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Progesterone administration fails to protect albino male rats against photostress-induced retinal degeneration

I. KÁLDI[1], A. BERTA[2]

[1]Department of Ophthalmology, Kenézy Hospital, Debrecen
[2]Department of Ophthalmology, Medical and Health Science Center, University of Debrecen - Hungary

PURPOSE. *Female patients show better recovery after brain injury and lower incidence of vascular diseases before menopause. The aim of this study was to test the protective effect of female sexual hormones against photostress-induced photoreceptor apoptosis.*
METHODS. *Five week old male albino Sprague-Dawley rats were injected intraperitoneally with progesterone (60 mg/kg body weight) for 4 days. The control group was injected with the vehicle only (benzyl alcohol). Both groups were halved and one was stressed with light (2700 lux for 24 hours) and the other remained under the original dim cyclic light condition. For functional evaluation, baseline electroretinograms (ERGs) were recorded 7 days before light stress, with follow-up ERGs 5 days after the cessation of light exposure. Animals were sacrificed and their eyes enucleated for histology.*
RESULTS. *Light exposure caused pronounced decrease in the ERG a- and b-wave amplitudes compared to controls. However, in the light-stressed group, the difference in retinal function between progesterone-treated and nontreated animals was not statistically significant. The thickness of the outer nuclear layer and the length of rod outer and inner segments were significantly reduced in the light-stressed group, indicating loss of rod photoreceptor cells. Progesterone had no neuroprotective effect on rod cell structure.*
CONCLUSIONS. *The administration of progesterone did not prove to be protective against excessive light-caused retinal degeneration on male albino rats. The role of other sexual steroids and their interaction need to be clarified. (Eur J Ophthalmol 2004; 14: 306-14)*

KEY WORDS. *Progesterone, Light damage, Photoreceptor apoptosis, Electroretinogram, Outer nuclear layer, Rod inner and outer segment*

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INTRODUCTION

Several experimental studies carried out in humans, as well as *in vivo* experiments in animals, show a protective effect of female steroid hormones against cardiovascular, cerebrovascular, and other neurologic diseases. Premenopausal women show lower incidence of stroke (1-3). Estrogen therapy in postmenopausal women caused a decreased risk of heart disease (4). In women with partial epilepsy, there is a decreased frequency during the luteal phase of the menstrual cycle (5). Intravenous progesterone infusions had sim-

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ilar effect in cats with epilepsy, evoked by administration of penicillin (6). In a study on seven women with partial epilepsy, intravenous progesterone infusions were administered, reaching plasma concentration as during the luteal phase. Four of the seven patients showed a significant decrease in spike frequency (7). Roof et al (8) studied the effect of progesterone on experimental brain injury-induced cerebral edema in rats. They found that sexually mature females exhibited significantly less edema than males, and pseudopregnant females were virtually spared from postinjury edema. They concluded that the reduction of cerebral edema depended on the level of circulating progesterone. Furthermore, in another study, they proved that progesterone was effective in reducing edema when treatment was delayed until 24 hours after injury (9). Wright et al (10) induced bilateral medial frontal cortex injuries in male rats and found that the water content of progesterone-treated injured animal brain was significantly lower than that in injured control rats. Jiang et al (11) evaluated progesterone's neuroprotective effect on male rats, after induction of cerebral ischemia by middle cerebral artery occlusion (MCAO). Their results show that the administration of progesterone before or 2 hours after the onset of MCAO reduced ischemic cell damage and improved physiologic and neurologic functions 2 days after stroke. Alkayed et al (12) showed that progesterone and estrogen administration in reproductively senescent female rats reduced cortical infarct volume after 2 hours of MCAO. Striatal infarct was smaller in the estrogen, but not in the progesterone-treated group. In another experiment, age-matched male, female, and ovariectomized female rats were subjected to 2 hours of MCAO (1) and cerebral blood flow and infarction volume in the cerebral cortex and caudoputamen were determined. Female rats had smaller infarct size and higher blood flow than male and ovariectomized animals, proving that endogen estrogen improves stroke outcome.

In the literature, there are also publications showing that ovarian hormones are ineffective in stroke and neuronal cell death. Murphy et al (13) examined the effect of acute and chronic progesterone administration on ovariectomized female rats after MCAO and found that chronic progesterone administration alone significantly exacerbated caudate-putamen infarction. Cortical and total hemispheric infarction volumes were unchanged. RU 486 (mifeperistone) is a widely studied drug, with a potent progesterone and glucocorticoid receptor antagonist effect. The drug was tested and proved to be protective on rat primary hippocampal neurons, clonal mouse hippocampal cells, and organotypic hippocampal slice cultures against oxidative stress-induced neuronal cell death (14).

Hormonal influences on photostress-induced photoreceptor damage have also been studied. Female albino rats were either hypophysectomized (HYPEX) or ovariectomized (OVEX) before puberty. Operated and control rats were light-exposed for 45 days. The retinal damage in OVEX and HYPEX rats were significantly less than in intact rats (15). The same authors in a later study compared the effect of estrogen and/or progesterone administration in OVEX animals with ovariectomy alone (16). Continuous light-exposed, OVEX rats receiving 0.05 µg estradiol benzoate had significantly greater destruction of photoreceptor cells than OVEX oil-treated animals. Progesterone administration (2.5 mg/day) alone had no effect on light-induced photoreceptor degeneration.

Progesterone is a steroid hormone, the most important progestin. In the normal nonpregnant female, progesterone is secreted during the luteal phase of the menstrual cycle by the corpus luteum. During pregnancy, the hormone is secreted by the placenta. The main source of synthesis is cholesterol, circulating in blood.

Androgen, estrogen, and progesterone receptor mRNAs were found in several ocular tissues (i.e., retina/uvea, retina/choroid, and retinal pigment epithelial cells) in male and female rats, rabbits, and humans, which may present target organs for sexual hormones (17). Lanthier and Patwardhan (18) detected higher progesterone concentration in rat retina than in plasma, although its synthesis does not appear to be local (19). The dynamics of progesterone metabolism were studied by Robinson et al (20), who found that the disappearance of exogenous progesterone from blood was described by two half-lives of 0.5 and 11.7 min. After a single intravenous administration of 500 µg/kg body weight progesterone, the distribution and elimination phase half-lives were 0.13 ± 0.024 and 1.21 ± 0.21 hours in rats (21). These findings assured us that the drug reached the target organ, the retina.

The aim of our present study was to test the neuro-

protective effect of progesterone in the retina of male albino rats acutely exposed to bright continuous illumination under experimental conditions, previously shown to cause apoptosis of rod photoreceptor cells (22).

MATERIALS AND METHODS

Chemicals

Progesterone (4-Pregnene-3, 20 dione) and benzyl alcohol (vehicle) were purchased from Sigma (St. Louis, MO).

Animals

Albino Sprague-Dawley rats were born and raised under strictly controlled cyclic light conditions (12 hr ON, 12 hr OFF, 5-10 lux). In order to minimize the effect of endogen progesterone synthesis, male rats at the age of 5 weeks were chosen for the experiment. Animals were fed laboratory chew *ad libitum* and had free access to water.

Light damage regimen

Five-week-old male rats were divided into two groups. Within each group, half of the animals (n=5) were injected intraperitoneally with progesterone (60 mg/kg body weight in 20 µL benzyl alcohol) and the other half with the vehicle (benzyl alcohol) alone. The injections were given daily, around 9:00 am for 4 days. The third injection was given 30 minutes before light stress and the fourth injection was given immediately after removing the rats from the light stress. Control animals were injected in the same manner, but remained under the dim cyclic light. Light stress was for 24 hours in a box with reflecting surfaces, illuminated by three fluorescent tubes (cool white, 34 W), of illuminance intensity of 2700 lux at the level of rats' eyes. Animals were placed in the light box near the time of normal light onset. After a 24 hour exposure, animals were placed in the dark for 24 hours and then returned to their normal cyclic schedule. Baseline electroretinograms (ERGs) were recorded 4 days before starting the drug administration and follow-ups 5 days after the end of light stress. Animals were then killed and eyes enucleated for morphologic evaluation. In the unexposed groups, both ERG tests and enucleations were performed in parallel.

Electroretinography

Dark-adapted rats were anesthetized under dim red light with xylazine (6 mg/kg body weight) and ketamine (120 mg/kg body weight, VEDCO, Inc., St Joseph, MO) by intramuscular injection. AK-Dilate 10% was used for pupil dilatation. The active electrode was placed on the cornea and the reference electrode in the mouth. The ground was clipped to one of the rear legs. White light stimulus was delivered in 10 msec pulses in a Ganzfeld sphere (LKC Technologies, Gaithersburg, MD). Five stimuli were presented in an ascending order of intensity (- 40, - 24, - 8, 0, 10 dB) and with a 60-second interval between flashes.

Histology

Rats were anesthetized with CO_2 and killed by cervical dislocation. Enucleated eyes were placed in fixative (PerFix) and processed for histology. Five-micrometer-thick paraffin sections were cut through the optic nerve head (ONH) along the vertical meridian and stained with hematoxylin and eosin. The thickness of the outer nuclear layer (ONL) and the length of the rod inner segment (RIS) plus rod outer segment (ROS) were measured at 0.5 mm distances from the ONH to the superior and inferior ora serrata.

Statistical analysis

Results were plotted as a mean ± SD. Unpaired t-test was used for assessing significant differences across groups for the ERG data. Paired t-test was used where appropriate for comparison of baseline and follow-up ERGs of the same animal. For comparison of ERG results, we used the amplitudes measured at the brightest flash intensity (10 dB). Values below p=0.05 were reported as significant.

RESULTS

Retinal function. Baseline ERGs were recorded 7 days before and follow-up ERGs 5 days after the 24-hour light stress. Figure 1 displays the a- and b-wave curves for the four experimental groups. Twenty four–hour 2700 lux illumination caused severe decrease in both a- and b-wave amplitudes. There was a significant difference ($p<0.05$) between light-damaged groups and

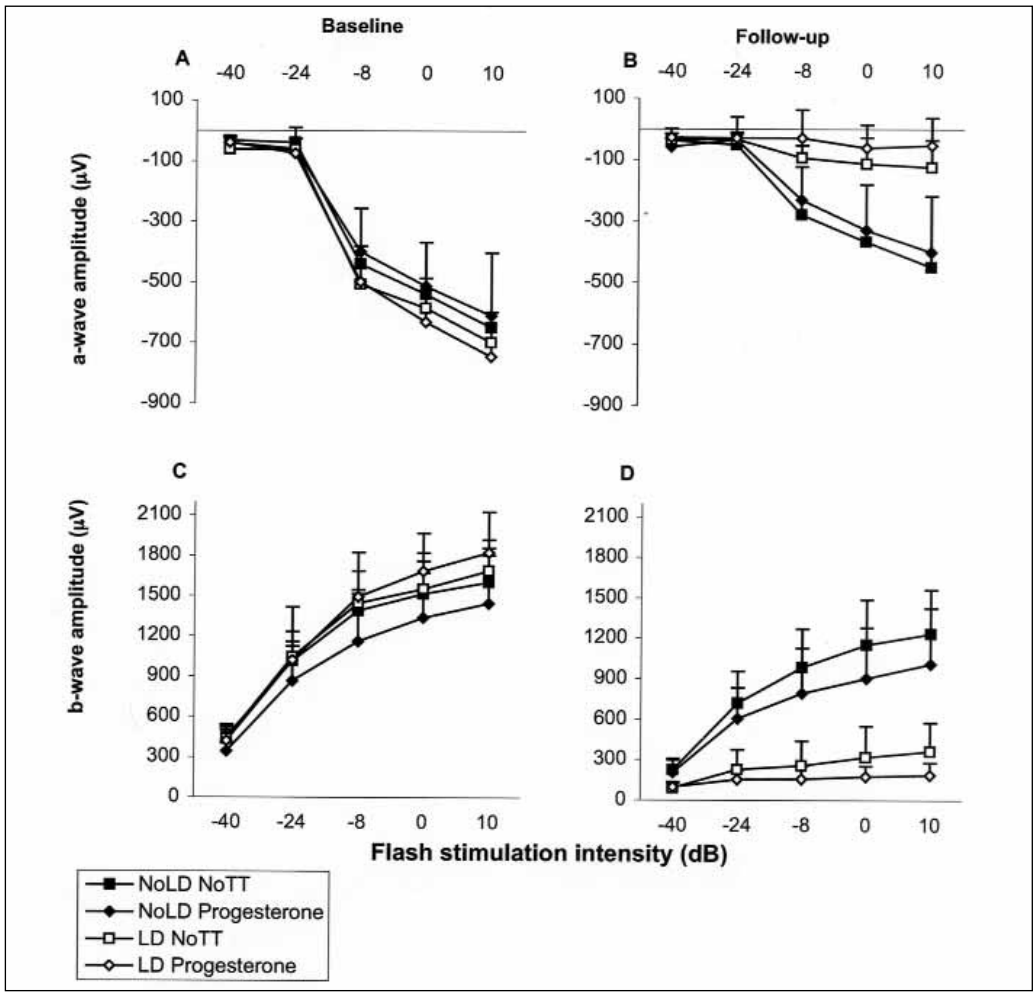

Fig. 1 - *Electroretinogram (ERG) sensitivity curves before and after exposure to 2700 lux for 24 hr. Effect of progesterone and vehicle.* **a)** *Baseline a-wave amplitudes;* **b)** *Follow-up a-wave amplitudes;* **c)** *Baseline b-wave amplitudes;* **d)** *Follow-up b-wave amplitudes. NoLD = Not light damaged; LD = Light damaged; Prog = Progesterone treated; NoTT = Not (vehicle) treated. Not Light damaged, vehicle treated (black boxes); not light damaged, progesterone treated (black diamonds); light damaged, vehicle treated (open boxes); light damaged, progesterone treated (open diamonds). Values are µV ± SD (n=5).*

nontreated groups in both the a- and b-wave values. When comparing follow-up ERG data of light-stressed progesterone treated and light-stressed nontreated rats, the difference was not statistically significant (a-wave: p=0.12; b-wave: p=0.08). These results show that the administration of progesterone did not provide functional protection against retinal damage caused by light stress.

Retinal structure. The morphology of the retinas of the four experimental groups is shown in Figure 2. Micrographs were taken about 1 mm from the ONH on the superior retina. The retinas of nonexposed animals were intact both in the progesterone-injected and in the control groups (Fig. 2, a and b). In the light-exposed groups, however, the retinas were severely damaged (Fig. 2, c and d). The rod inner and outer segments were shortened and disorganized. The ONL was thin, with only one to two rows of nuclei preserved. We measured the thickness of ONL and the length of RIS+ROS along the vertical meridian from the optic nerve head to the ora serrata, superiorly and inferiorly. The results in Figure 3 show that the retinal degeneration is the most severe in the superior hemisphere, in both light-damaged groups. The average ONL thickness and RIS+ROS length for each group was calculated. The difference between the progesterone-treated, exposed and the nontreated, exposed groups' morphologic data was not statistically significant (ONL: p=0.61; RIS+ROS: p=0.51). These results are in concordance with those of the ERG tests, which collectively show that progesterone is not protective against retinal degeneration caused by excessive light exposure.

DISCUSSION

Photoreceptor apoptosis induced by excessive light exposure in animals is a model to study human

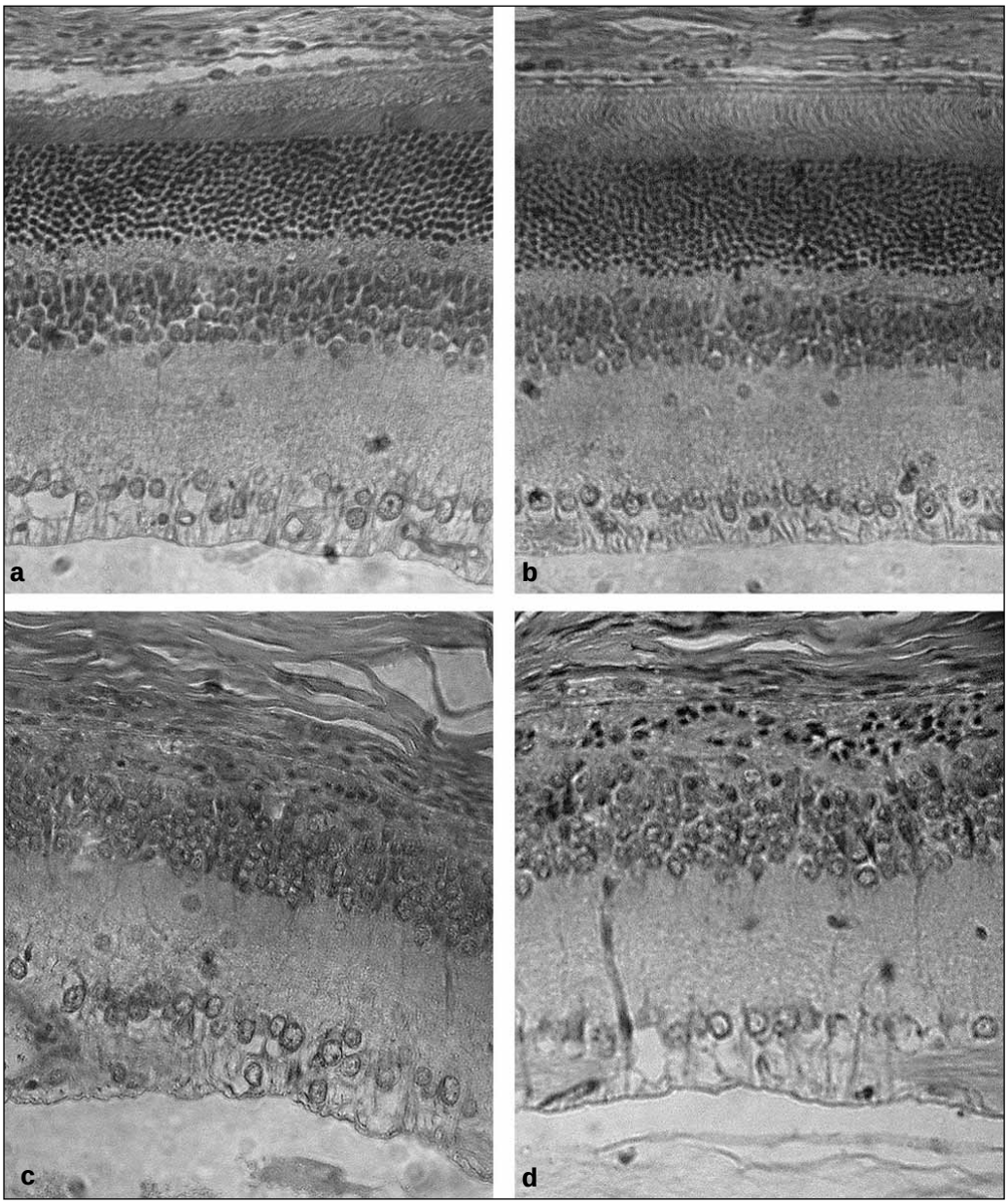

Fig. 2 - *Light micrographs of rat retinas. Sections were taken 1 mm from the optic nerve head along the superior meridian.* **a)** *Not light damaged, vehicle treated;* **b)** *Not light damaged, progesterone treated;* **c)** *Light damaged, vehicle treated;* **d)** *Light damaged, progesterone treated.*

retinal degeneration (22). The final common pathway is apoptosis (23-25), although the molecular etiology of light damage is unclear. A variety of factors influence the severity of retinal damage caused by photostress (26): light (27, 28), genotype (29), diet (30-33), age (34), eye pigmentation (35, 36), body temperature (37), pre-exposure light history (38, 39), retinal location (35), time of exposure relative to light cycle (40), and level of stress and sex hormones (41, 42). The latter observation and the fact that premenopausal women have lower incidence of stroke and the beneficial effect of estrogen therapy on postmenopausal women's heart disease morbidity raised the question of the importance of female sexual hormones in protection of cells from stress.

There have been many studies in which attempts were made to block the intracellular signaling cascade that leads to apoptotic photoreceptor cell death. It was suggested that light induces the formation of free radicals (33, 43-45). Experiments using antioxidant agents such as ascorbic acid (46, 47) and dimethyl thiourea (48) protected albino rats from

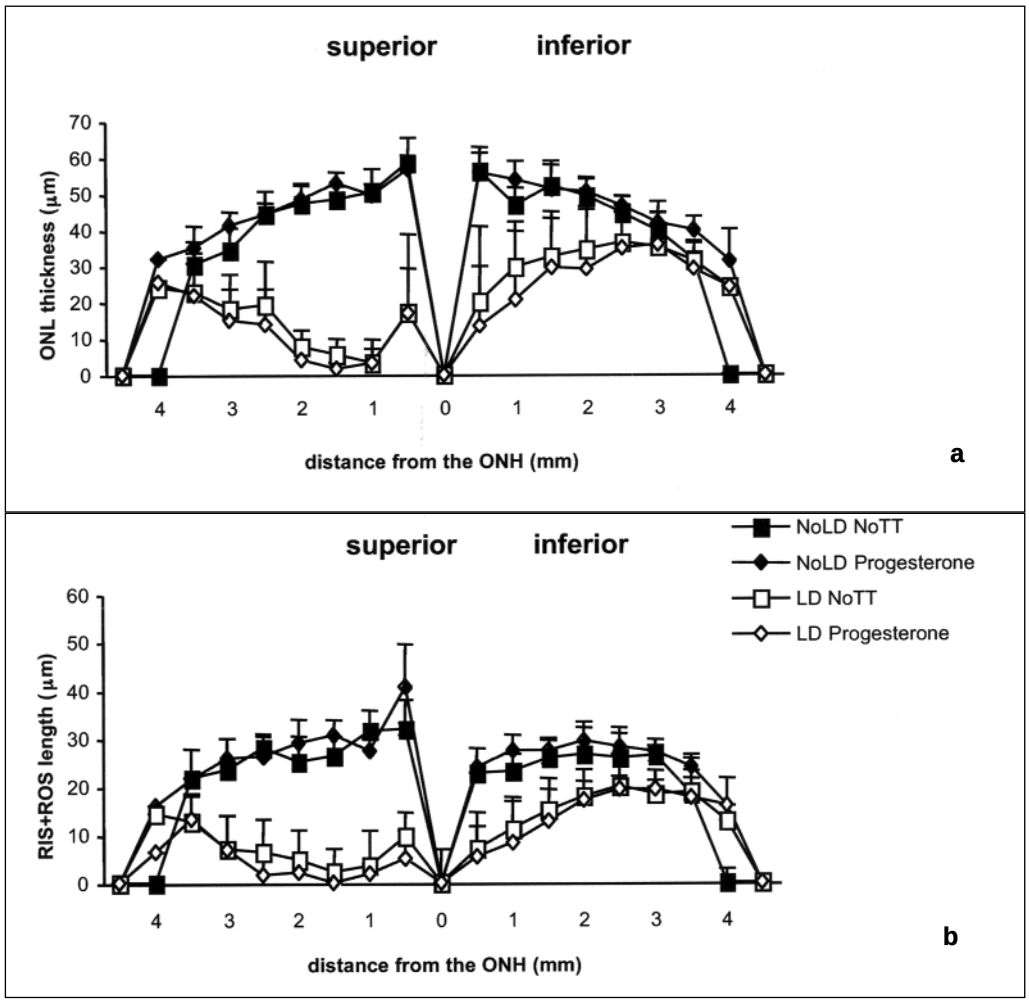

Fig. 3 - a) *Outer nuclear layer thickness,* **b)** *Rod inner segment + rod outer segment length are plotted as a function of distance from the optic nerve head. Measurements were made at 0.5 mm intervals along the vertical meridian to the superior and inferior ora serrata. These results are expressed as mean thickness/length ± SD (n=5). Not light damaged, vehicle treated (black boxes); not light damaged, progesterone treated (black diamonds); light damaged, vehicle treated (open boxes); light damaged, progesterone treated (open diamonds).*

light damage. Phenyl-N-tert-butylnitrone (49, 50), Ginkgo biloba (51), and methylprednisolone (52) also were protective. In our previous study, we found that the nitric oxide synthase inhibitor N^G–nitro-L-arginine-methyl ester (L-NAME) provided significant morphologic and pronounced functional protection on wild-type albino rats against acute light damage (53). Studies on the effect of vitamin E on light damage produced controversial data (54-56). However, the evidence to date favors a beneficial effect of antioxidant therapy on light-induced apoptosis.

Different endogenous cytokines were identified as photoreceptor rescue factors (57-59). A number of exogenous molecules, such as growth hormones and cytokines, have been shown to protect retinal photoreceptors from light-induced apoptosis (60-64).

In our present study we tested the effect of progesterone, one of the female steroid hormones, on acute light stress of the male albino rat retina. Five-week-old, dim cyclic light reared male albino rats were exposed to acute light stress (2700 lux for 24 hours). Half of the animals were injected with progesterone (60 mg/kg body weight) for 3 days before light damage and immediately after finishing light exposure. The control group was injected with the vehicle (benzyl alcohol) alone. The same number of animals stayed under the original dim cyclic lighting condition and was injected with either progesterone or vehicle. Baseline ERGs were recorded before light exposure and follow-up ERGs were taken after cessation of light stress.

Our functional data (Fig.1) showed that illumination caused statistically significant decrease in ERG amplitudes in both light-damaged groups, although there was no significant difference between exposed-progesterone treated and exposed-vehicle treated an-

imals' data. These results are supported by our structural data, which showed no difference in ONL thickness and length of RIS+ROS between treated or non-treated experimental animals (Fig. 3). The retinal degeneration of light-stressed rats was the most severe in the superior hemisphere, as previously shown by us (53) and others (26). However, the electrophysiologic and structural findings detailed above do not support the hypothesis that progesterone administration provides protection against light-induced retinal degeneration in male albino rats.

There are a number of publications reporting on progesterone's beneficial effect in women with partial epilepsy (7) and in the outcome of brain injury in rats (8-10). Progesterone also was protective after experimental stroke (11,12), although in the latter study striatal infarct was smaller in the estrogen- but not in the progesterone-treated group. A retrospective study on postmenopausal women and the risk of heart disease was compared between individuals taking combined progestin-estrogen and estrogen therapy alone (4), and found that the addition of progestin did not attenuate the cardioprotective effect of postmenopausal estrogen therapy.

In our study, we could not show any neuroprotective effect of progesterone on light-induced apoptosis in rod photoreceptor cells, in agreement with the report of Olafson and O'Steen (15).

It seems probable that light-induced retinal stress, contrary to other stresses (i.e., brain injury, stroke, epileptic seizures), cannot be ameliorated by progesterone administration. Although in this study progesterone alone was not protective, it is possible that the interaction between the female steroid hormones progesterone and estrogen is responsible for the neuro- and cardio-protective effects, and similar experiments designed for the prevention of photostress-induced retina damage may clarify the contribution exerted by additional factors. In this regard, Yu et al (65) have recently shown that estrogen administered to ovariectomized rats protected against light-induced apoptosis.

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Reprint requests to:
Ildikó Káldi, MD
Kenézy Hospital
Debrecen, Bartók 2-26
4043, Hungary
kappelmayer@jaguar.dote.hu

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