PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **189**, 211–216 (1988)

Underfeeding and Exposure to Short Photoperiod Alters Rat Pineal and Harderian Gland Lysosomal Enzyme Activities[1] (42800)

M. K. VAUGHAN, M. NORDIO, P. J. CHENOWETH,
J. P. CHAMBERS,* AND R. J. REITER

*Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284; and *Brain Research Laboratory, Division of Life Sciences, University of Texas at San Antonio, San Antonio, Texas 78285*

Abstract. Harderian gland (HG) weight and lysosomal enzyme activity were evaluated after 21-day-old female rats were singly caged in a long (LP; 14:10 LD) or short (SP; 8:16 LD) photoperiod and fed on one of two dietary regimens (fed *ad libitum* or 50% underfed) for 50 days; an additional fed and an underfed group of animals in LP were injected every afternoon with 100 μg melatonin. Absolute HG weights were significantly lower in all underfed groups compared to their respective fed controls or to the LP fed control group. Absolute HG weights of underfed rats in SP were significantly lower than the underfed rats in LP. Relative HG weights (mg/100 g body wt) were significantly higher in the underfed saline or melatonin-treated groups compared to their respective fed controls; however, HG of the underfed SP group were not different from SP-fed controls. No significant differences in HG acid phosphatase, hexosaminidase, and β-glucuronidase activities were observed in any of the treatment groups maintained in LP. Acid phosphatase, hexosaminidase, and β-glucuronidase activities were significantly elevated in HG of underfed animals maintained in SP compared to their respective fed controls or to the LP-underfed group. Both the underfed control and the underfed-melatonin treated groups had lower pineal protein values than their respective fed groups; underfed animals in 8:16 LD had similar pineal protein values compared to those of the fed control group in SP. Significant effects of photoperiod and underfeeding with no interaction between these variables were observed on pineal acid phosphatase. The fed group maintained in 8:16 LD had significantly higher acid phosphatase activity than the fed group kept in 14:10 LD. In conclusion, underfeeding resulted in severely reduced body weights and absolute Harderian gland weights. Increased activity in certain lysosomal enzymes occurred in both the pineal and Harderian gland and in some instances this was dependent upon the light cycle and dietary regimen to which the animals were exposed.

Underfeeding is a potent pineal potentiating factor in the rat which, when combined with light deprivation or melatonin treatment, depresses gonadal maturation (1–3). The effects of short photoperiod can be prevented by pinealectomy in underfed animals (2, 3); thus, short photoperiod must alter the net output of pineal substances affecting reproduction or alter the sensitivity of target tissues in underfed animals. Since pineal protein values are depressed in underfed animals receiving saline or daily injections of the pineal hormone melatonin (MEL), we proposed that normal catabolic processes might be enhanced by the combination of melatonin treatment or short photoperiod in underfed animals. Since lysosomal enzymes play a major role in the catabolism of tissues and fluctuate with the photoperiod in several tissues (4, 5) including the pineal (6), we determined if the activities of four acid hydrolases in the rat pineal were increased following 50 days of exposure to short photoperiod, melatonin treatment, and/or underfeeding.

The Harderian gland (HG), a large retrobulbar exocrine gland, synthesizes a variety of substances including porphyrins (protoporphyrin IX and tricarboxylic porphyrins), lipids (wax esters, phospholipids, cholesterol, cholesterol esters, and triglycerides), and melatonin (7–11). There is considerable speculation concerning the function of the gland but the synthesis of the indoleamine melatonin and the manipulation of the mor-

---

[1] This work was supported by NSF Grant PCM-8410592 and NIH Grants RR08194 and GM07717.

0037-9727/88 $1.50

phology and biochemistry of the gland by photoperiod and/or hormones (sex steroids, pituitary hormones) suggest that it, like the pineal, might be involved in the responses to photoperiod (12–17). In the course of our studies on the environmental factors which influence the Harderian gland and the pineal, we noted and report herein that a combination of underfeeding and short photoperiod significantly inhibited HG absolute weight more then underfeeding alone. This potentiating effect of underfeeding to the effects of short photoperiod is a pattern reminiscent of that described above for the pineal and we determined if similar mechanisms (i.e., increased activity of catabolic enzymes) might also be involved in this organ.

Materials and Methods. *Animals.* Twenty-one-day-old female rats (Charles River) were caged singly in a room providing either long (14:10 LD; lights on 0600 hr) or short (8:16 LD; lights on 0800 hr) photoperiod. One group in each photoperiod was fed *ad libitum* while a corresponding group received only 50% of the food of its fed control. Additionally, one fed and one underfed group in 14:10 LD received a sc injection of 100 μg melatonin (Sigma Chemical Co., St. Louis, MO) daily between 1400 and 1600 hr. Melatonin was dissoved in absolute alcohol and diluted with saline. All animals were decapitated between 0830 and 1130 hr after 50 days of treatment. Both Harderian glands were weighed, frozen on solid CO_2, and maintained at −70°C until assayed.

Preparation of homogenates. Tissues were thawed in 1.0 ml cold distilled water and homogenized on ice using glass homogenizers. Homogenates were centrifuged (5 min, 2000 rpm) in order to remove cellular debris and the resulting supernatants serve as the source of enzymes for the various assays.

Enzyme assays. All assays were carried out at 37°C and contained the following constituents in final volume of 0.1 ml: 0.2 *M* sodium acetate buffer adjusted to the specified pH, 5 μl of Harderian gland homogenate or 10 μl pineal homogenate as a source of enzyme, and the appropriate substrate. The following substrates (Sigma Chemical Co., St. Louis, MO) were used at the final concentration and pH indicated: α-mannosidase, 4-methylumbelliferyl α-D-mannopyranoside (1.5 m*M*, pH 5.0); acid phosphatase, 4-methylumbelliferyl phosphate (5.6 m*M*, pH 5.0); and β-glucuronidase, 4-methylumbelliferyl β-*D*-glucuronide (1.5 m*M*, pH 5.0). 4-Methylumbelliferyl-*N*-acetyl-β-D-glucosaminide (5.0 m*M* dissolved in 0.08 *M* citrate-phosphate buffer, pH 4.4) was used to determine hexosaminidase activity. Assays were terminated by addition of 2.9 ml 0.1 *M* ammonium hydoxide–glycine buffer, pH 10.5. The release of 4-methylumbelliferone from fluorogenic substrates was measured in identical fashion as previously described (18). Hydrolysis of fluorogenic substrate is expressed as nanomoles substrate hydrolyzed per minute per milligram protein. Fluorogenic assays are linear with respect to time of hydrolysis and protein assayed. All assays were run in triplicate.

Protein determination. Protein was determined by the method of Bradford (19) using bovine serum albumin as a standard.

Statistics. Data from the groups maintained in 14:10 LD were analyzed using a two-way ANOVA with injection and feeding as the two variables. Since melatonin injections had no significant main effects on pineal or HG enzyme activity or HG weight, the respective two groups of fed or underfed animals in 14:10 LD were pooled and a second two-way ANOVA was run using photoperiod and feeding as the two variables. A Student's *t* test was used for intercomparisons among means.

Results. Body weights ($P < 0.01$) and absolute HG weights ($P < 0.01$) (Fig. 1) were significantly inhibited by limiting access to food for 50 days; no effect of photoperiod alone or interaction of photoperiod and underfeeding was observed on either of these weights. Absolute HG weights were significantly lower in all underfed groups compared to those of their respective fed group ($P < 0.001$) or the fed control group in 14:10 LD ($P < 0.001$) (Fig. 1). Absolute HG weights of underfed rats in 8:16 LD were significantly lower ($P < 0.01$) than those of the underfed rats in 14:10 LD (Fig. 1).

Relative HG weights were significantly higher in the underfed 14:10 LD controls ($P < 0.001$) or melatonin-treated animals (P

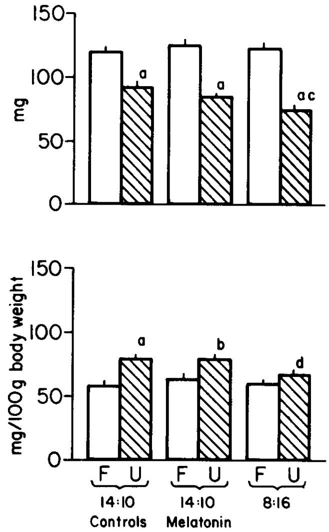

FIG. 1. Absolute (top panel) and relative (bottom panel) Harderian gland weights of fed (F) or underfed (U) rats kept in 14:10 LD or 8:16 LD and treated with saline (controls) or melatonin. Means ± SEM are indicated. a, $P < 0.001$; b, $P < 0.01$ vs respective fed group. c, $P < 0.01$ vs underfed 14:10 LD treated controls. d, $P < 0.05$ vs underfed 14:10 LD treated controls and underfed melatonin-treated group. Significant main effect ($P < 0.01$) of feeding on absolute HG weight. Significant main effects of photoperiod ($P < 0.01$), feeding ($P < 0.01$), and their interaction ($P < 0.05$) on relative HG weight.

< 0.01) compared to those of their respective fed controls. However, HG of the underfed 8:16 LD group were not different from those of their respective fed controls (Fig. 1) and significant effects of underfeeding ($P < 0.01$), photoperiod ($P < 0.01$), and their interaction ($P < 0.01$) were noted.

Underfed animals maintained in 8:16 LD had higher acid phosphatase, hexosaminidase, and β-glucuronidase activities than did corresponding fed controls in 8:16 LD and this was reflected as a significant main effect of underfeeding for all three enzymes and interaction with photoperiod (acid phosphatase and hexosaminidase only) (Fig. 2).

Both the underfed control ($P < 0.05$) and the underfed-melatonin ($P < 0.05$) treated groups had lower pineal protein values than their respective fed groups resulting in a significant main effect of underfeeding alone. Underfed animals in 8:16 LD had similar pineal protein values compared to those of the fed control group in short photoperiod.

Pineal acid phosphatase activity was significantly higher in the melatonin-treated group ($P < 0.01$) compared to its respective fed group. The fed group maintained in 8:16 LD had significantly higher acid phosphatase ($P < 0.05$) activity than the fed group kept in 14:10 LD (Fig. 3). Significant main effects of undernutrition were observed on pineal hex-

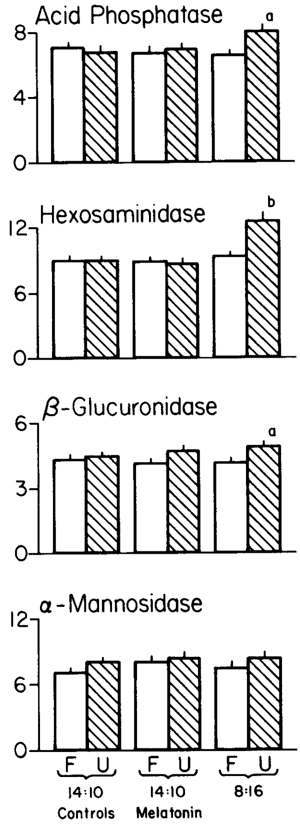

FIG. 2. Harderian gland acid phosphatase (ACP), hexosaminidase (HEX), β-glucuronidase (GLU), and α-mannosidase activities expressed as nM/min/mg protein. a, $P < 0.02$; b, $P < 0.05$ vs fed group in 8:16 LD. Enzyme activities are for fed (F) or underfed (U) rats kept in 14:10 LD or 8:16 LD and treated with saline (controls) or melatonin. Means ± SEM are indicated. Significant main effects of feeding on ACP ($P < 0.05$), GLU ($P < 0.01$), and HEX ($P < 0.01$). Significant main effects of photoperiod on HEX ($P < 0.01$). Significant interaction of feeding and photoperiod on ACP ($P < 0.05$) and HEX ($P < 0.01$).

osaminidase ($P < 0.01$) and pineal β-glucuronidase ($P < 0.01$) activity but no effect of photoperiod or its interaction with underfeeding was observed.

α-Mannosidase activity in either the pineal or the Harderian gland was unaffected by any treatment (Fig. 2).

Discussion. Environmental factors such as photoperiod length provide a reliable cue which photoresponsive species utilize to program certain metabolic and reproductive processes (2). On the other hand, underfeeding is a condition in which sufficient caloric intake to maintain normal growth and homeostasis varies according to food availability which, in the wild, may vary considerably depending on environmental factors and population density. In some species, e.g., the rat, the combination of reduced photoperiod and underfeeding leads to a synergistic effect in which body weight and reproductive function are markedly affected more than with either treatment alone; pinealectomy of underfed animals exposed to reduced photoperiod negates the retardation in genital development suggesting that pineal hormones, such as melatonin, are involved (1–3). In the present experiment, we investigated the interaction of underfeeding with either a reduced photoperiod or daily injection of melatonin on pineal protein content and lysosomal enzyme activity in prepubertal female rats to determine if there is an interaction in these two variables which could affect pineal metabolism and ultimately produce the dysfunctional reproductive system. Whereas underfeeding alone significantly lowered pineal protein values, no interaction with photoperiod or melatonin was noted. In the face of these lowered overall protein values, however, acid phosphatase levels were higher in underfed animals and, additionally, a significant main effect of photoperiod was noted but there was no interaction between photoperiod and undernutrition. Thus, both underfeeding and photoperiod may exert independent effects on these aspects of pineal metabolism, but, overall, the net output of the gland and/or sensitivity of its target organs may be altered in such a way as to allow the ultimate synergistic inhibitory effects on reproduction.

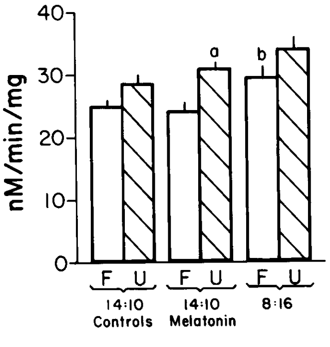

FIG. 3. Acid phosphatase activity (nM/min/mg protein) in pineal glands of fed (F) or underfed (U) rats kept in 14:10 LD or 8:16 LD and treated with saline (controls) or melatonin. Means ± SEM are indicated. a, $P < 0.01$ vs. respective fed group. b, $P < 0.05$ vs. fed 14:10 LD controls. Significant main effects of photoperiod ($P < 0.01$) and feeding ($P < 0.01$).

The proteolysis of tissues, e.g., liver, attendant to starvation is thought to be mainly, if not exclusively, a lysosomal process (20, 21). Considerable reduction in hepatic protein content can occur rapidly in deprivation-induced proteolytic states (22) although the recruitment of certain enzymes may be dependent upon such factors as the length of underfeeding as illustrated by the fact that cellular acid phosphatase activity is not enhanced during the initial 36 hr of fasting (23). Extrapolating to the tissues examined in the present study, activation of lysosomal enzymes undoubtedly contributed to the decrease in pineal protein content as evidenced by a clear effect of underfeeding alone on acid phosphatase activity in the pineal. Yet, perhaps more interestingly, other pineal acid hydrolases (hexosaminidase, β-glucuronidase, and α-mannosidase) were not affected by this underfeeding regimen. This may be because of differential activation of certain enzymes within the lysosome over the 50-day period of undernutrition or perhaps the relatively minor role that these enzymes play in pineal metabolism.

Absolute Harderian gland weight was considerably reduced in all underfed groups compared to fed controls. However, when these weights were expressed relative to body weight, Harderian gland weight increased in underfed animals maintained in long photoperiod, suggesting that the hormonal/metabolic millieu present in this condition promotes a sparing effect on weight and possibly on lysosomal enzyme activity. In short photoperiod, though, this sparing effect was not maintained and relative HG weight declined commensurate with body weight, and, concomitantly, this was accompanied by increased activity of three lysosomal enzymes. Since HG weight and function are affected by pituitary hormones, the mechanism(s) involved in these synergistic effects of photoperiod and undernutrition may be related to the hormonal imbalance which these treatments produce in the endocrine system (2, 3). Interestingly, in the reproductive and thyroid systems short photoperiod and afternoon injections of melatonin elicit comparable results; however, in the present study, this was not the case. A similar discrepancy in Harderian gland physiology was noted recently where daily melatonin injections in the LSH/SsLak female hamster did not simulate the effect of short photoperiod on melatonin content and *N*-acetyltransferase activity (24). Such results may implicate an effect of photoperiod independent of melatonin on the gland. Alternatively, the time course of gonadal degeneration induced by short photoperiod or melatonin treatment varies and, thus, the factors which affect Harderian gland weight may have been differentially affected.

---

1. Sorrentino S, Reiter RJ, Schalch DS. Interactions of the pineal gland, blinding and underfeeding on reproductive organ size and radioimmunoassayable growth hormone. Neuroendocrinology **7**:105–115, 1971.
2. Reiter RJ, Vaughan MK, Vaughan GM, Sorrentino S, Donofrio RJ. The pineal gland as an organ of internal secretion. In: Altschule MD, Ed. Frontiers of Pineal Physiology, Cambridge, MIT Press, pp54–174, 1975.
3. Blask DE, Nodelman JL, Leadem CA, Richardson BA. Influence of exogenously administered melatonin on the reproductive system and prolactin levels in underfed male rats. Biol Reprod **22**:507–512, 1980.
4. Bhattacharya RD. Circadian and seasonal rhythm of β-glucuronidase in rat liver. Cell Mol Biol **31**:305–307, 1985.
5. Litynska A. Seasonal changes in the circadian activity rhythm of light (LD) and completely dark (DD) regimen in the mouse submandibular gland in the presence of light-dark (LD) and completely dark (DD) regimen. Physiol Bohem **33**:447–456, 1984.
6. Vaughan MK, Chambers JP, Tsin ATC, Vaughan GM, Reiter RJ. Pineal and retinal lysosomal enzyme rhythms. Brain Res **417**:321-326, 1987.
7. Brownschiedle CM, Niewenhuis RJ. Ultrastructure of the Harderian gland in male albino rats. Anat Rec **190**:735–754, 1978.
8. Carriere R. Ultrastructural visualization of intracellular porphyrin in the rat Harderian gland. Anat Rec **213**:496–504, 1985.
9. Kennedy GY, Jackson AH, Kenner GW, Suckling CJ. Isolation, structure and synthesis of a tricarboxylic porphyrin from the Harderian gland of the rat. FEBS Lett **6**:9–11, 1970.
10. Murawski U, Jost U. Unsaturated wax esters in the Harderian gland of the rat. Chem Phys Lipids **13**:155–158, 1974.
11. Reiter RJ, Richardson BA, Matthews SA, Lane SJ, Ferguson BN. Rhythms in immunoreactive melatonin in the retina and Harderian gland of rats: Persistence after pinealectomy. Life Sci **32**:1229–1236, 1983.
12. Vaughan MK, Chenoweth PC, Sabry I, Chambers JP, Reiter RJ. Lysosomal enzymes in the rat Harderian gland are altered by either bromocriptine treatment or hypophysectomy and hormone replacement therapy. Proc Soc Exp Biol Med **187**:469–473, 1988.
13. Figge FHJ, Davidheiser RH. Influence of hypophysectomy on porphyrin synthesizing enzyme activity of rat Harderian glands and livers. Proc Soc Exp Biol Med **100**:64–66, 1960.
14. Johnston HS, McGadey J, Thompson GG, Moore MR, Breed WG, Payne AP. The Harderian gland, its secretory ducts and porphyrin content in the Plains mouse (*Pseudomys australis*). J Anat **140**:337–350, 1985.
15. McMaster KM, Hoffman RA. Harderian gland regulation of sexual "type" by gonads and pineal gland. Biol Reprod **31**: 579–585, 1984.
16. Shirama K, Furuya T, Takeo Y, Shimizu K, Maekawa K. Influences of some endocrine glands and of hormone replacement on the porphyrins of the Harderian glands of mice. J Endocr **91**:305–311, 1981.
17. Ulrich R, Yuwiler A, Geller E, Wetterberg L. Effects of sex hormones and environmental lighting on rat Harderian gland porphyrins. J Endocr **63**:99–102, 1974.
18. Chambers, JP, Elbein AD, Williams JC. Nojirimycin—A potent inhibitor of purified lysosomal α-glucosidase from human liver. Biochem Biophys Res Commun **107**:1490–1496, 1982.

19. Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. Anal Biochem **72**:248–254, 1976.
20. Mortimore GE, Pösö R. Lysosomal pathways in protein degradation: Regulatory role of amino acid. Fed Proc **43**:1289–1294, 1984.
21. Mortimore GE, Ward WF. Internalization of cytoplasmic protein by hepatic lysosomes in basal and deprivation-induced proteolytic states. J Biol Chem **256**:7659–7665, 1981.
22. Mortimore GE, Hutson NJ, Sarmacz CA. Quantitative correlation between proteolyse and macro- and micro-autophagy in mouse hepatocytes during starvation and refeeding. Proc Nat Acad Sci USA **80**:2179–2182, 1983.
23. James J, VanNoorden CJF, Bosch KS, Frederiks WM. Cytochemical determination of acid phosphatase activity in isolated rat hepatocytes during starvation-induced proteolysis. Histochem J **17**:1027–1032, 1985.
24. Menendez-Pelaez A, Santana C, Howes KA, Sabry I, Reiter RJ. Effects of photoperiod or exogenous melatonin administration on the activity of *N*-acetyltransferase and hydroxyindole-*O*-methyltransferase and the melatonin content of the Harderian gland. J Pineal Res **5**:293–300, 1988.

Received March 25, 1988. P.S.E.B.M. 1988, Vol. 189.
Accepted July 11, 1988.