Short communication

Secretory Response to Light in Rat Harderian Gland: Possible Photoprotective Role of Harderian Porphyrin

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The mammalian Harderian gland is a large orbital exocrine lipid secretion gland present in most species with the exception of primates and carnivores. Rodent Harderian glands are increasingly used as a model for porphyrin biosynthesis since porphyrins were described to be present and stored in the gland (Derrien and Turchini 1924; Payne et al. 1985) and complete synthesis of protoporphyrin IX in the gland was proved (Tomio and Grinstein 1968). Excretion of protoporphyrin into the conjunctival sac has been described by Towbin et al. (1945).

The physiological function of the Harderian gland remains unknown (Hoffman et al. 1985). It has been speculated that the gland lubricates the cornea (Davis 1929), modulates reproductive functions (Hoffman 1971), or produces pheromones (Payne 1977). The gland may play a role in the retinal-pineal axis (Wetterberg et al. 1970), both the Harderian and the pineal gland being affected by photoperiods (Reiter et al. 1983).

Lighting variations influence the porphyrin content in rodent Harderian glands (Wetterberg 1972). Effect of environmental lighting on porphyrin synthesis in rat liver has been described by Magnus et al. (1974). However, little is known about effects of light on porphyrin synthesis in the Harderian gland. Also, little attention has been paid to effects of light on the Harderian porphyrin secretion.

The aim of the present study was to examine the influence of a sudden illumination on the Harderian porphyrin secretion in rat.

Animals used in the experiments were male Wistar rats 10—14 weeks old, weighing 250—300 g. Animals used in the first experiment were kept in light-dark (10 : 14), illumination period between 7 a.m. and 5 p.m. Light sources as common for laboratory animal houses (fluorescent light) were used, its intensity ranged between 40 and 50 lx within the cages. Animals used in the second

experiment were first kept under the same conditions as above; they had been kept in complete darkness for additional 7 days before the start of the experiment. The light source used in these experiments was a group of 4 standard Tesla 20 W cool fluorescent lamps 0.6 m in length, with a distance of 50 mm between each two of them. The source was placed 0.5 m above the bottom of the cage with the animals. Light intensity in the cage was 3000 lx ("intense light"). Animals of the "dark" group were killed in a dark room illuminated by soft red light (0.5 lx). In both experiments animals were killed by decapitation between 10 p.m. and 12 p.m.

Porphyrins were determined spectrofluorimetrically as methylesters (Falk 1964) at 605 nm upon excitation at 407 nm. Dimethylprotoporphyrin was used as reference substance since a concurrent HPLC assay revealed that protoporphyrin made up over 95 % of the total porphyrin content.

Analysis of variance and *t*-test were used to process the data statistically.

In the first experiment, the decrease of porphyrin content in pairs of Harderian glands was determined after 5; 15; and 30 min of intense lighting, and compared with porphyrin content in glands of the rats killed in the dark. The animals were decapitated, the glands were rapidly removed, weighed, placed in cold methanol and kept in cool (0°C) until measurements (in the next morning). UV lamp was used to check the completeness of the gland removal. Pairs of glands were homogenized in methanol and porphyrins were esterified in methanol : sulphuric acid (9 : 1) (Table 1).

Table 1. Total porphyrin contents and porphyrin concentrations in pairs of Harderian glands of male rats killed in dark, and after various intervals of lighting, and significance of decrease as compared with the dark group.

Time of lighting (min)	Number of animals	Harderian gland porphyrin			
		content (μg ± SD)	*p*	concentration (μg/g tissue ± SD)	*p*
0	21	122.9 ± 34.3	—	481.1 ± 136.2	—
5	9	110.6 ± 27.5	NS	359.3 ± 126.0	NS
15	9	79.6 ± 25.6	<0.01	308.7 ± 139.2	<0.01
30	10	66.6 ± 24.6	<0.001	239.7 ± 135.3	<0.001

In the second experiment, the amount of porphyrins in the conjunctival sac was determined in 10 rats kept in dark for 7 days and killed in soft red light, and in 9 rats kept in dark for 7 days and killed after 2 min of intense lighting. Conjunctival moisture was collected with a strip of filter paper placed in the inner canthus of each eye for 30 seconds after decapitation; then, the strip was

dipped in methanol : sulphuric acid (9 : 1). Samples from both eyes of each animal were pooled and values per 1 eye were calculated.

Mean value ($\pm$ SD) in the dark was 27.0 ± 23.0 ng/eye, that after 2 min lighting was 114.7 ± 24.9 ng/eye. The difference was significant ($p < 0.001$, $t = 7.55$).

The results show an immediate increase in porphyrin secretion into the conjunctival sac after sudden lighting. However, exact direct quantification of porphyrin secretion rates into the conjunctival sac is difficult since unknown amounts flow into the nasolacrimal canal. Bearing in mind the yet unknown synthesis rate, the estimation of the decrease of porphyrin content in the gland would be useful to quantify secretion rates. The amounts of porphyrins in the conjunctival sac seem to be too small to result in a significant decrease in their content in the gland after 5 minutes of lighting.

Little interest has been taken in Harderian porphyrin secretion as yet. A phenomenon termed chromodacryorrhea, i.e. secretion of red tears from the Harderian gland, has been studied (Towbin et al. 1945) after injection of lethal acetylcholine doses.

Complete evolution of the gland and its porphyrins in species adapted to dark environment (e.g. rats) has been emphasized by Joó and Kahán (1975). The retinas of albino rats undergo degenerative changes if the animals are exposed continuously to ordinary animal room intensities of fluorescent light (Noell et al. 1966; O'Steen 1970). Taking into account that porphyrins show an intense absorption in both visible and UV range, one possible explanation for the increased secretion of Harderian porphyrin after light exposure might be a photoprotective role of Harderian porphyrin, i.e. a function of light filter. Provided this is true, the primary physiological role of Harderian porphyrin might be adaptation of the eye to light and dark.

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