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ENDOGENOUS GLUCOCORTICOIDS PARTICIPATE IN RETINAL DEGENERATION DURING CONTINUOUS ILLUMINATION

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Continuous illumination (CI) induces an oxidative stress of the retina which is involved in light-induced retinal degeneration (LIRD). As the increase of glucocorticoids (GC) could also collaborate in the damage, adrenalectomized (ADX) and sham-operated rats (control, CTL) were submitted to CI, and their eyes were studied at light and electron microscopic levels. After CI, ADX retinas were significantly thicker than CTL retinas. Retinal alterations appeared earlier and were severer in CTL than in ADX retinas. Corticosterone levels increased gradually in the sera of CTL rats along CI. These results suggest that adrenalectomy attenuates LIRD, supporting the hypothesis.

Keywords Corticosterone, degeneration, glucocorticoid, illumination, photoreceptor, retina

INTRODUCTION

Continuous illumination induces retinal degeneration of photoreceptors (PRs) both in adult (Gorn & Kuwabara, 1967; Kuwabara, 1966; Noell, Walker, Kang, & Berman, 1966) and in immature rats (Pecci Saavedra & Pellegrino de Iraldi, 1976). Membrane disorganization and tubular transformation of the PR discs characterizes the degenerative process which is followed by the phagocytosis of PR segments by the retinal pigment epithelial cells (RPE) (Pecci Saavedra & Pellegrino de Iraldi, 1976).

Lifetime cumulative effect of light and the experimental exposure to UV radiation produce free radicals which generate the photic damage of the retina (Organisciak et al., 1998). Reactive oxygen species (ROS) peroxidate membrane phospholipids, modify important proteins involved in signal transduction, induce DNA damage, and trigger an apoptotic pathway. In fact, apoptotic markers such as procaspase-3 (Wu, Gorman, Zhou, Sandra, & Chen, 2002), caspase-1 (Grimm, Wenzel, Hafezi, & Reme, 2000), and DNA fragments (Abbler, Chang, Full, Tso, & Lam, 1996) were detected in rodent retinas using different models of CI. In humans, the oxidative stress has been postulated as an important factor in the pathophysiology of age-related macular degeneration (AMD) (Beatty, Koh, Phil, Henson, & Boulton, 2000) and retinitis

pigmentosa, while apoptosis was observed in AMD and pathologic myopia (Xu, Li, & Tso, 1996).

Although oxidative stress is an important etiological factor of LIRD, stress hormones such as GC could also be involved in it. Continuous illumination is a stressful event that alters circadian rhythm and increases levels of GC (Persengiev & Kanchev, 1991). High GC levels have been shown to be deleterious to hippocampal pyramidal neurons. The atrophy of dendrites of CA3 neurons is an early marker of damage induced by GC (Mc Ewen, 1994). Lifetime exposure to GC is a determinant of neuronal death during aging in the rat (Sapolsky, 1985a, 1985b; Sapolsky, Krey, & Mc Ewen, 1985), and GCs modulate the severity of neuronal damage after seizures or ischaemia (Stein & Sapolsky, 1988). Adrenalectomy or the use of metirapone, an inhibitor of the synthesis of GC, protects neurons of the CA3 hippocampal region from kainite-induced seizures (Stein & Sapolsky, 1988).

The actions of GC are mediated by specific GC receptors (GR) that belong to the superfamily of nuclear receptors which include the receptors of the thyroid hormone, vitamin D, and retinoids. One GR subtype, named type I receptor, plays an important role in programmed cell death (Mc Ewen, 1994; Schimmer Y Parker, 1996). In human eyes, type I GR was detected in all retinal layers, where it plays an important role in the function and homeostasis of the organ (Suzuki et al., 2001). Meanwhile, type 2 GR was detected in the ciliary body epithelium, where it is involved in aqueous humor production (Suzuki et al., 2001).

In order to verify the hypothesis that during CI an increase of GC could collaborate with LIRD, ADX and CTL rats were exposed to different periods of CI (0, 1, 2, 5, and 7 days). Structural and ultrastructural alterations of their retinas were studied, and retinal thicknesses in both rat groups were determined along the different periods of CI. Also corticosterone (CORT) levels were determined in both rat groups. Preliminary results of this investigation were previously communicated to the Association for Research in Vision and Ophthalmology (ARVO) annual meeting (Julián, López, Coirini, & López-Costa, 2004) and to the XVII International Congress of Eye Reserch (López-Costa et al., 2006).

MATERIAL AND METHODS

Materials

Ketamine (Ketalar®, Parke Davis) and chloral hydrate (Anedra) were used.

Sucrose was from Sigma Chemical, and mono and dibasic sodium phosphates were from Anedra. Optical coherence tomography (OCT) compound (Tissue-Tek®) was bought from Sakura Finetek, and gelatin was from J. T. Baker. Absolute alcohol, lead nitrate, sodium citrate, sodium chloride, hematoxiline, eosine, and toluidine blue were from Merck. Paraformaldehyde was from ICN Biochemicals, and glutaraldehyde, propylene oxide, Durcupan®, and DPX (Distyrene Plasticizer Xylene) were from Fluka Chemie AG. Osmium tetroxide and uranyl acetate were from Electron Microscopy Sciences.

CORT antibody was a generous gift from Dr. B. Belanger, Laval University, Quebec, Canada.

Methods

Forty male Sprague Dawley albino rats (body weight 250 g, age 60 days) were used in this study. Rats were bred in the facilities of Instituto de Biología y Medicina Experimental (IBYME) under light/dark cycles of 12/12 hrs (light on 7 a.m.) with water and food ad libitum. Half of the animals were adrenalectomized (ADX) by bilateral approximation under ketamine anesthesia (50 mg/kg) The other half, used as control (CTL), were sham-operated. Animals were submitted to experimental procedures 18 hrs after surgery. During all experimental procedures ADX animals were supplied with 0.9% NaCl as drinking fluid. Surgery was performed at early evening (6 p.m.). Animal care was in accordance with the National Institutes of Health *Guidelines for the Care and Use of Laboratory Animals* and the principles presented in the *Guidelines for the Use of Animals in Neuroscience and Behavioral Research* by the Society for Neuroscience.

Animal Model of Continuous Illumination

Four different experiments were performed. For each experiment 10 animals (5 CTL and 5 ADX) were used. Two rats (1 CTL and 1 ADX) were euthanised without CI being submitted to them, and their eyes were processed (time point 0), while the remaining 8 rats (4 CTL and 4 ADX) were simultaneously placed in an open white acrylic box of 60 cm × 60 cm × 60 cm with 12 halogen lamps (12 V 50 W each) located on top. White light spectrum (400–700 nm) was used, and lighting level (12,000 lx) was determined using an analogical Gossen illuminance meter. The lighting box was kept in an air-conditioned room, and temperature was monitored and maintained at 24°C approx. All the animals received food and fluids ad libitum.

In each experiment, 2 illuminated rats (1 ADX and 1 CTL) were killed randomly (e.g., at each time point) after 1, 2, 5, and 7 days of CI. The nomenclature used in this paper to mention each illuminated rat condition is either ADX-1, ADX-2, ADX-5, and ADX-7 or CTL-1, CTL-2, CTL-5, and CTL-7, depending on the surgical procedure (adrenalectomy or sham operation) and the time of light exposition. Beside, in each experiment, 1 nonilluminated ADX rat (ADX-0) and 1 nonilluminated CTL rat (CTL-0) were euthanized to obtain their retinas (time point 0).

Schedule of Continuous Illumination

Tissue Processing and Histological Procedure for Light and Electron Microscopy. The eyes of 30 rats (15 ADX and 15 CTL) were used to perform light microscopy (LM), and the eyes of 10 rats (5 ADX and 5 CTL) were used to obtain semithin sections for LM and ultrathin sections for electron microscopy (EM).

For light microscopy, 30 rats from the first three experiments (15 ADX and 15 CTL) were deeply anesthetized at the different timepoints with chloral hydrate (350 mg/kg), and their eyes were removed; the cornea and lenses were cut off, and the remaining tissues with a cupule shape were fixed by immersion in a solution containing 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 24 hrs. Eyes were immersed in a solution containing 30% sucrose in 0.1 M phosphate buffer and were embedded in Tissue-Tek. The frozen eyes were cut along the vertical meridian at –20°C using a Lauda Leitz cryostat, and sections (thickness 15 μm) were stained with hematoxiline-eosine. Sections were mounted on gelatin-coated glass slides and covered with a coverslip using DPX. Sections were observed with a 40X objective using a Zeiss Axiophot LM, and retinal thicknesses were determined (as described in the next section).

The last set of experimental animals (5 ADX and 5 CTL) were processed to obtain semithin sections for LM and ultrathin sections for EM. In this case eyes were fixed by immersion in a solution containing 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 24 hrs. The eyes were cut in four pieces along two perpendicular meridians. The tissue blocks containing schlerotica, choroid, and retina were postfixed in 1% osmium tetroxide (O_4Os), dehydrated, and embedded in Durcupan. Semithin sections (thickness 0.5–1 μm) were obtained with a Reichert-Jung ultramicrotome (model Ultracut E) and were stained with toluidine blue. Sections were mounted using DPX and were observed and

photographed using a Zeiss Axiophot LM. Ultrathin sections (thickness 70–90 nm) were counterstained with lead citrate and were observed and photographed using a Zeiss 10C EM.

Image Analysis

The entire thicknesses of CTL and ADX rat retinas from the PR layer to the ganglion cell layer (GCL) were measured using a computerized image analyzer (VIDAS-Kontron, Zeiss) attached to an LM (Axiophot, Zeiss). Six retinas of the control ($n = 3$) and six retinas of experimental rats ($n = 3$) were analyzed at each time point. Three different sections per retina were measured 10 times at different points, randomly selected in the posterior pole retina (Rapp & Williams, 1980). A total number of 180 determinations per experimental condition was obtained. The same analysis was performed in CTL and ADX animals. All the quantified sections were from the same region of the eye. Results were expressed as mean $\pm$ standard deviation.

Statistical Analysis

Data were averaged and statistically analyzed using the Student-Newman-Keuls multiple comparison test, and $p < .05$ was considered significant. The statistics program Graphpad InStat V2.05a was used.

CORTICOSTERONE Determination

Blood samples were taken at the different time points by intracardiac puncture before the rats were killed. After the clot formation the supernatant serum was used to determine CORT concentrations.

CORT levels were determined by Radioimmunoassay (RIA) using a specific antibody. Sera (15 μl) were extracted with 300 μl of dichloromethanol. Tubes were centrifuged at 30,000 rpm at 4°C. Aqueous phase was discarded, and organic phase was evaporated at 35°C. Precipitate was resuspended in 150 μl of phosphate buffer (pH 7.4), and duplicate samples of 20 μl were measured. Calibration curve was established with increasing concentration of CORT (35–100 pg).

RESULTS

Light Microscopy

Adrenalectomy itself did not alter the histology of the retinas, and no significant differences in retinal thickness were observed between ADX-0 and CTL-0 rats ($p > .05$) (Figure 1).

In CTL-1 condition, we observed the disarrangement of outer photoreceptor segments (OPS) and dense pycnotic nuclei in the outer nuclear layer (ONL) (Figure 2A) accompanied by a decrease of retinal thickness (Figure 1).

ADX-1 rats, which were submitted to the same period of CI, also showed some pycnotic nuclei in ONL (Figure 2B). Retinal thickness of ADX-1 rats was significantly thicker than that determined in CTL-1 rat retinas ($p < .001$), (Figure 1).

Along illumination (2 and 5 days) PR degeneration and the number of pycnotic nuclei in ONL increased in both animal groups (CTL and ADX). The changes were accompanied by a reduction of the retinal thickness, which was more pronounced in CTL rats than in ADX rats (data not shown).

After 7 days of CI, CTL-7 rats showed the severest degeneration of PRs. Cellular debris was observed between the ONL and the RPE, and vacuolar inclusions were detected in the RPE. The ONL showed a huge number of pycnotic nuclei (Figure 2C, 2E).

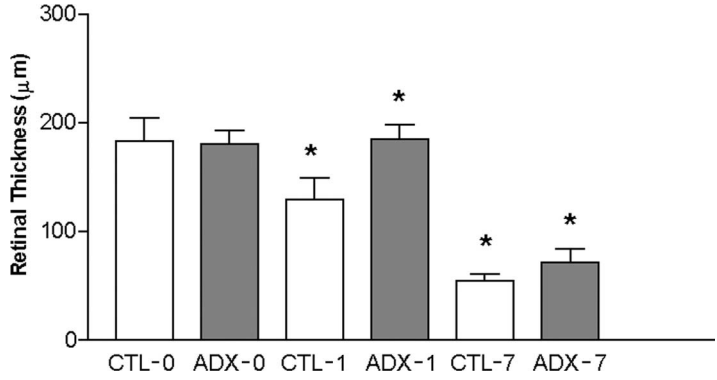

Figure 1. Retinal thickness of CTL and ADX rats before (CTL-0 and ADX-0) and after 1 day (CTL-1, ADX-1) or 7 days (CTL-7 and ADX-7) of continuous illumination. The differences between CTL-0 and ADX-0 are not significant ($p > .05$). The differences between CTL-1 and ADX-1 ($p < .001$) as well as those between CTL-7 and ADX-7 ($p < .001$) are highly significant (asterisks). Values represent the mean ± standard deviation (*SD*) of six retinas at each time point.

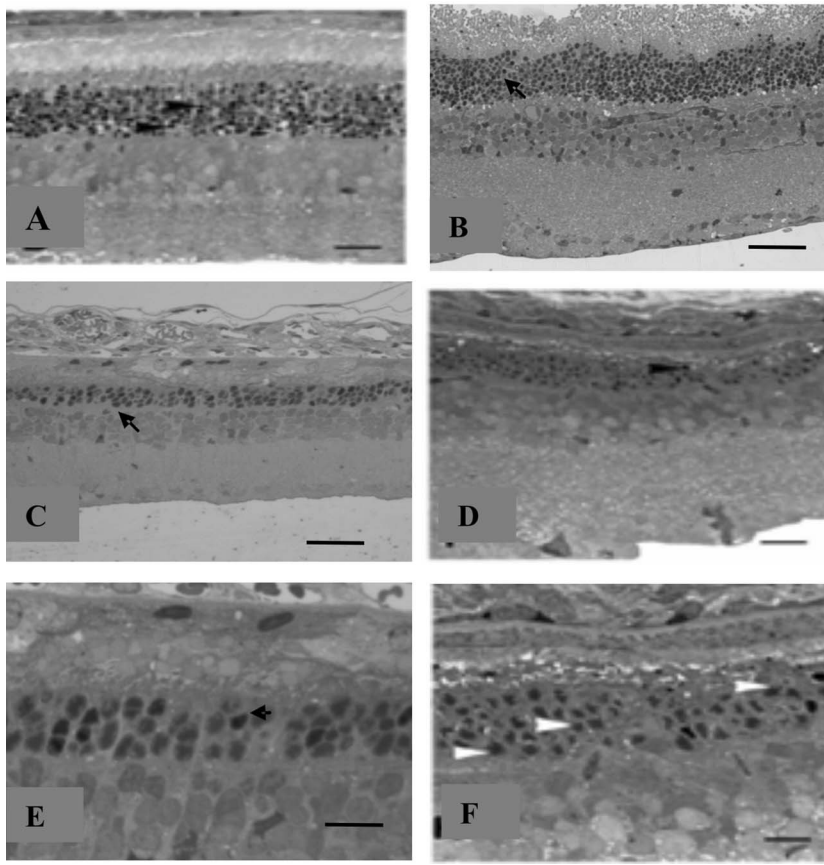

Figure 2. Semithin sections of retinas stained with toluidine blue. (A) Retina of a CTL-1 rat. Observe the disorganization of OPS. Pycnotic nuclei (arrowhead) may be observed in the ONL. (B) Retina of an ADX-1 rat. The ONL also shows some pycnotic nuclei (arrowhead). (C and E) Retina of CTL-7 rat. (C) A severe degeneration of photoreceptors and the presence of cellular debris corresponding to outer and inner photoreceptors may be observed. The decrease of retinal thickness is accompanied by the presence of some pycnotic nuclei in the ONL (arrowhead). (E) A higher magnification shows pycnotic nuclei (arrowhead) in the ONL. Retinal pigment epithelial cells appear swollen. (D and F) Retina of an ADX-7 rat. (D). Observe the severe degeneration of outer and inner photoreceptor segments, the decrease of retinal thickness, and a pycnotic nucleus in the ONL (arrowhead). (F) High magnification of the retina showing degeneration of outer and inner photoreceptor segments; some cellular debris and irregular nuclei (arrow) may be observed in the ONL. Compare changes and thickness with C and E. Scale bars: (A) 30 μm, (B) 40 μm, (C) 30 μm, (D) 30 μm, (E) 30 μm, (F) 12 μm, and (G) 12 μm.

Table 1. Blood CORTICOSTERONE levels of CTL and ADX rats

Days of CI	0 (Non-illuminated)	1	2	5	7
CTL	16.52 ± 1.79*	33.50 ± 7.35*	47.92 ± 7.19*	53.81 ± 1.00*	62.06 ± 4.18*
ADX	0	0	0	0	0

*Values are expressed in ng/ml.

Meanwhile ADX-7 rats showed similar structural alterations to those observed in CTL-7 rats (Figure 2D, 2F). Degeneration of PRs and pynotic nuclei were also observed in the ONL.

An important reduction of retinal thickness was observed in both condtions, CTL-7 and ADX-7, but ADX-7 retinas were significantly thicker than CTL-7 retinas ($p < .001$) (Figure 1).

CORTICOSTERONE levels increased along the different periods of CI in CTL rats but remained undetectable in ADX rats at any time point of CI (Table 1). An analysis of CORT concentration clearly shows that CORT level peaks quickly in the first couple of days of CI. It is 2.03 times higher in the sera of CTL-1 rats compared to CTL-0 rats ($p < .01$) and 2.9 times higher in the sera of CTL-2 compared to CTL-0 rats ($p < .01$) (Table 1). CORT serum levels remained high in CTL-5 and CTL-7 rats (3.2 and 3.75 times higher than CTL-0 respectively, $p < .01$) (Table 1). This increase in CORT serum levels correlated with the observed damage of the retina in CTL animals. On the opposite, CORT levels were not detected in the sera of ADX rats under any condition.

Electron Microscopy

Illuminated Control Rats (Sham-Operated). Retinas of illuminated CTL rats showed RPE edema at the different time points of CI. The RPE cells contained intracytoplasmic heterophagosomes (HP), whose quantity peaked in CTL-2 condition (Figure 5A).

The OPS exhibited a sustained degeneration. First the disarrangement and the tubular transformation of disks was observed in CTL-1 (Figure 4A) and in CTL-2 (Figure 5C). It increased in CTL-5 (Figure 6C), and its maximal point of ultrastructural damage was observed in CTL-7 (Figure 8A right corner, 8C upper left corner), when the debris of severely degenerated OPS was observed between the ONL and the RPE.

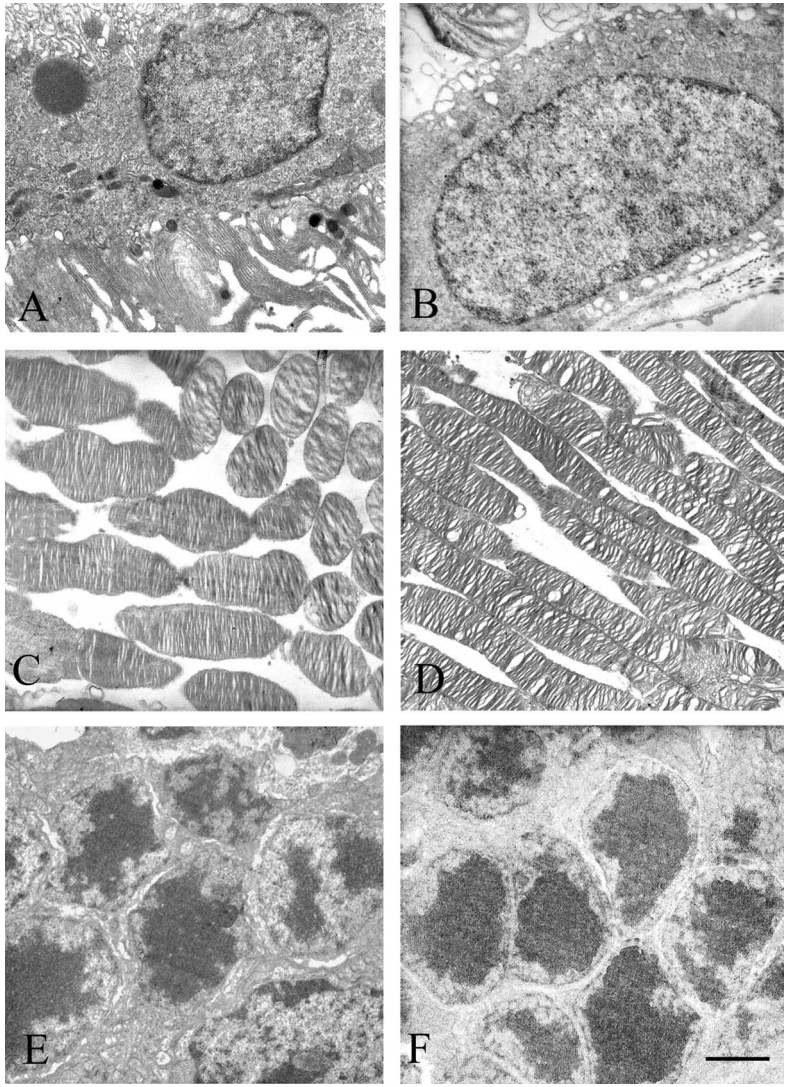

Figure 3. (A) Electron micrograph of an RPE cell of a CTL-0 rat. Typical epithelial ultrastructure with microvilli is observed. Some lysosomes are detected in the cell cytoplasm. (B) Electron micrograph of an RPE cell of an ADX-0 rat. An epithelial cell with a big nucleus is observed. Some clear vesicles are detected in the cell cytoplasm. (C and D) The OPS of CTL-0 and ADX-0 rats respectively. The ultrastructure of the disks is preserved in both cases. Minor alterations of disks are artifactual and are observed in both conditions. (E and F) The ONL of CTL-0 and ADX-0 rats respectively. The characteristic heterochromatin of photoreceptor nuclei is observed in both cases. No differences are observed between both conditions. Scale bars: (A, B, C, D, E, and F) 0.5 μm.

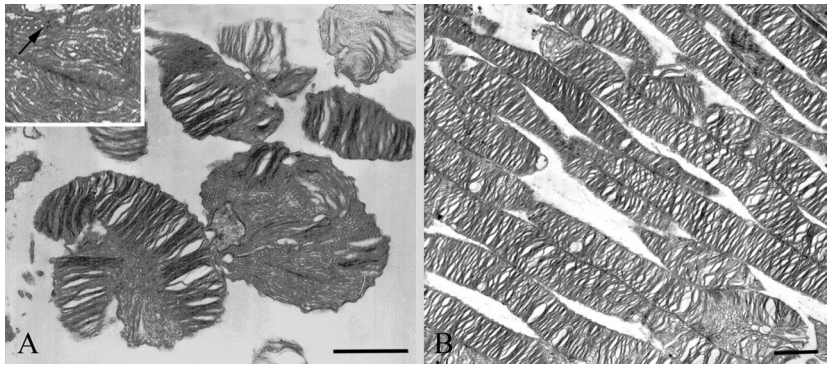

Figure 4. The OPS of (A) CTL-1 and (B) ADX-1 rats. Disorganization and tubular transformation of the disks is observed in both cases. Insert in A shows higher magnification of tubular transformation. Alterations in CTL-1 are more notorious than those in ADX-1. Scale bars: (A and B) 0.5 μm.

Pycnotic nuclei, nuclear fragmentation and apoptotic bodies were present in the ONL, peaking in CTL-2 (Figure 5E, 5F).

Severe ultrastructural alterations of the mitochondria were observed in CTL-5 rat retinas (Figure 7A)

After 5 days of CI, degeneration of ganglion cells and an increase of electronic density of Müller cell processes were observed (Figure 7C, 7E)

Adrenalectomized Rats

RPE cells exhibited a less pronounced phagocitic activity than CTL. The maximal RPE alterations were observed at ADX-5 (Figure 6B).

The OPS underwent a degenerative phenomenon, similar to that occurring in CTL rats (Figure 4B, 5D, 6D), with the maximal ultrastructural alteration in ADX-7 rats (Figure 8B bottom, 8D).

After 2 days of CI, ONL contained pycnotic nuclei and apoptotic bodies, but this degenerative phenomenon was less severe in ADX-2 rats than that observed in CTL-2 rats (Figure 5G). On days 5 and 7 of CI, macrophages migrated into the ONL (Figure 6F, 8D).

After 5 days of CI, inner nuclear layer (INL) cells exhibited swollen mitochondriae (Figure 7B). Nevertheless, ultrastructural changes were less pronounced than those observed in CTL-5 rats (compare with Figure 7A). Cellular degeneration appeared in the GCL, but the increased electron density in Müller cell processes was not observed in ADX-5 rats (Figure 7D).

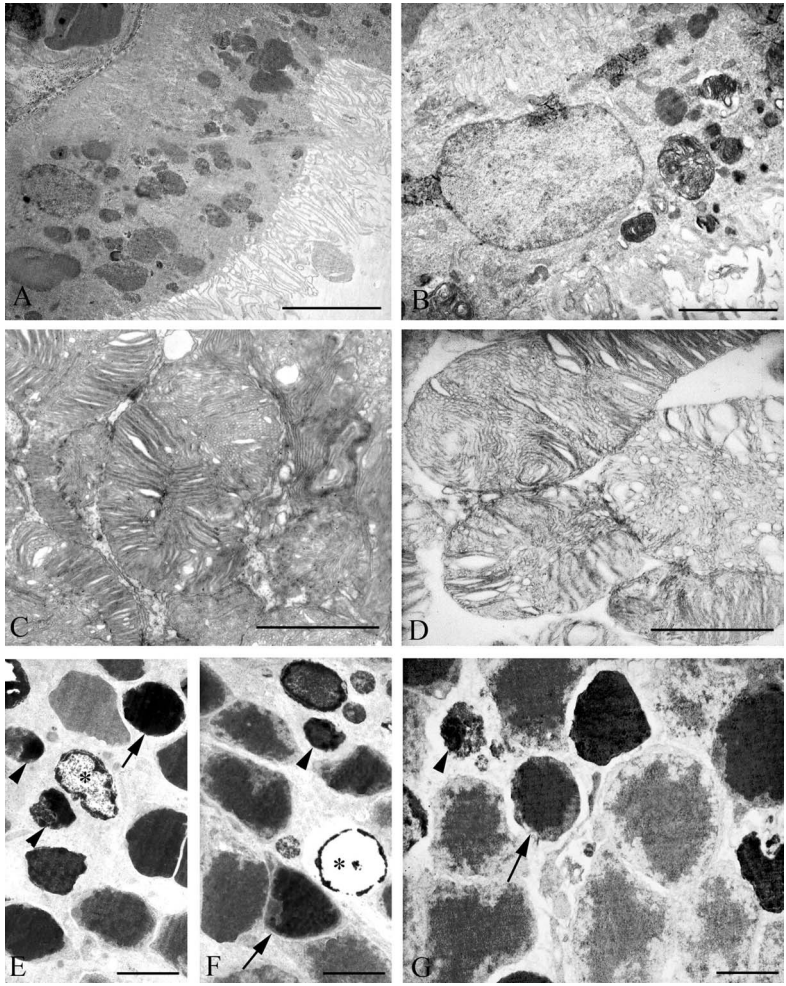

Figure 5. (A) Electron micrograph of an RPE cell of a CTL-2 rat. Epithelial cells are swollen, and a notoriously high number of lysosomes are observed in the cell cytoplasm. On the apical surface of the cell there are thin microvilli. (B) Electron micrograph of an RPE cell of an ADX-2 rat. The cell is swollen, and big lysosomes with heterogeneous content (HP) are observed in the cell cytoplasm. Disorganized microvilli are observed on the apical surface of the cell. (C and D) The OPS of CTL-2 and ADX-2 rats respectively. Disorganization and tubular transformation of the disks is severer than those observed in Figure 4. (E and F) The ONL of CTL-2 rats. Pycnotic nuclei (arrow), severely damaged nuclei (asterisk), and apoptotic bodies (arrowhead) are observed. (G) The ONL of ADX-2 rats. A pycnotic nucleus (arrow) and an apoptotic body (arrowhead) are observed. Changes are not so severe as those observed in CTL-2 rats (compare with E and F). Scale bars: (A) 1 μm, (B) 1 μm, (C) 0.5 μm, (D) 0.5 μm, (E) 1 μm, (F) 1 μm, and (G) 1 μm.

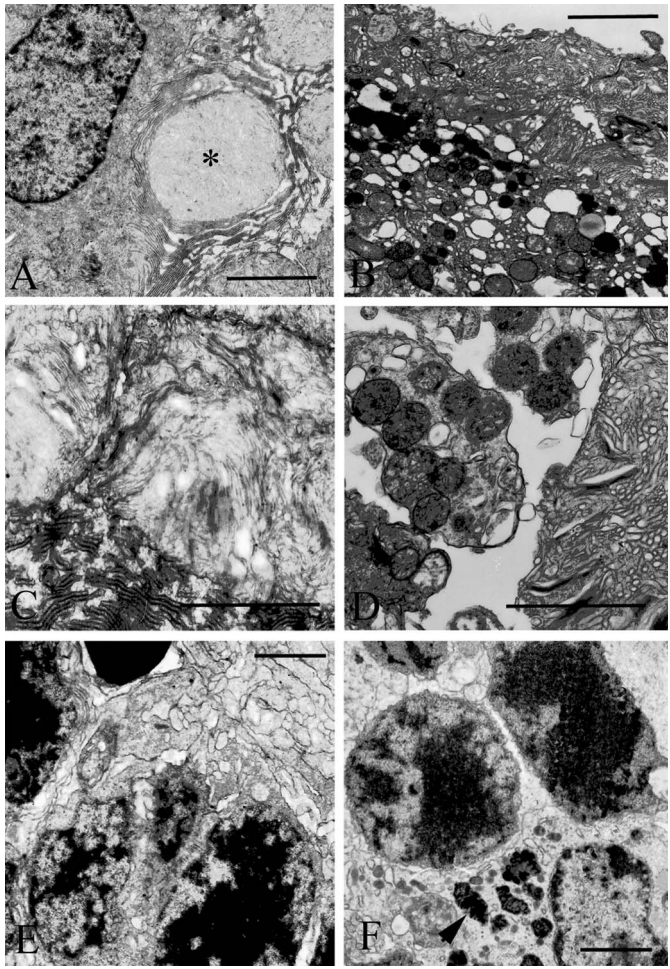

Figure 6. (A) Electron micrograph of the RPE of CTL-5 rats. An outer photoreceptor segment with tubular transformation (asterisk) of the disks is surrounded by microvilli of the RPE. (B) An RPE cell of an ADX-5 rat. Epithelial cells are swollen, and a big number of dense and heterogeneous lysosomes and clear vacuolae are observed in the cell cytoplasm. On the apical surface of the cells disorganized microvilli as well as debris of outer PR segments are observed. (C) Severely damaged outer photoreceptor segment of a CTL-5 rat is observed. (D) Outer and inner photoreceptor segments of an ADX-5 rat. Tubular transformation of the disks is observed in the outer photoreceptor segment. (E) The ONL of a CTL-5 rat. A pycnotic nucleus is observed in this layer. Apoptotic bodies are already removed in this layer. (F) The ONL of an ADX-5 rat. A macrophage (arrowhead) with HP in its cytoplasm is observed migrating in this layer, while it removes apoptotic bodies. Scale bars: (A) 1 μm, (B) 1 μm, (C) 0.5 μm, (D) 0.5 μm, (E) 1 μm, and (F) 1 μm.

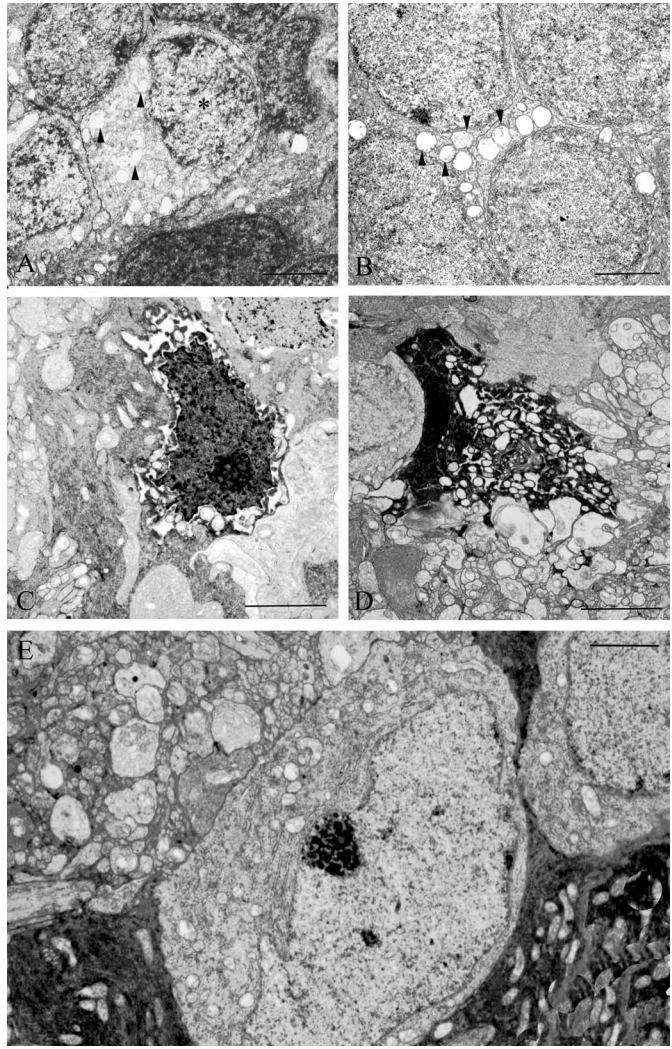

Figure 7. (A) The INL of a CTL-5 rat. Severe mitochondrial alterations, clear vacuolae, and a nucleus with irregular shape are observed. (B) The INL of an ADX-5 rat. Mitochondrial alterations are observed, but the cell ultrastructure is better preserved than in CTL-5 rats (compare with A). (C) The GCL of CTL-5 rats. A degenerated neuron with an increase of the electrondensity of the chromatin and vacuolar degeneration of the cytoplasm is observed. (D) The GCL of an ADX-5 rat. A degenerated cell with a small nucleus and an increase of density of the chromatin is observed. The cytoplasm shows a huge number of clear vacuolae/vesicles. (E) The GCL of a CTL-5 rat. A ganglion cell with a nucleus of irregular shape and its nucleolus contacting the nuclear envelope is detected. Dense processes of Müller cells surround the GC suggesting a reactive increase of gliofilaments. Scale bars: (A) 1 μm, (B) 1 μm, (C) 2 μm, (D) 2 μm, and (E) 1 μm.

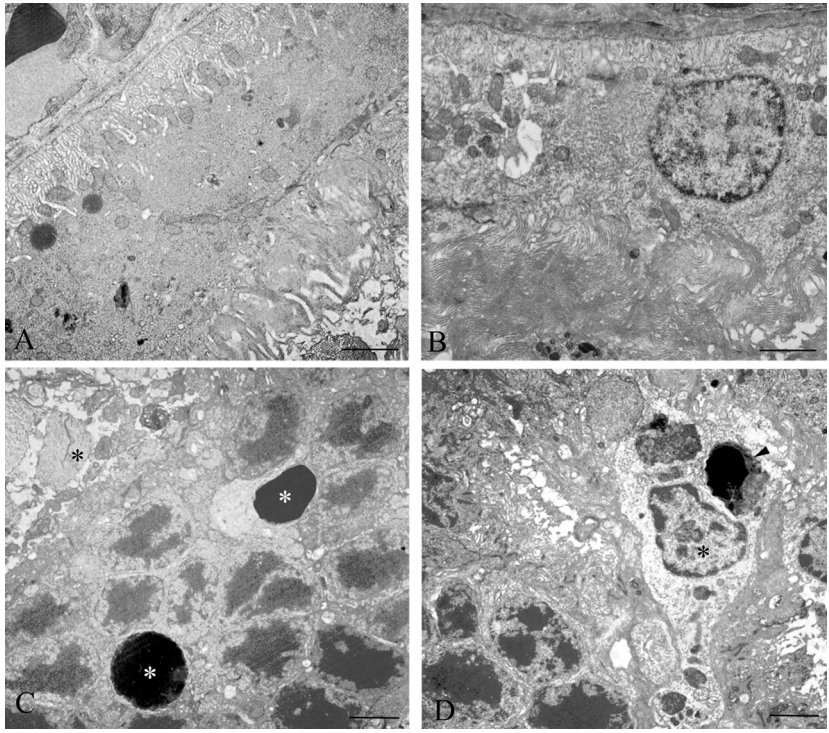

Figure 8. (A and B) RPE cells of CTL-7 and ADX-7 rats, respectively. Swollen epithelial cells with microvilli contacting degenerated photoreceptors are observed in both cases. (C and D) The ONL of CTL-7 and ADX-7 rats, respectively. Picnotic nuclei (asterisks) are observed in C. A macrophage with HP in its cytoplasm is observed migrating between the ONL and photoreceptor segments in D. Scale bars: (A and B) 1 μm and (C and D) 1 μm.

Lymphocytes were not observed in the ONL in any case (neither in ADX nor in CTL rats) along CI, supporting the hypothesis that apoptotic death is involved in this layer during LIRD and independently of the surgical procedure.

DISCUSSION

Continuous illumination is not a physiological condition, but its effects resemble those of the cumulative lifetime light exposure involved in retinal degenerative diseases such as AMD (Ambati, Ambati, Yoo, Ianchulev, & Adamis, 2003). It was demonstrated that apoptosis is the common final pathway involved in PR degeneration during AMD (Xu et al., 1996).

Previous works have shown that light toxicity induces free radical formation involved in lipid peroxidation and cell damage (Beatty et al., 2000; Capani et al., 2003; Piehl et al., 2004; Tanito, Yoshida, Kaidzu, Ohira, & Niké, 2006). Although it is difficult to quantify the total lifetime light exposure in humans, it was suggested that radiation damages the RPE and induces structural and functional alterations of PRs. Some of these modifications originate early AMD characteristic lesions. Peroxidation of polyunsaturated fatty acids (PUFA) that belong to outer PR segments are not degraded completely by RPE cells, and so extracellular material accumulates on Bruch's membrane originating soft drusen (Ambati et al., 2003).

The retinal degeneration observed in our model is characterized by severe PR alterations, apoptotic cell death in the ONL, and the reduction of retinal thickness, which were detected in all the studied conditions. There is an inverse correlation between the extension of the illumination exposure period and the retinal thickness (Figures 1, 2). However changes were not so severe in ADX rats compared to CTL rats (Figures 1, 2). Beside, the presence of apoptosis, detected by EM (Figure 5E–5G), is in agreement with previously mentioned observations using different models of CI (Abbler et al., 1996; Grimm et al., 2000; Xu et al., 1996; Wu et al., 2002).

Our EM results showed that PR degeneration increased along CI, being maximal by day 7 in ADX and CTL rats. RPE cells were swollen and contained HP in their cytoplasms because they play an active role in the phagocytosis of outer photoreceptors segments. This process was maximal in CTL-2 rats (Figure 5A) and ADX-5 rats (Figure 6B).

Pycnotic nuclei, nuclear fragmentation, and apoptotic bodies were detected in the ONL, peaking at day 2 in both CTL (Figure 5E, 5F) and ADX rats (Figure 5G), but the magnitude of this process was bigger in CTL rats than in ADX rats. Mitochondrial alterations were observed in the INL, but ultrastructural alterations were severer in CTL-5 rats (Figure 7A) than in ADX-5 rats (Figure 7B).

Macrophages migrating between the RPE and photoreceptor segments and into the ONL were observed in ADX rats (Figures 6F, 8D), but lymphocytes were not observed in any condition (ADX and CTL rats), confirming that cell death occurs by apoptosis in the ONL during LIRD.

As was previously demonstrated, the continuous exposure to light alters circadian rhythm and induces high levels of GC (Persengiev & Kanchev, 1991). Our results confirmed that CI increases CORT levels in CTL rats, while CORT levels were undetectable in ADX rats. The central hypothesis of the present work is that high intensity CI is a stressful event by itself and

that the induced high GC levels could collaborate with LIRD. Our results showed that adrenalectomy attenuated LIRD because retinal thicknesses from illuminated ADX rats were thicker than those observed in illuminated CTL rats and because ultrastructural damage was less severe at the same time point in ADX rats compared to CTL rats. In order to obtain the same injury in ADX rat retinas, illumination had to be prolonged, so damage was delayed in ADX rats compared to CTL rats. We concluded that the increased GC levels could be playing a damaging/proapoptotic role in the retinas. However, adrenalectomy did not inhibit retinal damage completely. Degeneration was significantly reduced in this condition, showing that the high GC level is one of the intervening factors involved in the process (see Figure 1; compare Figure 2A, 2C, 2E with Figure 2B, 2D, 2F). These results are in agreement with the previous ones in other areas of the central nervous system (CNS) (Mc Ewen, 1994; Sapolsky, 1985a, 1985b; Stein & Sapolsky, 1988).

In accordance with the present results, a previous work performed on surgical animals and animals with ether-induced stress showed that adrenalectomy has an antagonistic action on light-induced PR damage (O'Steen & Donnelly, 1982). However, we did not submit the rats to an additional stressful condition, as we consider that illumination is itself a stressful event. In fact, CORT determination performed on sham-operated rats in our work clearly demonstrated an increase in the serum concentration of the hormone during CI, while CORT was undetectable in ADX rats as expected (Table 1). The obtained results strongly suggest that the observed differences between ADX and CTL rat retinas are probably due to the demonstrated hormonal differences. Although the action of other steroid hormones and catecholamines cannot be ruled out and is discussed below.

Our results also agree with a recent research (Tanito & Anderson, 2006) which was performed on BALB/c mice, adrenalectomized or sham operated at the age of 28 days and kept under dim (5 lx) or bright (400 lx) cyclic light for 2 weeks. After submitting the animals to damaging light exposure (3000 lx) for 24 hrs, the authors observed that a- and b-wave amplitudes of the ERG and the ONL thicknesses and areas were significantly higher in adrenalectomized mice than in sham mice. However the mentioned paper (Tanito & Anderson, 2006) did not detect variations of corticoid levels between animal groups. Probably the higher intensity of light used in our work is responsible of the obtained difference, or the specificity of our antibody is better.

The retina may be a target of GC, as type I GRs were reported in all the layers of human retina (Suzuki et al., 2001), adding support to the hypothesis. But why is degeneration so marked in outer retina when GRs are present in

all retinal layers? One possibility is that it is due to the vulnerability of PR phospholipids to light-induced peroxidation (Tanito et al., 2006). The other reason could be that the presence of a GC-induced tumor necrosis factor (TNF) receptor (as demonstrated) in RPE and PR inner segments (Li et al., 2004). Both, the existence of TNF receptors in the RPE and the PR lipid composition could explain why the damaging effects of light are severer in outer retina, although GRs have been reported in all retinal layers (Suzuki et al., 2001).

The effects of GC could be related with a decrease of melatonin during CI. Melatonin is an output signal of an endogenous circadian clock and exerts a neuroprotective role in the CNS (Cagnoli, Atabay, Kharlamova, & Manev, 1995; Chen, Lin, & Chiu, 2003; Erol et al., 2004; Giusti et al., 1996; Mesenge et al., 1998; Skaper, Ancona, Facci, Franceschini, & Giusti, 1998) and in the retina (Liang, Green, Wanq, Alssadi, & Godley, 2004). Melatonin receptors were reported in the inner retina of guinea pig (dendrites, axons, and cell bodies of ganglion cells and subpopulations of amacrine cells) (Fujieda et al., 2000) and in the inner and outer retinas (rod and cone photoreceptor cells) of *Xenopus laevis* and humans (Meyer et al., 2002; Savaskan et al., 2002; Wiechmann & Wirsig-Wiechmann, 2001). Melatonin inhibits GC-induced apoptosis in thymocytes (Hoijman et al., 2004; Presman, Hoijman, Ceballos, Caligniana, & Pecci, 2006) and in cerebellar neurons (Persengiev, 2001). The mechanism involved is the inhibition of the nuclear traslocation of GR (Presman et al., 2006). As illumination inhibits melatonin synthesis in vivo (Depres-Brummer, Levi, Metzger, & Touitou, 1995) and in vitro (Ivanova & Iuvone, 2003), then GRs may be traslocated to the cell nuclei, and GCs could exert their proapoptotic actions on retinal cells. The GC dependent apoptotic pathway could involve the classical up-regulation of Bax expression and a decrease of p53 as has been demonstrated in other CNS neurons (Almeida et al., 2000).

On the other hand, an antiapoptotic effect of dexametasone on light-induced degeneration has also been described (Wenzel et al., 2001). In addition, many studies in the literature report protective effect of increased GC on LIRD. In these models, the GC levels were increased by either fasting or addition of dexamethasone or methylprednisolone (Wenzel et al., 2001; Fu, Lam, & Tso, 1992). This opposite result could be due to the use of different models of illumination, different steroid concentrations, and differences between exogenous (dexametasone) and endogenous steroids (the main endogenous steroid in the rat being CORT) (Singer & Stack-Dunne, 1954). Corticoids may have a biphasic effect, antiapoptotic or proapoptotic, depending on the hormonal concentration. In the hippocampus, for example, trkB mRNA

expression increased in CA3 and dentate gyrus after the administration of low doses of CORT (30 microgram s.c.), while its expression was unaffected by higher doses (300 and 1000 micrograms s.c.) (Schaaf, Hoetelmans, de Kloet, & Vreugdenhil, 1997).

In addition, it must be remarked that adrenalectomy also removes the source of adrenaline, noradrenaline, and circulating catecholamines. GCs modulate the expression of adrenergic receptors and sensitize choroid vessels to the actions of catecholamines. Catecholamine-induced vasoconstriction is potentiated by the up-regulation of adrenergic receptors induced by GC, and it could contribute to the observed LIRD. So adrenalectomy could be playing an additional protective role by removing the source of circulating catecholamines.

In summary, light exerts a deleterious effect on the retinas of both sham-operated and adrenalectomized animals. However, the damage is severer on the retinas of nonadrenalectomized animals. Continuous illumination of a nocturnal animal such as the rat is a stressful condition that stimulates the secretion of GC by the adrenal glands as is shown in this paper. It is possible that steroid hormones potentiate LIRD or trigger a damage additional to that induced by light. This hypothesis is in agreement with the deleterious action of GC observed in the CNS.

A more general conclusion is that the increased GC secretion during the stressful event of chronic retinal degenerative diseases could cooperate with retinal damage. The role of steroid hormones in the pathophysiology of retinal degenerative diseases should be reassessed, and current treatments using high doses of corticoids should be reconsidered. However, further research is needed to better clarify steroid-induced structural and functional changes of the retina in animal models of degenerative retinal diseases.

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