PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 187, 469–473 (1988)

Lysosomal Enzymes in the Rat Harderian Gland Are Altered by Either Bromocriptine Treatment or Hypophysectomy and Hormone Replacement Therapy[1] (42690)

M. K. VAUGHAN, P. C. CHENOWETH, I. SABRY, 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, The University of Texas at San Antonio, San Antonio, Texas 78285*

Abstract. Four groups of adult male hypophysectomized rats were injected subcutaneously twice daily between 0800–0900 hr and 1600–1700 hr with either saline diluent, 150 μg sheep prolactin and/or growth hormone (GH); intact rats received either saline or 150 μg bromocriptine twice daily. After 4 days of treatment, lysosomal enzyme assays revealed significant elevations in both acid phosphatase and α-mannosidase enzyme activities in the Harderian glands of saline-injected hypophysectomized rats compared to those in intact controls. β-Glucuronidase levels were depressed and hexosaminidase activity unaffected by hypophysectomy treatment alone compared to intact controls. Lysosomal enzyme activities in hypophysectomized animals treated with prolactin were not different from the hypophysectomized control animals. However, treatment with GH alone or in combination with prolactin had a significant inhibitory effect on β-glucuronidase, hexosaminidase, and α-mannosidase enzyme activities in the Harderian gland of hypophysectomized animals. Bromocriptine treatment in intact rats only elevated acid phosphatase activity. In summary, the patterns of responses did not reveal a role for prolactin in the control of Harderian gland lysosomal enzyme activities by the pituitary. However, some of the influence on this target system may be exerted by growth hormone.

The Harderian gland, a compound tubulo-alveolar gland located deep in the orbital cavity of the rat, is rich in porphyrins and a mixture of wax esters contained in lipid secretory vacuoles (1–4). The gland has been implicated in various behavioral and hormonal aspects of reproduction, and gender-related anatomical and biochemical differences have been noted in several species (2, 5–7). Although many of these differences appear to depend on sex steroids, several pituitary hormones including prolactin and growth hormone (GH) affect the weight and function of the gland (8–10); additionally, hypophysectomy causes a significant decrease in weight and histological changes in the gland (8). Since hormones have been shown to play a prominent role in the induction of lysosomal enzymes in target tissues (11), we investigated the effects of hypophysectomy and replacement therapy with prolactin and GH on lysosomal hydrolase enzymes to determine if they were affected by the surgical and/or hormone treatment. Additionally, the effects of treatment with bromocriptine, a drug known to inhibit prolactin secretion, are described.

Materials and Methods. *Animals.* Forty-eight adult male Sprague–Dawley rats (≈150 g) were hypophysectomized by the supplier (Harlan, Houston) and shipped with 24 intact animals to our facility. Animals were maintained in a light (14:10 LD; lights on 0600 hr) and temperature (22 ± 2°C)-controlled room and given access to food and water *ad libitum.* Hypophysectomized (Hypox) animals were separated randomly into four treatment groups: (1) saline, (2) prolactin, (3) GH, and (4) GH + prolactin. Beginning 1 week after hypophysectomy, rats were injected sc twice daily between 0800 and 0900 hr and between 1600 and 1700 hr for 4 days with 150 μg of sheep prolactin (Sigma Chemical Co.) and/or with GH (Raben type, Nutritional Biochemical Co.). The intact animals were separated into two groups and treated twice daily sc for 4 days with either saline or 150 μg bromocriptine

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

0037-9727/88 $1.50

mesylate (2-bromo-α-ergocryptine methane sulfonate, Sigma). All animals were killed at midnight and a piece of the Harderian gland was removed and quickly frozen on solid CO_2. Glands were 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 served as the source of enzyme 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 tissue homogenate as a source of enzyme, and the appropriate substrate. The following substrates 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 hydroxide-glycine buffer, pH 10.5. The release of 4-methylumbelliferone from fluorogenic substrates was measured in a fashion identical to that previously described (12). Hydrolysis of fluorogenic substrates 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 using bovine serum albumin as a standard.

Statistics. Data were analyzed using a one-way ANOVA. A Student–Newman–Keuls test was used for intercomparisons among means.

Results. Significant elevations in acid phosphatase ($P < 0.01$) and α-mannosidase ($P < 0.001$) activities were noted in Hypox rats compared with those of their intact controls (Fig. 1). On the other hand, β-glucuronidase levels were depressed ($P < 0.05$) and hexosaminidase activity unaffected by hypophysectomy treatment alone (Fig. 1). Treatment with prolactin did not alter any of the significance levels attributable to hypophysectomy alone. However, treatment with GH alone or in combination with prolactin had a significant inhibitory effect on β-glucuronidase ($P < 0.05$), hexosaminidase ($P < 0.01$), and α-mannosidase ($P < 0.05$) enzyme activities and no effect on acid phosphatase levels compared to the enzyme activities in hypophysectomized animals. In the case of α-mannosidase activity, GH hormone treatment suppressed the rise caused by hypophysectomy to presurgical control levels.

Bromocriptine treatment caused an eleva-

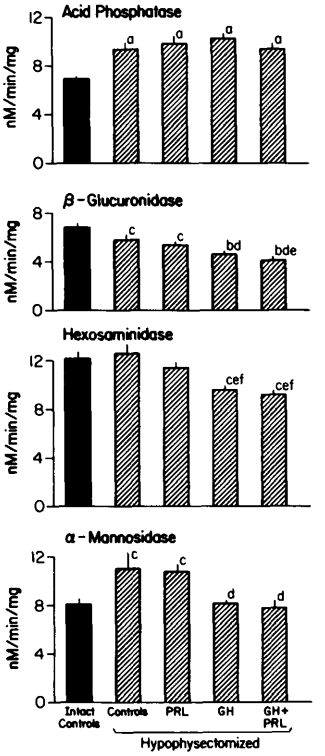

FIG. 1. Harderian gland lysosomal enzyme activities (acid phosphatase, β-glucuronidase, hexosaminidase and α-mannosidase) in hypophysectomized (Hypox) rats treated for 4 days with either diluent (Controls), prolactin (PRL), growth hormone (GH) or GH + PRL. Intact rats were given saline. a, $P < 0.01$; b, $P < 0.001$; c, $P < 0.05$ vs intact animals. d, $P < 0.05$; f, $P < 0.01$ vs Hypox group. e, $P < 0.01$ vs Hypox + PRL. Enzymes are nmole/min/mg protein. Means ± SEM are indicated.

tion of acid phosphatase enzyme activity ($P < 0.01$) compared to the activity in glands of the controls. Bromocriptine treatment had no effect on either β-glucuronidase, hexosaminidase, or α-mannosidase enzyme activity (Table I).

Discussion. It is clear from the present experiment that hypophysectomy and GH treatment alter the enzyme activity of several Harderian gland acid hydrolases. Although the exact function of these specific hydrolases within the gland is unknown, lysosomal enzymes play an important role in the mediation of hormonal signals, in the disposal of excess hormone vesicles, and in the enzymatic degradation of intracellular macromolecules whose byproducts are recycled for metabolic use or export (11, 13). Lysosomal enzymes are also clearly involved in the programmed cellular lytic processes characteristic of holocrine secretion (14), one of three (holocrine, merocine, apocrine) mechanisms described for the Harderian gland (3, 15, 16). Though lysosomal enzymes form a part of the secretion of the lacrimal gland, this has not yet been specifically examined in the Harderian gland (17, 18).

From the literature, it is evident that the weight and histological appearance of the Harderian gland are responsive to both pituitary and steroid hormones (8, 19, 20). Initial experiments in which pituitary extracts were given to produce exophthalmos described the stimulatory effects of pituitary extracts on the weight and glycosaminoglycan content of the retrobulbar eye tissue which was predominantly Harderian gland (19–21). Several pituitary hormones (thyrotropin, GH, prolactin, α-melanocyte-stimulating hormone) have specific effects on either weight or function of the gland (21–23). Hypophysectomy alone causes a reduction in Harderian gland weight; histologically, the glands from these surgically treated animals had smaller cells, larger lumina, and appeared to contain less stainable intracellular lipid (8). Ebling and co-workers (9) also noted a 25% decrease in Harderian gland weight due to hypophysectomy and this was partially prevented by treatment with GH. Undoubtedly, lysosomal enzymes were likely involved in the catabolic processes induced by this surgical procedure. Interestingly, in an experiment by Lorincz and Lancaster (20), GH treatment had no effect on Harderian gland weight if the hypophysectomized animals were additionally castrated. This observation suggests that the weight of the Harderian gland is influenced by many hormonal agents.

In the present experiment both hypophysectomy and GH treatment induced biochemical changes within the gland. Interestingly, hypophysectomy increased (acid phosphatase and α-mannosidase activities), decreased (β-glucuronidase activity) or had no effect (hexosaminidase activity) on the enzymes. These nonparallel changes in the activities of these enzymes may be due to heterogeneity of lysosomes and/or a differential effect of hypophysectomy on their function and/or disposition. Such nonparallel changes have also been observed in the lacrimal gland (17, 18) and the liver (24).

In a study reported by Ebling and coworkers (9), GH administration partially attenuated the decrease in weight of the Harderian gland induced by hypophysectomy. In the present experiment, administration of GH alone or in combination with prolactin to hypophysectomized animals generally resulted in a decrease in the activity of three (β-glucuronidase, hexosaminidase, α-mannosidase) lysosomal enzymes compared to

TABLE I. BROMOCRIPTINE TREATMENT AND HARDERIAN LYSOSOMAL ENZYMES

Treatment	*N*	ACP	β-Gluc	Hex	α-Man
Control	12	6.87 ± 0.34	6.83 ± 0.42	12.12 ± 0.67	8.07 ± 0.60
Bromocriptine	13	8.98 ± 0.56*	7.74 ± 0.42	13.32 ± 0.60	8.43 ± 0.64

Note. ACP, acid phosphatase; β-Gluc, β-glucuronidase; Hex, hexosaminidase; α-Man, α-mannosidase. Enzyme activities expressed as nmole/min/mg protein. Means ± SEM are indicated.

* $P < 0.01$ vs controls.

the activity observed in the gland of hypophysectomized controls. Since lipids make up approximately 21% of the weight of the rat Harderian gland (4), changes in their systhesis and/or excretion could influence the weight of the gland. If indeed these enzymes play a role in the excretion of lipids and/or other substances from the acini and are involved in hypophysectomy-induced catabolism, then a decrease in enzyme activity caused by GH treatment might lead to retention of lipids and an overall anabolic effect with the previously described increase in the weight of the gland.

Glycosaminoglycans are found in the Harderian gland and stimulation with pituitary homogenates or thyrotropin increase their [^{35}S]sulfate uptake (19, 21, 23) particularly in hypophysectomized animals (23). Since glycosaminoglycans contain a substrate for β-glucuronidase, a decrease in this enzyme in Hypox or Hypox + GH-treated animals as noted in the present experiment may be reflected in their content and/or turnover.

Administration of prolactin had no effect on enzyme activity whereas injection of the prolactin antagonist, bromocriptine, stimulated only acid phosphatase. Prolactin has been shown to have a stimulatory effect on both mitosis and secretory activity in the Harderian gland of the chick (10). In the present rat model, hypophysectomy-induced changes in lysosomal activity are not likely due to deprivation of prolactin for the reasons listed. (i) The prolactin-inhibitory dopamine agonist bromocriptine caused at least as much rise in acid phosphatase as hypophysectomy alone but had no significant effect on α-mannosidase which also rose after hypophysectomy. (ii) Prolactin administration did not reverse the hypophysectomy-induced rise in acid phosphatase and α-mannosidase. (iii) The fall of β-glucuronidase activity after hypophysectomy was not offset by prolactin administration, nor was it mimicked by bromocriptine. On the other hand, GH may play a role in part of the Harderian lysosomal response to hypophysectomy in that GH administration reversed the changes in α-mannosidase. That the changes in the other enzymes did not fit such a pattern for the role of GH suggests that effects of the pituitary on the Harderian gland are mediated also through means other than GH secretion and that diverse avenues of hormonal activity regulate lysosomal function.

1. Brownscheidle CM, Niewenhuis RJ. Ultrastructure of the Harderian gland in male albino rats. Anat Rec **190**:735–742, 1975.
2. 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 Endocrinol **91**: 305–311, 1981.
3. Carriere R. Ultrastructural visualization of intracellular prophyrins in the rat Harderian gland. Anat Rec **213**:496–504, 1985.
4. Murawski U, Jost U. Unsaturated wax esters in the Harderian gland of the rat. Chem Phys Lipids **13**:155–158, 1974.
5. Diioria DP, Nadakavukaren MJ. Prevention by pinealectomy of short photoperiod induced ultrastructural changes in the hamster Harderian gland. Anat Rec **210**:449–452, 1984.
6. McMasters KM, Hoffman RA. Harderian gland regulation of sexual type by gonads and pineal gland. Biol Reprod **31**:579–585, 1984.
7. Thiessen DD, Harriman AE. Harderian gland exudates in the male *Meriones unguiculatus* regulate female proceptive behavior, aggression and investigation. J Comp Psychol **100**:85–87, 1986.
8. Boas NF, Bates RW. Role of the thyroid and anterior pituitary glands in the maintenance of the Harderian glands of the rat. Endocrinology **55**:601–612, 1954.
9. Ebling FJ, Ebling E, Randall V, Skinner J. The effects of hypophysectomy and of bovine growth hormone on the response to testosterone of prostate, preputial, Harderian and lachrymal glands and of brown adipose tissue in rats. J Endocrinol **66**:401–406, 1975.
10. Gupta SK, Maiti BR. Action of prolactin on the Harderian gland of the chick. Gen Comp Endocrinol **45**:289–292, 1981.
11. Szego CM. Lysosomal function in nucleocytoplasmic communication. In: Dingle JT, Dean RT, Eds. *Lysosomes in Biology and Pathology.* Amsterdam, North Holland, Vol. IV:pp.385–477, 1975.
12. 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.
13. Farquhar MG. Lysosome function in regulating secretion and disposal of secretory granules in cells of the anterior pituitary gland. In: Dingle JT, Fell HB, Eds. *Lysosomes in Biology and Pathology.* Amsterdam, North-Holland, Vol. II:pp.462–482, 1969.
14. Brandes D, Bertini F, Smith EW. Role of lysosomes

in cellular lytic processes. II. Cell death during holocrine secretion in sebaceous glands. Exp Mol Pathol **4**:245–265, 1965.
15. Watanabe M. An autoradiographic, biochemical, and morphological study of the Harderian gland of the mouse. J Morphol **163**:349–365, 1980.
16. Johnston HS, McGadey J, Thompson GG, Moore MR, Breed WG, Payne AP. The Harderian gland: Its secretory duct and porphyrin content in the plains mouse (*Pseudomys australis*). J Anat **140**:337–350, 1985.
17. Van Haeringen NJ, Glasius E. Characteristics of acid hydrolases in human tear fluid. Ophthalmol Res **8**:367–373, 1976.
18. Van Haeringen NJ, Glasius E. Lysosomal hydrolases in tears and the lacrimal gland: Effect of acetylsalicylic acid on the release from the lacrimal gland. Invest Ophthalmol Vis Sci **19**:826–829, 1980.
19. Wegelius O, Naumann J, Brunish R. Uptake of ^{35}S-labelled sulfate in the Harderian and the ventral lacrymal glands of the guinea pig during stimulation with ophthalamotrophic pituitary agents. Acta Endocrinol **30**:53–60, 1959.
20. Lorincz AL, Lancaster G. Anterior pituitary preparation with tropic activity for sebaceous, preputial and Harderian glands. Science **126**:124–125, 1957.
21. Singh SP, McKenzie JM. The influence of thyrotropin on Harderian gland glycosaminoglycans of the mouse. Proc Soc Exp Biol Med **135**:478–482, 1976.
22. Ebling FJ, Ebling E, Randall V, Skinner J. The synergistic action of α-melanocyte stimulating hormone and testosterone on the sebaceous, prostate, preputial, Harderian and lachrymal glands, seminal vesicles and brown adipose tissue in the hypophysectomized-castrated rat. J Endocrinol **66**:407–412, 1975.
23. Singh SP, McKenzie JM. Effects of thyrotropin on ^{35}S-sulfate uptake by mouse Harderian gland. Endocrinology **85**:952–955, 1969.
24. Davies M. The heterogeneity of lysosomes. In: Dingle JT, Dean RT, Eds. *Lysosomes in Biology and Pathology*. Amsterdam, North-Holland, Vol. IV:pp.305–348, 1975.

Received September 29, 1987. P.S.E.B.M. 1988, Vol. 187.
Accepted December 11, 1987.