pretreatment was capable of activating retinal GR and of inducing its translocation to the nucleus during light exposure, providing the prerequisite for inhibition of AP-1 activity by GR.

DISCUSSION

Stress-induced secretion of CS and application of DEX activated retinal GR. Activated GR can inhibit AP-1,[15–18] which in turn is essential for the induction of photoreceptor apoptosis by light.[23] GR-mediated inhibition may occur in the nucleus of retinal cells by a protein–protein interaction of both transcription factors.[16–18,38] Thus, induction of GR activity prevents light-induced retinal degeneration by interference with AP-1–dependent steps of apoptosis induction.

AP-1–Dependent Steps of Light-Induced Photoreceptor Apoptosis Blocked by Activation of GR

DEX induced an almost complete inhibition of AP-1 when applied before light exposure. Nevertheless, a minimal increase in AP-1 activity was observed after application of DEX or food deprivation (Figs. 3, 7). This remaining AP-1 activity, however, appears insufficient to mediate photoreceptor cell death in general, or it may represent commitment to apoptosis in a small number of photoreceptors undetectable by our functional or morphologic analysis.

Among the proteins that can constitute the AP-1 complex (c-Fos, Fra-1, Fra-2, Fos-B and c-Jun, and Jun-B and Jun-D),[22] c-Fos is a major component of light-induced AP-1.[23,25] AP-1 containing c-Fos, however, appears particularly amenable to inhibitory cross talk with GR.[38] Thus, activation of GR by DEX

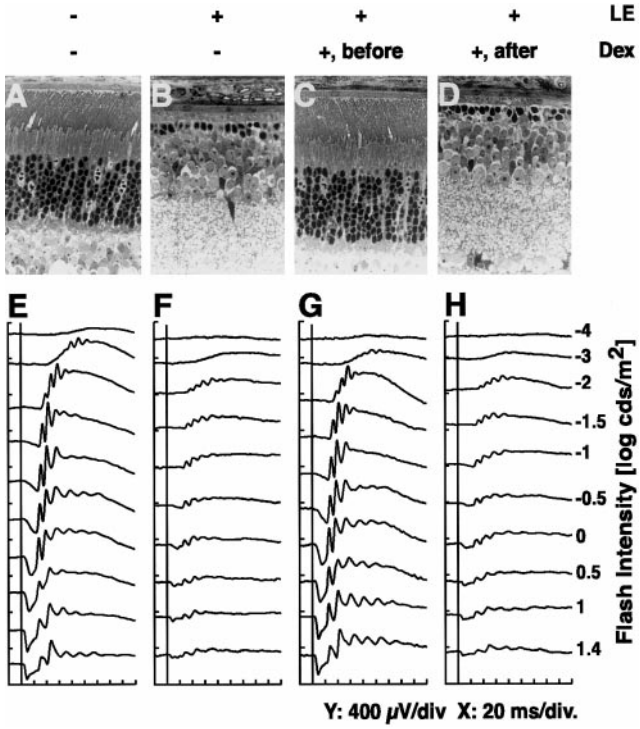

FIGURE 6. DEX-mediated protection of retinal function and morphology. Retinal sections and ERGs were from unexposed (LE−) mice or light-exposed (LE+) mice. Mice received either 52 mg/kg DEX (Dex+) or saline injections (Dex−). ERG data and morphology were obtained from the same eye 10 days after the respective treatment. Exemplary retinal morphology (**A**) and scotopic ERG intensity series (**E**) in a control animal (i.e., without light exposure and DEX application). Morphology (**B**) and ERG (**F**) in a light-damaged animal (i.e., with light exposure but no steroid application). Morphology (**C**) and ERG (**G**) in a protected animal (i.e., with light exposure and steroid pretreatment; before). Morphology (**D**) and ERG (**H**) in a mouse with delayed steroid application (i.e., with light exposure and steroid application after light exposure; after). In mice that received no DEX before light exposure, the outer nuclear layer (ONL) of large central areas was reduced to one or two rows of photoreceptor nuclei (**B**). Rod outer segments (ROS) were almost completely absent, and the ERG was strongly reduced (**F**). Remaining activity may represent peripheral regions of the retina, which are less affected in light damage. In contrast, retinas of mice pretreated with DEX showed regular morphology (**C**), and the ERG was normal (**G**). In comparison, mice treated with the same dose of DEX after light exposure (**D**, **H**) showed no evidence of protection against light damage. Representative of three independent experiments.

may have resulted in an inhibitory protein–protein interaction between the GR and c-Fos–containing AP-1.[16–18,38]

Alternatively, DEX may also decrease transcription of c-*fos*,[39–41] thus depleting an essential constituent of AP-1 in light induced apoptosis.[23–25] However, in line with other reports,[42] DEX pretreatment increased even c-*fos* expression (not shown). Similarly, the gene of the major partner of c-Fos in light-induced AP-1, c-Jun,[25,43] was expressed at higher levels after DEX treatment (not shown). Thus, the absence of an AP-1 response after light appears to be based solely on the inhibitory cross talk of GR and AP-1.

Other functional properties of the retina, which may also influence light damage susceptibility of photoreceptors, were not affected by treatment with DEX. Also, in fasted mice, results indicated that the observed protection was specifically brought about by GR and AP-1 interference. Food deprivation did not influence retinal function or rhodopsin metabolism and did not induce an energy deficiency that prevented apoptosis.

Timing of GR-Mediated Photoreceptor Rescue

Light exposure increases retinal AP-1 activity within 15 minutes[23] and induced photoreceptor loss had occurred at as early as 20 minutes of exposure (Fig. 5B). Therefore, timing of GR-mediated inhibition of AP-1 may be crucial for protecting the retina. GR may have to be activated and translocated before an AP-1 response sufficient to mediate apoptosis can be induced.

Indeed, findings in food deprivation and DEX experiments support this assumption. Only when CS levels were raised before light exposure and only when DEX was applied before light exposure were retinal function and morphology protected. Under both conditions, we found elevated nuclear GR levels before or immediately after light exposure. Elevation of CS induced by light exposure also resulted in an increase of nuclear GR (Fig. 3B). However, the increase was observed only after light exposure (Fig. 7). This light-induced activation of GR, similar to application of DEX after light exposure, was insufficient to rescue photoreceptors (Figs. 5H, 6D, 6H). Thus, only when activated during the phase of increasing AP-1 activity may GR prevent AP-1–dependent steps of apoptosis induction. Once sufficient AP-1 complexes have been activated, an induction of GR activity may no longer interfere with the signal cascade triggered by AP-1. Therefore, a rescue of retinal cells after AP-1 has been activated may be possible only by interfer-

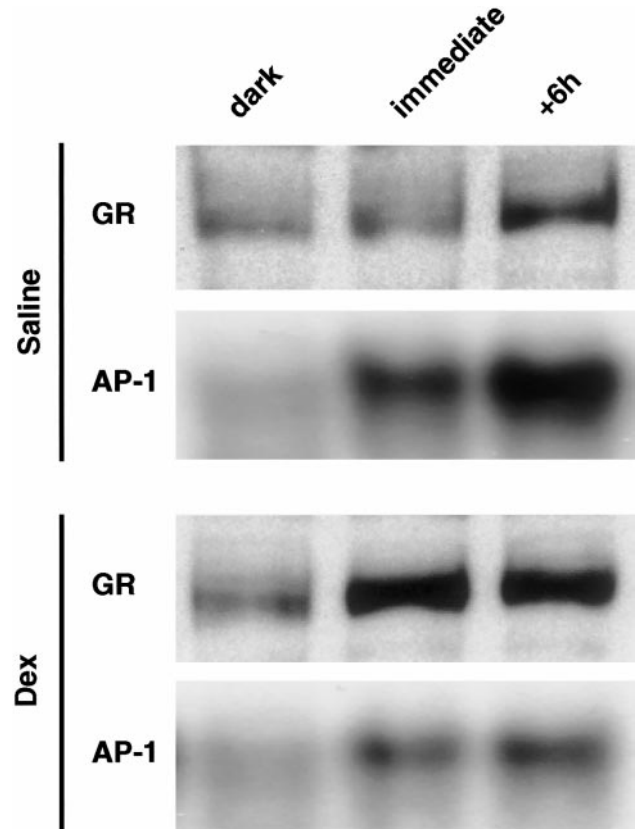

FIGURE 7. Nuclear GR immunoreactivity and AP-1 activity in retinal nuclear extracts after DEX treatment. GR immunoreactivity increased in nuclear extracts of saline-injected mice between the end of light exposure (immediate) and 6 hours thereafter. In these animals AP-1 activity increased during light exposure to reach a peak 6 hours thereafter. In mice pretreated with DEX, nuclear GR levels had already increased during light exposure and remained elevated thereafter. Under these circumstances the normally observed strong increase in AP-1 DNA–binding activity was abolished, and photoreceptors resisted light exposure. Representative of three independent experiments.

ing with the currently unknown effector cascades downstream of AP-1.

Conclusions

Because glucocorticoids have a long-standing and successful record for application in human patients, new steroid-based treatments may be envisioned for the prevention of those blinding retinal diseases in which light is a cofactor.[1–9] Additionally, application of DEX may be beneficial for the prevention of retinal damage that may be induced during intraocular surgery where high doses of light are applied for prolonged periods.[44] Apart from the retina, steroids may also be beneficial for treating excitotoxicity-induced brain lesions, in which interference with AP-1 activation has also been shown to be neuroprotective.[45]

Acknowledgments

The authors thank Gabi Hoegger, Coni Imsand, Dora Greuter, and Karin Mai for skilled technical assistance and Theo Seiler for continuous support.

References

1. Organisciak DT, Li M, Darrow RM, Farber DB. Photoreceptor cell damage by light in young Royal College of Surgeons rats. *Curr Eye Res.* 1999;19:188–196.
2. Sanyal S, Hawkins RK. Development and degeneration of retina in rds mutant mice: effects of light on the rate of degeneration in albino and pigmented homozygous and heterozygous mutant and normal mice. *Vision Res.* 1986;26:1177–1185.
3. Chen J, Simon MI, Matthes MT, Yasumura D, LaVail MM. Increased susceptibility to light damage in an arrestin knockout mouse model of Oguchi disease (stationary night blindness). *Invest Ophthalmol Vis Sci.* 1999;40:2978–2982.
4. Chen CK, Burns ME, Spencer M, et al. Abnormal photoresponses and light-induced apoptosis in rods lacking rhodopsin kinase. *Proc Natl Acad Sci USA.* 1999;96:3718–3722.
5. Wang M, Lam TT, Tso MO, Naash MI. Expression of a mutant opsin gene increases the susceptibility of the retina to light damage. *Vis Neurosci.* 1997;14:55–62.
6. LaVail MM, Gorrin GM, Yasumura D, Matthes MT. Increased susceptibility to constant light in nr and pcd mice with inherited retinal degeneration. *Invest Ophthalmol Vis Sci.* 1999;40:1020–1024.
7. Cideciyan AV, Hood DC, Huang Y, et al. Disease sequence from mutant rhodopsin allele to rod and cone photoreceptor degeneration in man. *Proc Natl Acad Sci USA.* 1998;95:7103–7108.
8. Cruickshanks KJ, Klein R, Klein BE. Sunlight and age-related macular degeneration: The Beaver Dam Eye Study. *Arch Ophthalmol.* 1993;111:514–518.
9. Simons K. Artificial light and early-life exposure in age-related macular degeneration and in cataractogenic phototoxicity. *Arch Ophthalmol.* 1993;111:297–298.
10. Remé CE, Grimm C, Hafezi F, Marti A, Wenzel A. Apoptotic cell death in retinal degenerations. *Prog Retina Eye Res.* 1998;17:443–464.
11. Cox G. Glucocorticoid treatment inhibits apoptosis in human neutrophils: separation of survival and activation outcomes. *J Immunol.* 1995;154:4719–4725.
12. Messmer UK, Winkel G, Briner VA, Pfeilschifter J. Glucocorticoids potently block tumour necrosis factor-alpha- and lipopolysaccharide-induced apoptotic cell death in bovine glomerular endothelial cells upstream of caspase 3 activation. *Br J Pharmacol.* 1999;127:1633–1640.
13. Pagliacci MC, Migliorati G, Smacchia M, Grignani F, Riccardi C, Nicoletti I. Cellular stress and glucocorticoid hormones protect L929 mouse fibroblasts from tumor necrosis factor alpha cytotoxicity. *J Endocrinol Invest.* 1993;16:591–599.
14. Yang Y, Mercep M, Ware CF, Ashwell JD. Fas and activation-induced Fas ligand mediate apoptosis of T cell hybridomas: inhibition of Fas ligand expression by retinoic acid and glucocorticoids. *J Exp Med.* 1995;181:1673–1682.
15. Feng Z, Marti A, Jehn B, Altermatt HJ, Chicaiza G, Jaggi R. Glucocorticoid and progesterone inhibit involution and programmed cell death in the mouse mammary gland. *J Cell Biol.* 1995;131:1095–1103.
16. Jonat C, Rahmsdorf HJ, Park KK, et al. Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. *Cell.* 1990;62:1189–1204.
17. Schule R, Rangarajan P, Kliewer S, et al. Functional antagonism between oncoprotein c-Jun and the glucocorticoid receptor. *Cell.* 1990;62:1217–1226.
18. Yang-Yen HF, Chambard JC, Sun YL, et al. Transcriptional interference between c-Jun and the glucocorticoid receptor: mutual inhibition of DNA binding due to direct protein–protein interaction. *Cell.* 1990;62:1205–1215.
19. Gottlicher M, Heck S, Herrlich P. Transcriptional cross-talk, the second mode of steroid hormone receptor action. *J Mol Med.* 1998;76:480–489.
20. Karin M. New twists in gene regulation by glucocorticoid receptor: is DNA binding dispensable? *Cell.* 1998;93:487–490.
21. Reichardt HM, Kaestner KH, Tuckermann J, et al. DNA binding of the glucocorticoid receptor is not essential for survival. *Cell.* 1998;93:531–541.
22. Karin M, Liu Z, Zandi E. AP-1 function and regulation. *Curr Opin Cell Biol.* 1997;9:240–246.
23. Wenzel A, Grimm C, Marti A, et al. c-Fos control animals the "private pathway" of light-induced apoptosis of retinal photoreceptors. *J Neurosci.* 2000;20:81–88.
24. Hafezi F, Steinbach JP, Marti A, et al. The absence of c-fos prevents light-induced apoptotic cell death of photoreceptors in retinal degeneration in vivo. *Nat Med.* 1997;3:346–349.
25. Hafezi F, Marti A, Grimm C, Wenzel A, Remé CE. Differential DNA binding activities of the transcription factors AP-1 and Oct-1 during light-induced apoptosis of photoreceptors. *Vision Res.* 1999;39:2511–2518.
26. Kueng-Hitz N, Grimm C, Lansel N, et al. The retina of c-fos+/+ and c-fos-/- mice: Electrophysiological, morphological and biochemical aspects. *Invest Ophthalmol Vis Sci.* 2000;41:909–916.
27. Suzuki T, Sasano H, Kaneko C, Ogawa S, Darnel AD, Krozowski ZS. Immunohistochemical distribution of 11beta-hydroxysteroid dehydrogenase in human eye. *Mol Cell Endocrinol.* 2001;173:121–125.
28. Yoshida K, Kawamura K, Imaki J. Differential expression of c-fos mRNA in rat retinal cells: regulation by light/dark cycle. *Neuron.* 1993;10:1049–1054.
29. Imaki J, Yamashita K, Yamakawa A, Yoshida K. Expression of jun family genes in rat retinal cells: regulation by light/dark cycle. *Brain Res Mol Brain Res.* 1995;30:48–52.
30. Biel M, Seeliger M, Pfeifer A, et al. Selective loss of cone function in mice lacking the cyclic nucleotide-gated channel CNG3. *Proc Natl Acad Sci USA.* 1999;96:7553–7557.
31. Tinnikov AA. Responses of serum corticosterone and corticosteroid-binding globulin to acute and prolonged stress in the rat. *Endocrine.* 1999;11:145–150.
32. Komori T, Fujiwara R, Shizuya K, Miyahara S, Nomura J. The influence of physical restraint or fasting on plaque-forming cell response in mice. *Psychiatry Clin Neurosci.* 1996;50:295–298.
33. Wenzel A, Remé CE, Williams TP, Hafezi F, Grimm C. The *Rpe65* Leu450Met variation increases retinal resistance against light-induced degeneration by slowing rhodopsin regeneration. *J Neurosci.* 2001;21:53–58.
34. Ahn JH, Ko YG, Park WY, Kang YS, Chung HY, Seo JS. Suppression of ceramide-mediated apoptosis by HSP70. *Mol Cells.* 1999;9:200–206.
35. Gabai VL, Meriin AB, Mosser DD, et al. Hsp70 prevents activation of stress kinases: a novel pathway of cellular thermotolerance. *J Biol Chem.* 1997;272:18033–18037.
36. Barbe MF, Tytell M, Gower DJ, Welch WJ. Hyperthermia protects against light damage in the rat retina. *Science.* 1988;241:1817–1820.

37. Grimm C, Wenzel A, Hafezi F, Yu S, Redmond TM, Reme CE. Protection of Rpe65-deficient mice identifies rhodopsin as a mediator of light-induced retinal degeneration. *Nat Genet.* 2000;25: 63–66.
38. Lucibello FC, Slater EP, Jooss KU, Beato M, Muller R. Mutual transrepression of Fos and the glucocorticoid receptor: involvement of a functional domain in Fos which is absent in FosB. *EMBO J.* 1990;9:2827–2834.
39. Ekert P, MacLusky N, Luo XP, et al. Dexamethasone prevents apoptosis in a neonatal rat model of hypoxic-ischemic encephalopathy (HIE) by a reactive oxygen species-independent mechanism. *Brain Res.* 1997;747:9–17.
40. Hass R, Brach M, Kharbanda S, Giese G, Traub P, Kufe D. Inhibition of phorbol ester-induced monocytic differentiation by dexamethasone is associated with down-regulation of c-fos and c-jun (AP-1). *J Cell Physiol.* 1991;149:125–131.
41. Karagianni N, Tsawdaroglou N. The c-fos serum response element (SRE) confers negative response to glucocorticoids. *Oncogene.* 1994;9:2327–2334.
42. Briski KP, DiPasquale BM, Gillen E. Induction of immediate-early gene expression in preoptic and hypothalamic neurons by the glucocorticoid receptor agonist, dexamethasone. *Brain Res.* 1997; 768:185–196.
43. Hafezi F, Grimm C, Wenzel A, Abegg M, Yaniv M, Reme CE. Retinal photoreceptors are apoptosis-competent in the absence of JunD/AP-1. *Cell Death Diff.* 1999;6:934–936.
44. van Den Biesen PR, Berenschot T, Verdaasdonk RM, van Weelden H, van Norren D. Endoillumination during vitrectomy and phototoxicity thresholds. *Br J Ophthalmol.* 2000;84:1372–1375.
45. Yang DD, Kuan C-Y, Whitmarsh AJ, et al. Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the Jnk3 gene. *Nature.* 1997;389:865–870.