Journal of Cell and Animal Biology Vol. 3 (2), pp. 029-032, February, 2009
Available online at http://www.academicjournals.org/JCAB
ISSN 1996-0867 © 2009 Academic Journals

Full Length Research Paper

Ultra structural and histomorphometric study of retinal pigmented epithelium of retina in female cat under the effect of continuous light exposure and dark adapted

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Acceptad 31 December, 2008

The aim of this study was to evaluate the morphometry and ultra structure of retinal pigmented layer in continuous light exposed and dark adapted domestic female cats. Twenty four healthy adult cats were divided into three groups: control, continuous light exposed and continuous dark adapted groups. The eyes of animals were routinely fixed and studied by electron microscope. The results showed that in light exposed group, the mean of thickness and number of melanosome of retinal pigmented layer had significant increment when compared with control and dark adapted groups (P<0.05). Mitochondria, golgi apparatus and rough endoplasmic reticulum were also observed to increase in the light exposed group. Our finding have confirmed that high cell activity occur in cat retinal pigmented layer under the effect of 24 h continuous light.

Key words: Morphometry, ultra structure, retinal pigmented layer, cat.

INTRODUCTION

Retina plays an important role in visual system. Retina has several layers; one of the layers that are really crucial for visualization is retinal pigmented layer. Light rays falling on the eye pass through its refractive media (cornea, lens, anterior and posterior chambers) before reaching the retinal pigmented layer and visual receptor cells (the rods and cones) in the retina. The refractive media help focus the image on the retina (Banks, 1993).

Various histological studies were undertaken on retinal pigmented layer and photoreceptor structures of retina in different domestic animals and human at light and electron microscopic level (Braeckvelt, 1983, 1985, 1986a, b, 1988, 1990, 1992, 1993, 1996, 1998; Garcia and Dejuan, 1999). The best understood functions of the retinal pigmented epithelium include: transport of materials to and from the photoreceptors (Steinberg and Miller, 1973), architectural stabilization and effective orientation of photo receptor outer segments (Enoch, 1979), internal adhesion of the neurosensory retina (Zinn and Benjamin-Henkind, 1979), the storage and modification of vitamin A precursors of the visual pigments (Young and Bok, 1970) and the phagocytosis and lysosomal degradation of photoreceptor outer segment discs (Bok and Young, 1979).

As a consequence of these important functions, the retinal pigmented layer region has been investigated in a variety of animals and while this region is similar in all vertebrates, species differences are usually present (Nguyen-Legros, 1978; Kuwabara, 1979; Braeckvelt, 1983, 1985, 1986a, b, 1988).

Other scientists studied the fine structure of the photoreceptor layer in different animals such as in the butterfly fish (Braeckvelt, 1990), red-backed salamander (Braeckvelt, 1992), red–tailed hawk (Braeckvelt, 1993), barred owl (Braeckvelt, 1996), emu (Braeckvelt, 1998), black bass (Garcia and Dejuan, 1999), grenadier anchovy coilia nasus (Haacke et al., 2001), cat (Esfandiari et al., 2007, 2008). Retinal light damage in rats exposed to intermittent light and comparison with continuous light exposure was studied by Organisciak et al. (1989), they

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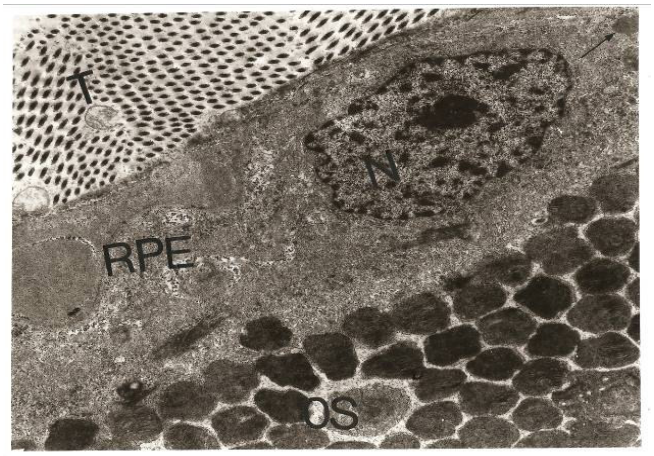

Figure 1. Micrograph of the retinal pigmented epithelium (RPE) in control group. N: nucleus of the retinal pigmented cell; T: tapetum; OS: outer segment; melanosome (thin arrow) (×5200).

concluded that intermittent light exposure exacerbates type 1 light damage in rats. White et al. (1987), studied degree of light damage to the retina with time of day of bright light exposure in albino rats.

However, no ultra structural evaluation of retinal pigmented layer under the effect of continuous light exposure and dark adapted has been investigated in cat. Therefore, present study was undertaken to describe the ultra structure of the retinal pigmented layer in retina of the female domestic cat under the influence of continuous light exposure and dark adapted.

MATERIALS AND METHODS

Animals

Twenty four adult female cats were obtained from animal house of Shiraz Medical University of Iran. Animals were randomly classified in three groups of control, light exposure and dark adapted. The animals were in the normal environmental conditions (12:12 light–dark cycle) for 4 weeks. Room temperature was kept at approximately 28°C. Exposure to light be accomplished by placing the animal's cages which were open at the top under white 60 W florescent bulbs. Bright light intensity measured with a power meter, was 500 - 600 lux. The bulbs hanged up on the wooden boxes (110 cm height). The wooden boxes were 120 cm wide × 170 cm long × 130 cm height. Each cage contained two animals. All studies were performed in accordance with the Guide to the Care and Use of Experimental Animals (Olfert et al., 1993).

Experimental design

1- Adult cats (8 in numbers) were exposed to continuous light for 24 h from 6:00 a.m. to 6:00 am the next day (light exposure group).
2- Adult cats (8 in numbers) were maintained in the darkness for 24 h from 6:00 a.m. to 6:00 a.m.the next day (dark adapted group).
3. Adult cats (8 in numbers) were in the normal environmental condition for 24 h from 6:00 a.m. to 6:00 a.m. the next day (normal group) (12:12 light-dark cycle).

Morphometric analysis was done using SPSS software, version 11.5 and was carried out using One Way ANOVA. The histological effects of continuous light exposure and dark adapted were evaluated with transmission electron microscope when compared with control group.

The animals were sacrificed by overdose of xylazine-ketamin injection. The eye balls were quickly removed. The cornea, lens and vitreous body were removed and opened at the equator then fixed for 4 h in 4% glutaraldehyde buffer to pH 7.3 with sodium caccodylate at 4°C. The posterior half of the eyeball was removed; the retina was separated near the optic nerve and fixed for addi-tional 1 h. Then, the tissue was washed in sodium caccodylate and cut into pieces less than 1 mm^2. The tissue was then post fixed for 1.5 h in 1% osmium tetroxide, washed briefly in distilled water, dehydrated through graded ethanol and then cleared in propylene oxide and embedded in Agar resin.

Semi thin sections of 0.5 µm thicknesses were obtained using ultra microtome (Reichert-jung, ultra cut Austria equipped with glass knives), stained with toluidine blue and morphometric studies on the semi thin sections were carried out using light microscope. Ultra thin sections of 60 nm thickness were obtained and collected on copper grids. These sections were stained in aqueous uranyl acetate and lead citrate and examined with Philips CM-10 transmission electron microscope.

Statistical analysis

Statistical analysis was done using SPSS software, version 11.5. Results are presented as mean ± SD. Statistical analysis was carried out using one way ANOVA. The $P<0.05$ was considered as statistically significant.

RESULTS

The findings showed that the retinal pigmented layer of the control group had simple cuboidal cells, characterized by heterochromatin nucleus (Figure 1). In addition, the cats in control group showed few numbers of melanosomes in the RPE (around nucleus), so two melanosomes by mean in 20 µm RPE length has been observed (Figure 1). In control group, the mean ± SD. of the thickness of retinal pigmented layer was 4.71 ± 0.03 by micrometer standard technique while that of the dark adapted group was 4.57 ± 0.08. Only two melanosomes were observed in 20 µm RPE length (Figure 2). Results in light exposure group showed high activity of this layer (Euchromatin nucleus and increased the rough endoplasmic reticulum, Golgi apparatus and Mitochondria), whereas the cell nucleus became more euchromatin and seven melanosomes were observed in 20 µm RPE length (Figures 3, 4). The thickness of layer was 7.59 ± 0.08 in light exposure group. Also, in this group the number of mitochondria, golgi apparatus and rough endoplasmic reticulum increased (Figures 4 and 5), whereas in control and dark adapted groups, these organelles were considerably limited.

DISCUSSION

Retinal pigmented layer in all domestic animals consisted

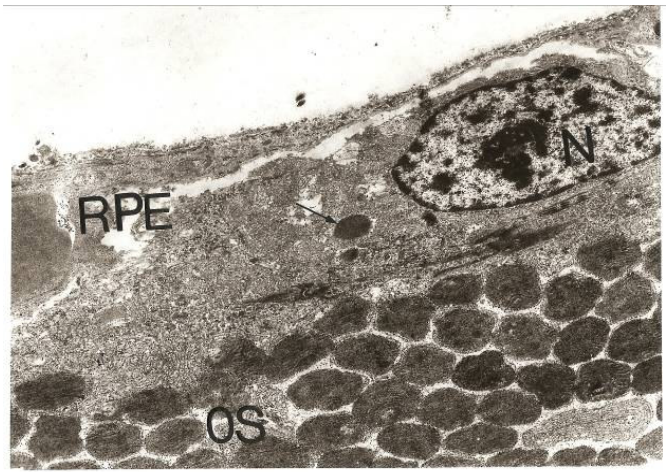

Figure 2. Micrograph of the retinal pigmented epithelium (RPE) in dark adapted group. N: nucleus of the retinal pigmented cell; OS: outer segment; melanosome (thin arrow) (×5200).

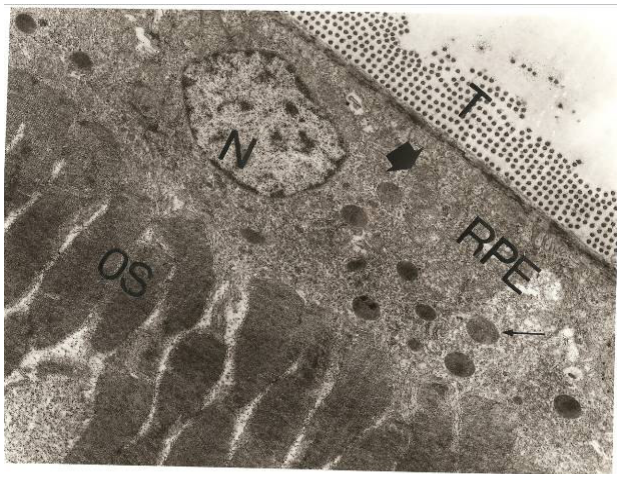

Figure 3. Micrograph of the retinal pigmented epithelium (RPE) in light exposure group. N: nucleus of the retinal pigmented cell; T: tapetum; OS: outer segment; melanosome (thin arrow); mitochondrion (thick arrow) (×5200).

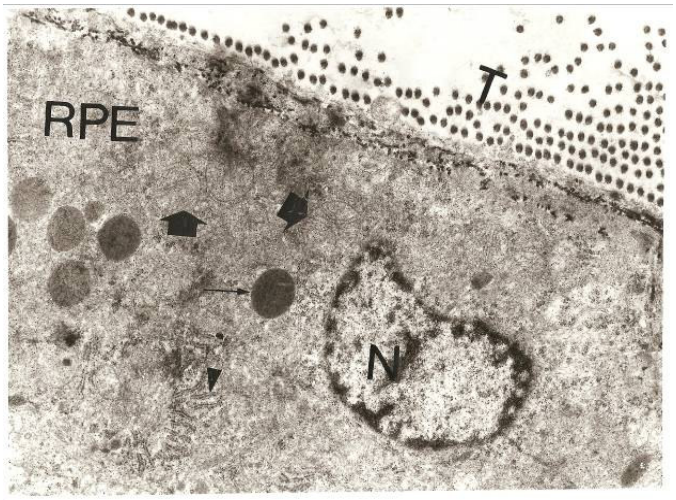

Figure 4. Micrograph of the retinal pigmented epithelium (RPE) in light exposure group. N: nucleus of the retinal pigmented cell; T: tapetum; melanosome (thin arrow); mitochondrion (thick arrows); rough endoplasmic reticulum (arrow head) (×5200).

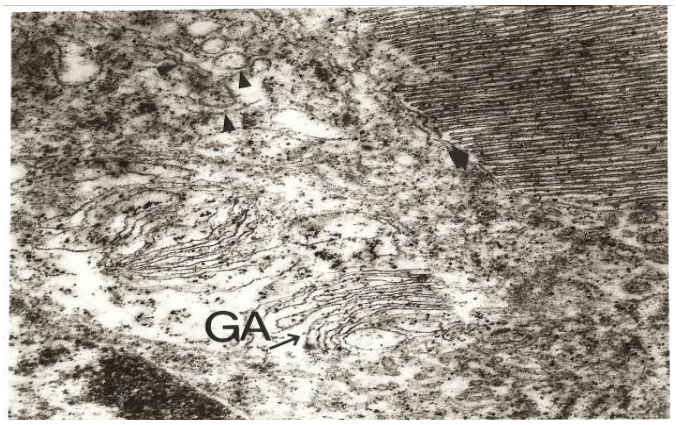

Figure 5. Micrograph of the retinal pigmented epithelium in light exposure group. GA: golgi apparatus (arrow); bimembranous disc of outer segment of photoreceptor cell (thick arrow); rough endoplasmic reticulum (arrow heads) (×39000).

of one row of cuboidal cells (Braeckvelt, 1984, 1985, 1986a, b, 1988; Nguyen-Legros, 1978; Kuwabara, 1979). In the present study, observations by transmission electron microscopy indicated that in control group, the structure of retinal pigmented layer in cat was similar to other mammals, while in light exposure group, the cell nucleus became more euchromatin in comparison with control group. These changes were due to increased activity of retinal pigmented cells in light exposure group (Banks, 1993).

Results obtained from this study showed that the increase in the number of melanosomes in light exposure group were higher in comparison with the other groups (with significant difference $P<0.05$), but there was no significant difference between control group and dark adapted group. Melanosomes of retinal pigmented layer prevent the scattering of light in other layers of retina and caused a clear image. Mitochondria, Golgi apparatus and rough endoplasmic reticulum were increased in light exposure group. This phenomenon showed that the production of protein was increased, because retinal pigmented layer has the responsibility of double membranous disc turnover of outer segment. Also, this layer plays an important role in forming scotopsin and retinal for visualization. Morphometrical analysis showed that the thickness of retinal pigmented layer has increased in light exposure group with significant difference in comparison with control and dark adapted groups ($P<0.05$).

Also, the thickness of this layer has decreased in dark adapted group with no significant difference in comparison with control group. Probably, retinal pigmented layer was activated in light exposure group.

ACKNOWLEDGMENTS

This study was conducted under sponsorship of Islamic Azad University-Abadeh branch. The authors are indebted to Ali Safavi (department of electronic microscope, School of Veterinary Medicine, Shiraz University) for their valuable assistances.

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