PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 187, 287–291 (1988)

Sexual Dimorphism in *N*-Acetyltransferase Activity, Hydroxyindole-*O*-methyltransferase Activity, and Melatonin Content in the Harderian Gland of Syrian Hamsters: Changes following Gonadectomy[1] (42666)

ARMANDO MENENDEZ-PELAEZ,[2] RUSSEL J. REITER,[3] JUAN M. GUERRERO,[4] MANUEL PUIG-DOMINGO,[5] AND KIMBERLY A. HOWES

Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78284-7762

Abstract. The activities of *N*-acetyltransferase (NAT) and hydroxyindole-*O*-methyltransferase (HIOMT) and the melatonin content of the Harderian glands of intact and gonadectomized male and female Syrian hamsters were studied. NAT activity in intact male Harderian glands was twice that of the female. Prepubertal or adult castrated males exhibited a decrease in NAT activity to a level comparable to that seen in the female. Testosterone implants in the castrated males led to a recovery of the original male NAT levels. Intact male hamsters had very low levels of Harderian HIOMT activity and melatonin content in comparison with the glands of the females. Prepubertal gonadectomy but not castration of adult males raised the levels of HIOMT activity and the melatonin content to those of the females. Bilateral ovariectomy had no effect on melatonin content, NAT activity, or HIOMT activity in the female hamster Harderian gland.

The Harderian gland is a tubuloalveolar gland that secretes lipids by means of a merocrine mechanism (1). Among the rodents, the Harderian gland of the Syrian hamster exhibits a remarkable sexual dimorphism both anatomically and biochemically. The pigmented glands of the females have only one glandular cell type, large amounts of intraluminal porphyrins, and high levels of the pineal hormone melatonin. In contrast, the pale yellow gland of the male is comprised of two morphologically different secretory cell types and possesses low levels of both porphyrins and melatonin (2). Bilateral gonadectomy converts the male type, both morphologically (2–4) and biochemically (5–8), into the female type. However, bilateral ovariectomy has little effect on Harderian gland structure and composition (9, 10). Since administration of testosterone prevents the morphological changes (2, 3) in castrated males and induces the masculinization of the gland in females (10), androgenic control of the Harderian glands has been proposed (6, 8). Moreover, it has been recently shown that androgen receptors exist in male hamster Harderian glands (11). Both red light exposure (12) and surgical blinding (5) have been shown to prevent the effects of gonadectomy in male hamsters, indicating that light may be an important modulator of Harderian gland physiology. Also, the exposure of albino rats to continual light leads to morphological regression of the Harderian glands (13). Finally, we have recently described the recovery of some of the male morphological characteristics after prolonged bilateral gonadectomy, indicating that the effects of gonadectomy in males may be transitory (4). The enzymes involved in melatonin synthesis, hydroxyindole-*O*-methyltransferase (HIOMT) and *N*-acetyltransferase (NAT), have been described in the hamster Harderian gland (14, 15). However, the activities of these two enzymes in male and female hamster after bilateral gonadectomy have not yet been determined. The purpose of the present studies was to examine potential changes in melatonin synthesis following surgical removal of the gonads.

[1] Research supported by NSF Grant DCB 8410592.

[2] Supported by a FICYT Postdoctoral Fellowship from the Principado de Asturias Autonomous Government (Spain).

[3] To whom reprint requests should be addressed.

[4] Supported by a grant from the Fulbright Commission.

[5] Supported by Spanish-American Grant CCA 8309-108.

0037-9727/88 $1.50

Materials and Methods. Pregnant Syrian hamsters were purchased from Sasco (Omaha, NE). Throughout the studies, the dams and offspring were housed under a light:dark cycle of 14:10 (lights on at 0600 hr). Offspring were weaned at 21–25 days of age and thereafter maintained five per cage with food and water provided *ad libitum.*

In the initial study, eight male and eight female 25-day-old hamsters were bilaterally gonadectomized while under ether anesthesia. Fifty days postcastration, at 1500 hr, glands were collected from both the gonadectomized and the intact control animals (eight animals/group). Harderian glands were removed, weighed, and immediately frozen on solid CO_2. Within 72 hr after collection, NAT and HIOMT activities were measured using radioenzymatic methods (16). The melatonin content of the glands was determined according to Rollag and Niswender (17). Protein content of the samples was measured by the method of Lowry *et al.* (18). The enzymatic activities and the immunoreactive melatonin were expressed as picomoles and picograms per milligram of protein, respectively.

In the second experiment, 16 adult male hamsters (70 days old) were bilaterally gonadectomized. After 20 days, eight castrated males received a subcutaneous implant of testosterone (1 mg testosterone in 24 mg beeswax). Fifteen days after the pellet implant, hamsters of the castrated group, the castrated + testosterone-implanted group, and an untreated control group (eight animals) were sacrificed at 1500 h. Harderian glands were collected and processed as in Experiment 1. Plasma testosterone was measured using a commercial kit from Diagnostic Products (Los Angeles, CA).

Data were analyzed using a one-way analysis of variance (ANOVA) and a *t* test.

Results. The NAT activity of the intact male Harderian glands was twice that of the intact female ($P < 0.001$, Fig. 1a). However, 50 days after castration, the NAT levels in the prepubertally gonadectomized males had decreased to a level comparable to that seen in the female Harderian gland. Adult male hamsters, castrated for 20 days, showed a similar decrease in NAT activity ($P < 0.001$, Fig. 2a).

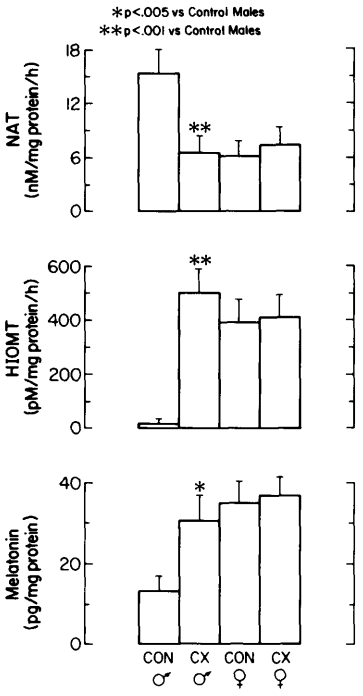

FIG. 1. NAT and HIOMT activities and melatonin content in the Harderian glands of Syrian hamsters. Castration (CX) of prepubertal male animals altered the levels of enzyme activities and melatonin content so that they were equivalent to those in females. Ovariectomy did not change these constituents in the Harderian gland.

The NAT activity in the Harderian glands of castrated males with testosterone implants was not significantly different from that of the intact males (Fig. 2a). The level of plasma testosterone in the implanted group was, however, low compared to that in the intact animals (Table I).

Intact male hamsters had undetectable levels of HIOMT activity in their Harderian glands; in contrast, the glands of females had high HIOMT activity (390 pmole/mg protein, $P < 0.001$, Fig. 1b). Bilateral gonadectomy of prepubertal males led to a significant increase in HIOMT activity 50 days later ($P < 0.001$), to the level seen in females. By comparison, the adult castrated males as well as the testosterone replacement group showed no HIOMT activity differences with respect to the intact animals (Fig. 2b). Female hamster Harderian glands contained more than twice the immunoreactive mela-

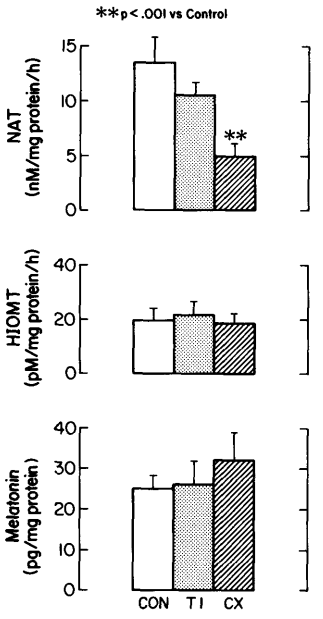

FIG. 2. Effects of castration (CX) of adult male hamsters on NAT and HIOMT activites and the melatonin content of the Harderian gland. Unlike castration in prepubertal males, castration after adulthood does not alter either HIOMT activity or melatonin level but NAT values are decreased; this change is reversed by a subcutaneous implant of testosterone (TI).

tonin concentrations than did these organs in intact males ($P < 0.005$, Fig. 1c). Melatonin concentrations in prepubertal castrated males increased to levels seen in the Harderian glands of females. Adult castrated males exhibited a statistically significant rise in Harderian gland melatonin concentrations (Fig. 2c). Unlike its effect in the male, bilateral gonadectomy had no effect on melatonin, NAT activity, or HIOMT activity levels inHarderian glands of female hamsters (Fig. 1).

Discussion. This is the first report to show a marked sexual dimorphism in the activities of NAT and HIOMT in the Harderian gland of the Syrian hamster. We also confirm herein the differences in melatonin levels, first observed by Hoffman and colleagues (8). NAT and HIOMT are the two enzymes involved in the conversion of serotonin to melatonin; this conversion has been thoroughly investigated in the pineal gland (19) but only sparingly in the Harderian gland. NAT activity has not been previously described in the Harderian gland of the Syrian hamster. A claim has been made that maximal HIOMT acitivity in the rat Harderian gland may require Mg^{2+} (20); this possibility was not tested in reference to HIOMT activity in the hamster Harderian gland. We have, however, characterized NAT activity in the hamster Harderian gland (A. Menendez-Pelaez *et al.* unpublished); this will be the subject of another report.

The drop in the Harderian gland NAT activity following castration of male hamsters and the restoration of the activity of this enzyme by subcutaneous testosterone implants support the idea that there may exist either a direct or an indirect androgenic control over Harderian NAT activity. A similar androgenic control has been proposed for porphyrin levels in the Harderian gland (6, 9). Conversely, in the female Syrian hamster, ovariectomy is without effect on the Harderian gland NAT activity, suggesting that ovary-derived steroids have little influence on the activity of the acetylating enzyme. The response of NAT to circulating testosterone in the present study suggests that NAT activity is differentially regulated in the Harderian and pineal glands. NAT activity in the pineal gland has been shown to be essentially independent of the status of the testes and testosterone (21, 22). Interestingly, androgen receptors have been found in both the Harderian gland (11) and the pineal gland (23), but they seem to be involved in the control of NAT activity only in the former.

The changes in Harderian gland HIOMT activity follow a pattern the inverse of NAT activity. In intact male animals, low levels of

TABLE I. RADIOIMMUNOASSAYABLE LEVELS OF TESTOSTERONE IN THE BLOOD OF INTACT AND CASTRATED MALE HAMSTERS

Treatment	Concentration (ng/ml ± SE)
Intact	3.61 ± 0.61
Castrated	ND[a]
Castrated + testosterone implanted	0.28 ± 0.04

[a] ND, not detectable.

HIOMT were accompanied by high NAT values. Castration of prepubertal animals led to a marked rise in HIOMT levels comparable to those of the intact females. However, castration of adult males had no effect on the HIOMT levels. A possible explanation may be that the stimulation of the Harderian gland by endogenously produced androgens during puberty may permanently alter the sensitivity of Harderian gland NAT activity to circulating gonadal hormones; hence, castration after adulthood may have consequences different from those of prepubertal gonadectomy.

It seems likely that the low HIOMT activity in the male hamster Harderian gland accounts for the reduced melatonin levels in these same organs. The close association of HIOMT activity and melatonin content of the gland in this as well as a previous study (14) indicates that in the male hamster Harderian gland, HIOMT activity may rate limit melatonin formation.

Similar to melatonin, the porphyrin concentration of the Harderian glands of female hamsters is much higher than that of males. It has been shown that Harderian porphyrin content is influenced by sexual hormones and lighting conditions (12, 24). Thus, visual function of the eye has been proposed to be necessary for the increases in porphyrin content in glands of castrated male hamsters (12). It also has been reported that the combination of blinding and castration maintains both the melatonin (8) and the porphyrin (5) concentrations in male hamster Harderian glands, while blinding alone has no effect. These facts indicate not only that sexual hormones influence the Harderian glands but also that visual input involving pineal secretory products may be important. This is supported by evidence that pinealectomy prevents the effect of blinding on the castrated male Harderian glands (6). Furthermore, a reciprocal relationship may exist between the hamster pineal and the hamster Harderian glands, since surgical removal of the Harderian glands has been shown to diminish the nocturnal rise in the melatonin content of the hamster pineal gland (25).

The specific cellular type in which melatonin synthesis occurs in the hamster Harderian glands remains unknown. As noted above, the cellular composition of the hamster Harderian glands varies according to the sex of the animal as well as with the gonadal status (2–4). Immunocytochemical studies of melatonin distribution in the hamster Harderian gland may provide information concerning which cells are involved in melatonin production.

The functions of the Harderian have been investigated frequently. Thus, the glands may be related to extraretinal photoreceptor (26), reproductive (2), and associated behaviors (27), and may be involved, by means of a lipoidal merocrine secretion, in lubricating the eye (28). Whether melatonin in the Harderian glands relates to any of these proposed functions is speculative; however, if melatonin is released from the Harderian glands, a possibility that seems unlikely (29), it could certainly modulate reproductive physiology as well as sexual behavior (30).

In conclusion, the hamster Harderian gland seems to provide an excellent model to study the hormonal regulation of the enzymes involved in extrapineal melatonin metabolism. Furthermore, investigations involving the pineal gland, the gonads, and the Harderian glands may provide additional insight into understanding melatonin physiology.

1. Sakai T. The mammalian Harderian gland: Morphology, biochemistry, function and phylogeny. Arch Histol Japan **44**:299–333, 1981.
2. Hoffman RA. Influence of some endocrine glands, hormones and blinding on the histology and porphyrins of the Harderian glands of golden hamsters. Amer J Anat **132**:463–478, 1971.
3. Lin WL, Nadakavukaren MJ. The androgenic effect on the fine structure of the Harderian gland in the male hamster. Cell Tiss Res **198**:119–127, 1979.
4. Menendez-Pelaez A, Lopez JM, Alvarez-Uria M, Howes KA, Reiter RJ. Cambios ultrastructurales en la glandula Harderiana del hamster Syrio tras gonadectomiz bilateral prolongada. Rev Biol Univ Oviedo, in press.
5. Clabough JW, Norvell JE. Effects of castration, blinding, and the pineal gland on the Harderian glands of the male golden hamster. Neuroendocrinology **12**:344–353, 1973.
6. Payne AP, McGadey J, Moore MR, Thompson G. Androgenic control of the Harderian gland in the male golden hamster. J Endocrinol **75**:73–82, 1977.
7. Hoffman RA, Jones JW. Concentrations of metals

in the Harderian glands of male and female hamsters. Comp Biochem Physiol **69A**:153–156, 1981.
8. Hoffman RA, Johnson LB, Reiter RJ. Harderian glands of golden hamsters: Temporal and sexual differences in immunoreactive melatonin. J Pineal Res **2**:161–168, 1985.
9. McMasters KM, Hoffman RA. Harderian gland: Regulation of sexual "type" by gonads and pineal gland. Biol. Reprod **31**:579–585, 1984.
10. Spike RC, Johnston HS, McGadey J, Moore MR, Thompson CG, Payne AP. Quantitative studies on the effects of hormones on structure and porphyrin biosynthesis in the Harderian gland of the female golden hamster. I. The effects of ovariectomy and androgen administration. J Anat **142**:59–72, 1985.
11. Vilchis F, Hernandez A, Perez AE, Perez-Palacios G. Hormone regulation of the rodent Harderian gland: Binding properties of the androgen receptor in the male golden hamster. J Endocrinol **112**:3–8, 1987.
12. Wetterberg L. Increase of Harderian gland porphyrin content in castrated male hamster is dependent on light and visual function. Life Sci **11**:541–546, 1972.
13. Reiter RJ, Klein DC. Observations on the pineal gland, the Harderian glands, the retina, and the reproductive organs of adult female rats exposed to continuous light. J Endocrinol **51**:117–125, 1971.
14. Pevet P, Balemans MGM, Legerstee WC, Vivien-Roels B. Circadian rhythmicity of the activity of hydroxyindole-*O*-methyltransferase (HIOMT) in the formation of melatonin and 5-methoxytryptophol in the pineal, retina, and Harderian gland of the golden hamster. J Neural Transm **49**:229–245.
15. Menendez-Pelaez A, Howes KA, Gonzalez-Brito A, Reiter RJ. *N*-Acetyltransferase activity, hydroxyindole-*O*-methyltransferase activity, and melatonin levels in the Harderian glands of the female Syrian hamster: Changes during the light:dark cycle and the effect of 6-parachlorophenylalanine administration. Biochem Biophys Res Commun **115**:1231–1238, 1987.
16. Champney TH, Holtorf AP, Steger RP, Reiter RJ. Concurrent determination of enzymatic activities and substrate concentrations in the melatonin synthetic pathway within the same pineal gland. J Neurosci Res **11**:59–65, 1984.
17. Rollag MD, Niswender GD. Radioimmunoassay of serum concentrations of melatonin in sheep exposed to different lighting regimens. Endocrinology **98**:482–489, 1976.
18. Lowry HD, Rosenbrough NJ, Farr AL, Randall NJ. Protein measurements with folin phenol reagent. J Biol Chem **193**:265–275, 1951.
19. Reiter RJ. Pineal indoles: Production, secretion and actions. In: McLeod RM, Müller EE, Eds. Neuroendocrine Perspectives. Amsterdam, Elsevier, Vol 3:pp345–372, 1984.
20. Vlahakes GH, Wurtman RJ. A Mg++ dependent hydroxyindole-*O*-methyltransferase in the rat Harderian gland. Biochim Biophys Acta **261**:194–198, 1972.
21. Cardinali DP. Hormone effects on the pineal gland. In: Reiter RJ, Ed. The Pineal Gland. Boca Raton, FL, CRC Press, Vol 1:pp244–272, 1981.
22. Reiter RJ, Trakulrungsi WK, Trakulrungsi C, Vriend J, Morgan WW, Vaughan MK, Johnson LY, Richardson BA. Pineal melatonin production: Endocrine and age effects. In: Klein DC, Ed. Melatonin Rhythm Generating System. Basel, Karger, pp143–154, 1982.
23. Cardinali DP, Nagle CA, Rosner JM. Metabolic fate of androgens in the pineal organ: Uptake, binding to cytoplasmic proteins and conversion of testosterone into 5-reduced metabolites. Endocrinology **95**:175–187, 1974.
24. Thompson GG, Hordovatzi X, Moore MR, McGadey J, Payne AP. Sex differences in haem biosynthesis and porphyrins content in the Harderian gland of the golden hamster. Int J Biochem **16**:849–852, 1984.
25. Panke ES, Reiter RJ, Rollag MD. Effect of removal of the Harderian glands on pineal melatonin concentrations in the Syrian hamster. Experientia **35**:1045, 1979.
26. Wetterberg L, Geller E, Yuwiler A. Harderian gland: An extraretinal photoreceptor influencing the pineal gland in neonatal rats? Science **167**:884–885, 1970.
27. Thiessen DD, Harriman AE. Harderian gland exudates in the male *Meriones unguiculatus* regulate female proprioceptive behavior, aggression, and investigation. J Comp Physiol **100**:85–87, 1986.
28. Davis FA. The anatomy and histology of the eye and orbit of the rabbit. Trans Amer Ophthalmol Soc **27**:401–441, 1929.
29. Vaughan GM, Reiter RJ. Pineal dependence of the Syrian hamster's nocturnal serum melatonin surge. J Pineal Res **3**:9–14, 1986.
30. Reiter RJ. The pineal and its hormones in the control of reproduction. Endocr Rev **1**:109–131, 1980.

Received June 29, 1987. P.S.E.B.M. 1988, Vol. 187.
Accepted November 4, 1987.