Effects of hypophysectomy, pituitary gland homogenates and transplants, and prolactin on photoreceptor destruction

W. Keith O'Steen and Sandra L. Kraeer

Prepubertal removal of the pituitary gland, which in young animals influences sexual maturation, reduces significantly the amount of retinal photoreceptor destruction when the rats are exposed to continuous illumination in adulthood. When crude pituitary gland homogenate is administered to adult rats hypophysectomized prior to puberty, photoreceptor destruction is more severe. Transplantation of whole pituitary glands to the kidney capsule of hypophysectomized rats also reduces the effect of pituitary gland removal and results in more extensive damage to receptor cells than found in hypophysectomized, adult animals. Hypophysectomized rats treated with prolactin had more severe retinal damage than untreated, hypophysectomized rats. The injection of pregnant mare serum and human chorionic gonadotropic hormones into hypophysectomized rats was not effective in reversing the protection afforded by hypophysectomy. Results of these studies indicate the hormones of the pituitary gland have a regulatory influence on the severity of light-induced, retinal photoreceptor damage in the rat.

Key words: retina, retinal damage, photoreceptors, pituitary gland, hormones, prolactin.

Photoreceptor destruction caused by exposure of albino rats to continuous fluorescent illumination[1-5] apparently is influenced by the age of animals.[6-8] The retinas of sexually immature rats are significantly more resistant to the damaging effects of light than those of sexually mature, adult rats or of rats over 7 weeks of age.[7] The gender of the animal does not appear to influence the rate of photoreceptor destruction, which also may be induced by exposure to incandescent lighting.[9] However, incandescent light exposure, in addition to damaging the photoreceptor cells, causes a reversible degeneration in the extraocular muscles, which is not related to the rat's gender or age.[10]

Light damage to photoreceptor cells in rats begins prior to puberty, which is indicated by the vaginal opening time at 42 days of age, but becomes significantly more severe in subsequent weeks during and immediately after puberty.[7] The removal of the ovaries or the pituitary gland prior to puberty, or the ovaries alone after

From the Department of Anatomy, Emory University School of Medicine, Atlanta, Ga.

This project was supported by United States Public Health Service grant EY 01566 from the National Institutes of Health.

Submitted for publication Feb. 23, 1977.

Reprint requests: Dr. W. Keith O'Steen, Department of Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, N. C. 27103.

puberty, decreases the destructive influence of light on photoreceptor cells, as indicated by the survival of more receptors in these animals.[11] The effects of postpubertal pituitary removal on light-induced receptor cell damage has not been examined at this time.

The purpose of the present study is to examine further the influence of the pituitary gland on light-induced photoreceptor damage. Rats hypophysectomized prior to puberty either have been injected with pituitary gland homogenate or have received a pituitary gland transplant to the kidney capsule. The influence of prolactin on photoreceptor survival has been investigated, since elevated levels of this hormone are produced by pituitary glands transplanted at sites distant to the hypothalamus.[12, 13] Pituitary gland–ovarian interactions and their influence on photoreceptor damage have been examined by stimulating the secretion of ovarian hormones in hypophysectomized rats by the injection of gonadotropic hormones.

Materials and methods

Intact, female albino rats (CD strain, Charles River Laboratories, Wilmington, Mass.) were hypophysectomized by the parapharyngeal approach at 21 days of age. They were maintained on ground Rat Chow during a 2-week postoperative period. Thereafter they received rat chow pellets and water ad libitum.

The animals were housed in clear polyethylene cages with wire tops, at 22° ± 1° C., with a diurnal cycle of 14 hr. of light at an intensity of 70 ft.-c. and 10 hr. of darkness. At the beginning of continuous light exposure, the rats were placed under cool-white fluorescent light with a spectral range of 397 to 732 mμ (maximum at 590 mμ) and an approximate intensity of 70 ft.-c. A total energy flux density of 210 μw/cm.^{2} mμ was measured with a model SR Spectroradiometer (Instrumentation Specialities Co., Lincoln, Neb.) at animal eye level. All animals receiving continuous illumination were exposed for 21 days, that is, from the ninth through the twelfth weeks of age, with the exception of the 5-week-old recipient rats described below.

Pituitary transplants. Pituitary glands from 9-week-old rat donors were transplanted by the dorsal approach to the kidney capsule of prepubertal 5-week-old and adult 9-week-old hypophysectomized (HYPEX) recipient animals. One group of eight HYPEX, 9-week-old rats received a transplant bilaterally, but since the results to be described later did not differ from those of animals receiving a single transplant, all other experiments involved rats with a unilateral transplant. The sham operation was identical and involved puncture and manipulation of the kidney capsule, but no pituitary tissue was transplanted. Adult 9-week-old recipient and sham control groups were exposed to continuous light from 9 to 12 weeks of age, and 5-week-old prepubertal groups were exposed from 5 to 8 weeks of age. All groups were autopsied at the end of the exposure period.

Pituitary homogenates. Pituitary glands were collected from 9- to 14-week-old donor rats and pooled to provide 6 mg. of pituitary tissue per hypophysectomized rat recipient per day. The glands were disintegrated in a glass homogenizer with sufficient physiological saline for a 0.2 ml. injection volume. Subcutaneous injections in the dorsal cervical region were given daily during the 21-day, continuous–light exposure period, which began when the rats were 9 weeks of age.

Hormone injections. Groups of 9-week-old HYPEX rats were given injections of either 30 I.U. of pregnant mare serum gonadotropin (PMS; CalBiochem, La Jolla, Calif.) in a single subcutaneous injection or 30 I.U. of PMS plus 10 I.U. of human chorionic gonadotropin (HCG; CalBiochem). HCG was administered 48 hr. after PMS. Another group of 9-week-old HYPEX rats was injected daily during the 21 days of continuous illumination with either ovine prolactin (NIH-P-S12, 2 mg./day intraperitoneally) or ovine prolactin and estradiol benzoate (0.1 μg/day subcutaneously; Sigma Chemical Co., St. Louis, Mo.). Continuous light exposure was begun at 9 weeks of age in these groups. They were autopsied at 12 weeks of age.

• • •

Six 9-week-old female rats, which were unoperated, injected daily with physiological saline for 21 days, and exposed to cyclic photoperiod, were included for comparison with the other control and experimental groups. They were autopsied at 12 weeks of age.

At the end of the light-exposure period, animals were weighed and autopsied. Ovaries were weighed, and they and the eyes were fixed in Bouin's solution. After the cornea had been trimmed and the lens removed, the remainder of the eye was dehydrated in alcohols, embedded in paraffin, and sectioned at 7 μ thickness on the anterior-posterior axis of the eye. Sections of the retina, including the optic nerve, were stained with Harris's hematoxylin and eosin. The follow-

Invest. Ophthalmol. Visual Sci.
October 1977

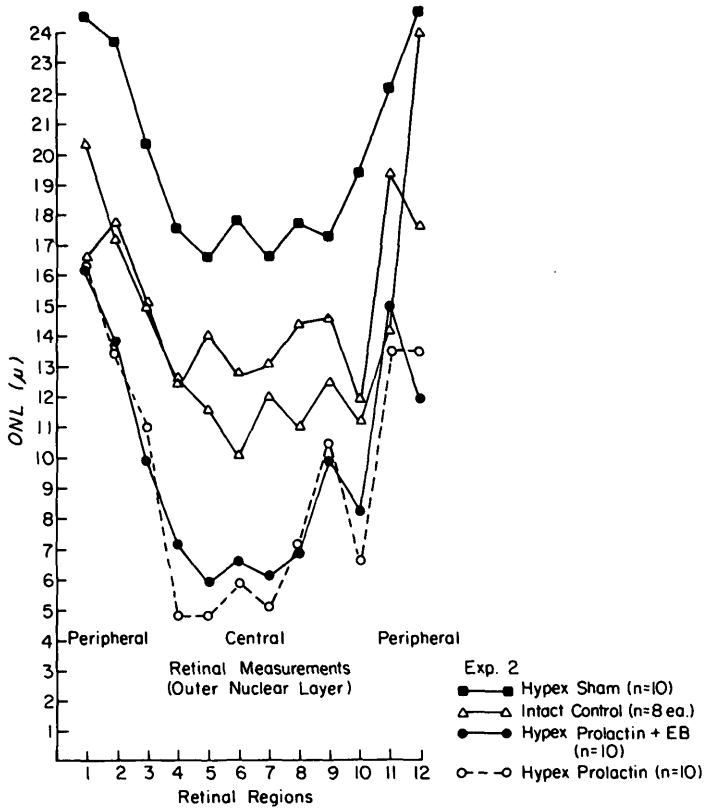

Fig. 1. Data from experiment 2, with an additional intact control group from experiment 1. Average measurements are depicted on each tissue section from each of 12 retinal loci extending from the peripheral (locus 1) through the central retina (loci 4 to 9) to the opposite peripheral retina (locus 12). In each group, maximal damage occurred in the central retina. HYPEX sham retinas were significantly thicker than those of the intact control groups, whereas HYPEX-prolactin retinas were significantly thinner than those of intact control or HYPEX-sham groups. *n*, Number of animals/group.

ing measurements were made on each retina with an ocular micrometer at 400× magnification: (1) outer nuclear layer (ONL) thickness, measured from the outer limiting membrane inward to include all photoreceptor nuclei, and (2) retinal thickness (RT), the distance from the outer limiting membrane to the inner margin of the ganglion cell layer. The two measurements were taken at 12 different loci around the circumference of each retinal section, beginning at the periphery (See Fig. 1 for example of data from each locus.) Loci were separated by approximately 540 μ. Statistical comparisons were based on a two-tailed, one-way analysis of variance[14] or an analysis by the Student t test.

Results

Experimental results on ONL thickness and entire RT. Table I is a summary of results of three experiments related to effects of the pituitary gland on photically induced retinal damage. The data are based on light microscopic measurements of the ONL thickness and entire RT of control and experimental female albino rats. A comparison of data from group 6 (intact, cyclic-light exposed) and group 5 (intact, continuous light exposed) indicates the severe degree of retinal destruction resulting from 3 weeks of exposure to low-intensity fluorescent light. Removal of the pituitary gland (HYPEX rats) significantly reduced retinal damage (groups 1 vs. 5), as previously described.[11] When a pituitary gland was transplanted to the kidney capsule of HYPEX rats, retinal damage was more severe than in HYPEX sham-operated rats (groups 1 vs. 2; groups

Table I. Summary of ONL thickness and RT measurements and of body and ovarian weights in intact and HYPEX rats

Treatment groups	*N*	*ONL thickness ± S.E.M. (μ)*	*RT ± S.E.M. (μ)*	*Body wt. (gm.)*	*Ovarian wt.*	
					mg.	*mg./100 gm. body wt.*
Experiment 1:						
1. HYPEX sham, L:L	8	19.09 ± 1.20	87.58 ± 4.18	68.15 ± 4.66	2.76	4.35
2. HYPEX transplant, L:L	26	14.94 ± 0.18†	82.80 ± 1.65*	94.31 ± 6.52	4.32	4.60
3. HYPEX PMS, L:L	12	17.68 ± 1.92	83.69 ± 1.24	59.93 ± 2.02	39.88	66.54
4. HYPEX PMS-HCG, L:L	14	19.88 ± 1.61	94.24 ± 2.63	64.50 ± 0.89	62.26	96.50
5. Intact control, L:L	8	15.22 ± 0.77*	95.94 ± 1.44	190.33 ± 11.95	49.10	25.80
6. Intact control, L:D	6	42.90 ± 0.70†	129.79 ± 1.06	178.27 ± 3.71	46.16	25.90
*p <0.02 (groups 5 vs. 1); †p <0.001 (groups 1 vs. 2 and 6 vs. all).						
Experiment 2:						
7. HYPEX sham, L:L	10	19.98 ± 0.89	71.21 ± 1.60	64.60 ± 2.94	2.98	4.62
8. HYPEX prolactin, L:L	10	9.77 ± 0.92*	71.61 ± 2.75	67.80 ± 7.02	4.13	6.09
9. HYPEX prolactin-EB, L:L	10	9.78 ± 0.80*	72.78 ± 1.25	61.40 ± 6.50	3.32	5.40
10. Intact control, L:L	8	14.57 ± 0.43†	76.32 ± 1.72	268.25 ± 15.96	96.22	35.87
*p <0.001 (groups 7 vs. 8 and 9 and 10 vs. 8 and 9), †p <0.01 (groups 7 vs. 10).						
Experiment 3:						
11. HYPEX sham, L:L (5 wk.)	12	17.88 ± 0.99	97.72 ± 2.41	67.80 ± 1.55		
12. HYPEX transplant, L:L (5 wk.)	16	13.97 ± 1.02*	92.95 ± 2.89	98.78 ± 10.33		
13. HYPEX sham, L:L (9 wk.)	8	16.87 ± 0.09	98.89 ± 4.93	69.44 ± 2.52	2.26	3.28
14. HYPEX transplant, L:L (9 wk.)	16	11.22 ± 1.03†	85.79 ± 2.38*	98.69 ± 8.13	5.20	5.13
15. HYPEX prolactin, L:L (9 wk.)	18	10.44 ± 0.05‡	77.14 ± 2.10‡	73.51 ± 7.80	2.49	3.48
16. HYPEX homogenate, L:L (9 wk.)	18	11.62 ± 0.13‡	77.04 ± 1.99‡	124.78 ± 12.84	67.49	52.88

*p <0.02 (groups 11 vs. 12 and 13 vs. 14); †p <0.01 (groups 13 vs. 14); ‡p <0.001 (groups 15 vs. 13 and 16 vs. 13).
L:L = Continuous light exposure; L:D = cyclic light exposure; EB = estradiol benzoate.

11 vs. 12; groups 13 vs. 14). Retinal photoreceptor damage in HYPEX rats with pituitary gland transplants was comparable to that in intact control rats exposed to continuous illumination (groups 2 vs. 5 and 10). PMS and PMS-HCG injections did not reverse the protection against retinal destruction afforded by hypophysectomy alone (groups 3 and 4 vs. 1).

Of interest is the observation that the mean ONL thickness of 5-week-old prepubertal rats receiving pituitary transplants and their control group, which were exposed to continuous light from 5 to 8 weeks of age, was greater than that of 9-week-old, sexually mature animals with pituitary transplants and their control group exposed from 9 to 12 weeks of age (group 11 > 13; group 12 > 14). The difference, however, was not statistically significant. In addition, the entire RT was significantly reduced in the 9-week-old rats with transplants (groups 13 vs. 14), but the retinal thickness of the 5-week-old transplanted animals (group 12), although reduced, did not differ significantly from that in the HYPEX sham-operated control group (group 11).

The ONL thickness and entire RT of animals receiving crude pituitary gland homogenates (6 mg./day, group 16) were significantly reduced as compared to those of the HYPEX sham-operated control rats (group 13).

The ONL thickness of rats receiving 2 mg./day prolactin was reduced significantly when compared to that of HYPEX saline-treated animals (groups 8 vs. 7; 15 vs. 13). The entire RT was significantly less in group 15 than in group 13, but this difference did not occur between groups 7 and 8. Results obtained from injecting 2 mg./day prolactin did not differ statistically from those after administration

of both 2 mg./day prolactin and 0.1 mg. of estradiol benzoate (groups 8 vs. 9); however, both of these groups had significantly reduced ONL's as compared to those of intact control animals (group 10) and of the HYPEX sham-operated group.

Experimental results on body weights, ovarian weights, and vaginal opening time. Table I also is a summary from three experiments of body weights and ovarian weights from groups of rats at autopsy. Ovarian weights have been corrected for the animal's body weight and are expressed as ovarian weight per 100 gm. of body weight.

Body weights of rats hypophysectomized at 21 days of age (groups 1, 7, 11, and 13) were significantly less than those of intact rats of the same age (groups 5, 6, and 10). Body weights of HYPEX rats receiving a pituitary transplant (groups 2, 12, and 14) or crude pituitary homogenates (group 16) were significantly increased as compared to those of sham-operated HYPEX rats (groups 1, 7, 11, and 13), but they were significantly reduced as compared to intact animals (groups 5, 6, and 10). The mean body weight of homogenate-injected rats (group 16) was greater than that of HYPEX pituitary-transplanted animals (groups 2, 12, and 14) and of HYPEX sham-operated animals (groups 1, 7, 11, and 13).

Mean body weights of HYPEX rats receiving prolactin (groups 8 and 15) or prolactin–estradiol benzoate (group 9) were not statistically different from those of HYPEX sham-operated rats. Body weights were significantly reduced by treatment with PMS (group 3) and PMS-HCG (group 4).

Ovarian weights of HYPEX rats given saline (groups 1, 7, and 13), transplanted with pituitary glands (2 and 14), injected with prolactin (groups 8, 15), or prolactin-estradiol (group 9) were significantly smaller than those of intact rats (groups 5, 6, and 10). Treatment of HYPEX rats with PMS or PMS-HCG significantly increased ovarian weights (groups 3 and 4), as did injections of crude pituitary gland homogenates (group 16), as compared with their HYPEX sham-operated and intact control groups (Table I).

Vaginal patency occurred 4 to 7 days (mean, 6.14 days) after PMS injection; 7 to 14 days (mean, 7.00 days) after PMS-HCG injection; 5 to 7 days (mean, 6.22 days) after pituitary homogenate injection; and 4 to 14 days (mean, 6.92 days) after pituitary transplantation to the kidney capsule. Vaginal patency did not occur in HYPEX sham-operated, saline-injected animal groups.

Discussion

Retinal photoreceptor destruction induced by exposure of albino rats to low-intensity fluorescent illumination is significantly more severe in sexually mature rats than in prepubertal animals. Severity of damage increases at a time period which is coincident with the transition of male and female rats into sexual maturity, that is, between the sixth and eighth weeks of age.[7] These observations led to the supposition that the susceptibility of the retina to damage by constant illumination may be associated with events of sexual maturation. An earlier experiment showed that removal of the ovaries or pituitary gland, which influences the progress of sexual maturation in young animals, resulted in decreased damage to photoreceptor cells or appeared to afford a significant degree of protection as compared to that in intact rats of the same age and after comparable exposure periods.[11] Therefore photoreceptor destruction seemingly is influenced by hormones secreted by the pituitary gland or by their target organs such as the gonads.

To test the reversibility of the apparent protection afforded by removal of the pituitary gland, a group of HYPEX rats was given, during exposure to continuous light, daily injections of a crude homogenate prepared from pituitary glands of mature rats. This treatment was adequate to stimulate (1) growth in the HYPEX rats, as

indicated by a significant increase in body weight over that of saline-injected HYPEX rats, and (2) ovarian enlargement, which was greater than that in saline-injected HYPEX and intact rats of the same age. In the pituitary homogenate–injected animals photoreceptor damage was significantly greater than that in HYPEX rats and equal to or greater than that in intact animal groups. Therefore substances in the pituitary homogenate apparently had an influence on the susceptibility of photoreceptors to photic damage.

Since several pituitary hormones such as gonadotropic hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH), and prolactin are associated with sexual maturation, groups of HYPEX rats were injected with PMS and PMS-HCG. PMS has FSH-like activity,[15] whereas HCG stimulates ovulation in PMS-primed prepubertal and HYPEX rats. Animals treated with PMS and PMS-HCG developed unusually large and apparently functional ovaries. Administration of these gonadotropins, as described, did not reverse the protection afforded to the photoreceptors by hypophysectomy. Therefore stimulation of the ovaries alone and the presence of circulating gonadotropins and ovarian steroids in HYPEX rats during the light exposure period did not appear to influence adversely photoreceptor destruction, even though removal of the ovaries from rats with intact pituitary glands previously was demonstrated to reduce the damaging effects of light exposure.[11]

Two approaches were used to examine the effects of prolactin on photically induced photoreceptor destruction: (1) transplantation of pituitary glands to a site distant from the hypothalamus and (2) injection of ovine prolactin during the light-exposure period. Everett[12, 13] demonstrated that separation of the pituitary gland from its hypothalamic control by transplanting it to the kidney capsule would initiate an elevated and prolonged period of prolactin secretion. Transplanted pituitaries were unable to secrete FSH and LH in quantities sufficient to maintain the ovaries. Retinas from HYPEX rats with functional transplants were significantly more severely damaged than those from HYPEX sham-operated animals, indicating that a gland producing elevated levels of prolactin, but reduced levels of other pituitary trophic hormones, could reverse the protection afforded by hypophysectomy and enhance photoreceptor destruction. A comparison of the appearance (atrophic, afollicular, and nonluteinized) and weights of ovaries from the pituitary transplanted groups with those of intact control groups indicated an absence, or only a minimal degree, of ovarian function.

Prolactin administration to HYPEX animals was the most effective method of reversing the protection of photoreceptor cells from light damage. The enhancement of retinal damage by prolactin treatment was best demonstrated in animal groups of experiment 3 (Table I), in which both ONL thickness and entire RT were significantly reduced when compared with retinas of HYPEX sham-operated groups. A highly significant reduction in ONL thickness was also observed in other prolactin-treated groups. After 21 days of prolactin administration, the ovaries, by the criteria mentioned above for pituitary transplant groups, were apparently nonfunctional or only minimally functional, in the absence of normal levels of pituitary gonadotropic hormones. Even though estrogen is known to be an important stimulus of prolactin secretion in rats,[16] the simultaneous administration of estrogen and prolactin (group 9) in the dosages used was no more effective in stimulating photoreceptor destruction than prolactin alone. The exact nature or mechanism of the prolactin effect, which might also account for the pituitary effect on retinal destruction, is unknown.

In summary, retinal photoreceptor destruction by light exposure is significantly more severe in adult, intact rats than in young, prepubertal animals or in those that have been hypophysectomized. Pituitary gland transplants to the kidney capsule

and injection of crude pituitary gland homogenates or the pituitary gland hormone, prolactin, reverse this protection from photic damage. Hypophyseal hormones therefore appear to have a regulatory influence on the severity of light-induced retinal photoreceptor damage in the rat.

REFERENCES

1. Noell, W. K., Walker, V. S., Kang, B. S., et al.: Retinal damage by light in rats, INVEST. OPHTHALMOL. **5**:450, 1966.
2. Gorn, R. A., and Kuwabara, T.: Retinal damage by visible light, Arch. Ophthalmol. **77**:115, 1967.
3. Kuwabara, K., and Gorn, R. A.: Retinal damage by visible light: an electron microscopic study, Arch. Ophthalmol. **79**:69, 1968.
4. Grignola, A., Orzalesi, N., Castellazzo, R., and Vittone, P.: Retinal damage by visible light in albino rats: an electron microscope study, Ophthalmologica **157**:43, 1969.
5. O'Steen, W. K.: Retinal and optic nerve serotonin and retinal degeneration as influenced by photoperiod, Exp. Neurol. **27**: 194, 1970.
6. Ballowitz, L., and Dammrich, K.: Retinaschaden bei Ratten nach einer Fototherapie, Z. Kinderheilkd. **113**:42, 1972.
7. O'Steen, W. K., Anderson, K. V., and Shear, C. R.: Photoreceptor degeneration in albino rats: dependency on age, INVEST. OPHTHALMOL. **13**:334, 1974.
8. Kuwabara, T., and Funahashi, M.: Light damage in the developing retina, Arch. Ophthalmol. **94**:1369, 1976.
9. O'Steen, W. K., and Anderson, K. V.: Photoreceptor degeneration after exposure of rats to incandescent illumination, Z. Zellforsch. **127**:306, 1972.
10. O'Steen, W. K., Shear, C. R., and Anderson, K. V.: Extraocular muscle degeneration and regeneration after exposure of rats to incandescent radiant energy, J. Cell Sci. **18**:157, 1975.
11. Olafson, R. P., and O'Steen, W. K.: Hormonal influences on photoreceptor damage: the pituitary gland and ovaries, INVEST. OPHTHALMOL. **15**:869, 1976.
12. Everett, J. W.: Luteotropic function of autografts of the rat hypophysis, Endocrinology **54**:685, 1954.
13. Everett, J. W.: Functional corpora lutea maintained for months by autografts of rat hypophysis, Endocrinology **58**:786, 1956.
14. Fisher, R. A.: Statistical Methods for Research Workers, Edinburgh, 1925, Oliver & Boyd, Publishers.
15. Cole, H. H.: On the biological properties of mare gonadotropic hormone, Am. J. Anat. **59**:299, 1936.
16. Ratner, A., Talwalker, P. K., and Meites, J.: Effect of estrogen administration in vivo on prolactin release by rat pituitary in vitro, Proc. Soc. Exp. Biol. Med. **112**:12, 1963.