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Sexual Differences in 5′-Deiodinase Activity in the Harderian Gland of Syrian Hamsters and the Effect of Pinealectomy: Regulation by Androgens

Amalia Rubio, Armando Menendez-Pelaez, Gerald R. Buzzell, Mary K. Vaughan, George M. Vaughan, and Russel J. Reiter

The University of Texas Health Science Center at San Antonio, Department of Cellular and Structural Biology, 7703 Floyd Curl Drive, San Antonio, Texas 78284-7762 (A.R., A.M.-P., M.K.V., G.M.V., R.J.R.); Department of Anatomy and Cell Biology, University of Alberta, Edmonton, Alberta T6G 2H7, Canada (G.R.B.)

Abstract Sexual differences on thyroxine 5′-deiodinase (5′-D) in the Harderian gland of Syrian hamsters were investigated. We compared the 24-h profile of 5′-D activity in male and female hamsters, observing a clear rhythm in males but not in females. Female values were always significantly higher than male ones. After pinealectomy day/night variations in male 5′-D activity at the time points studied were abolished, results that are in correlation with serum thyroid hormones. We also studied the regulation by androgen of the enzyme activity. Basal 5′-D activity increased in castrated males and levels fell when animals were implanted with testosterone or its product 5α-dihydrotestosterone (DHT). Female 5′-D activity was also inhibited by androgens. As only the addition of DHT in the presence of epitestosterone, an inhibitor of the conversion of testosterone on DHT, in castrated males was able to decrease 5′-D activity to the control animal levels, we suggest a probable direct effect of DHT by itself.

Key words: 5′-deiodinase activity, Syrian hamster, Harderian gland, testosterone, 5α-dihydrotestosterone, pinealectomy, rhythm

Thyroxine 5′-deiodinase (5′-D, E.C. 3.8.1.4.), an enzyme which is present in many tissues, is responsible for the conversion of thyroxine (T_4) to a more biologically active thyroid hormone, triiodothyronine or T_3 [Larsen et al., 1981]. Two isoenzymes are known to be involved in the process of converting T_4 to T_3, type I and type II. The type I enzyme chiefly maintains serum levels of the active hormone, and is primarily found in the liver [Visser et al., 1978] and kidney [Leonard and Rosemberg, 1978]. However, the type II enzyme has been localized in a variety of tissues, namely, in the pineal [Tanaka et al., 1986], Harderian glands [Guerrero et al., 1987], the anterior pituitary [Kaplan, 1980], brain [Crantz and Larsen, 1980], brown adipose tissue [Leonard et al., 1982], and epidermal keratinocytes [Kaplan, 1988], and seems to play an important role in maintaining intracellular levels of T_3 as a defense mechanism against low levels of serum T_3. In the pineal gland of rodents including the rat [Tanaka et al., 1986], and Swiss mouse [Rubio et al., 1991a], the activity of the type II isoenzyme is regulated by both the thyroid status and by the light-dark cycle. Thus, there is a gradual rise in pineal 5′-D activity after the onset of the dark period with peak values being reached roughly 6 h later [Tanaka et al., 1986; Guerrero et al., 1988a].

Type II 5′-D is also present in the rat Harderian glands [Guerrero et al., 1987]. In this organ, 5′-D activity shows a similar pattern of activation as in the pineal, and here it is also regulated by the light-dark cycle, with high values at night and basal levels during the day [Rubio et al., 1991b; Osuna et al., in press]. Rhythms in Harderian 5′-D activity have been also reported in the male Syrian hamster [Delgado et al., 1988; Guerrero et al., 1989].

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Address reprint requests to Dr. Russel J. Reiter, The University of Texas Health Science Center at San Antonio, Department of Cellular and Structural Biology, 7703 Floyd Curl Drive, San Antonio, TX 78284-7762.

The Harderian glands are tuboalveolar retroorbital glands, which in Syrian hamsters, exhibit marked sexual differences, not only in terms of the morphophysiology but in their metabolism as well.

Besides a gender difference in indole metabolism, porphyrin metabolism in the Harderian glands also shows dramatic sex-associated differences in the Syrian hamsters [Hoffman, 1971; Menendez-Pelaez et al., 1989]. Both, gonadal steroids and thyroid hormones have been implicated in the regulation of porphyrin synthesis by these lacrimal glands [Hoffman et al., 1989a].

In the present work, we compared the 24-h profile of 5′-D activity type II in male and female Syrian hamsters and its relationship with the pineal gland. Herein, we also investigate the possible sexual differences in activity of this enzyme and the influence of androgens on the 5′-D activity.

MATERIALS AND METHODS

Animals

Male Syrian hamsters (*Mesocricetus auratus*) weighing 75–100 g were purchased from Sasco (Omaha, NE). Animals received food and water *ad libitum* and were maintained under a regulated 14-h light, 10-h dark (LD 14:10) cycle during 2 weeks; the lights were turned off daily from 20.00 to 06.00h. On the day of the experiment, animals were euthanized by decapitation at the indicated times, and Harderian glands were quickly collected, frozen on solid CO_2, and stored at −70°C until assayed for 5′-D activity. Hamsters killed at night in the dark, were sacrificed under a dim red light.

Animals were pinealectomized using the standard technique described by Hoffman and Reiter [1965]. Castration of male hamsters was performed via a scrotal approach while under ether anesthesia. All androgens used were administrated by subcutaneous implant in 24 mg of beeswax.

Materials

All reagents were of analytical grade and obtained from commercial sources. T_3, D,L-dithiothreitol (DTT), 4-androsten-17β-ol-3-one (testosterone),5-androsten-17β-ol-3-one(5α-dihydrotestosterone),4-androsten-17α-ol-3-one (epitestosterone), and Dowex-50W, were purchased from Sigma (St. Louis, MO); $Na^{125}I$ was purchased from Amersham (Arlington Heights, IL). T_3 was iodinated with ^{125}I using the chloramine T method, as described by Nakamura et al. [1976], and purified through a 3 ml Sephadex LH-20 column. The purified tracer contained less than 2% free iodine and was immediately used for 5′-D analyses.

Analytical Procedures

The measurement of 5′-D activity was based on the release of radioiodine from the 5′ position of the radiolabeled [3′, 5′-^{125}I]T_4. Briefly, Harderian glands were homogenized in 1 ml using cold 0.1 M phosphate buffer, 1 mM EDTA, pH 7.0, and centrifuged for 15 min at 3,000 rpm. Thereafter 100 μl of the homogenate were immediately incubated in the presence of 40 mM DTT and 2 nM [3′,5′-^{125}I]T_4 as substrate (200 μl final volume). The substrate concentration was similar to the Km value described for 5′-D activity in the rat [Guerrero et al., 1987]. The reaction was started by the addition of the substrate and carried out for 60 min at 37°C. Control incubations were performed by omission of homogenates. The reaction was terminated by the addition of 100 μl of cold 2% bovine serum albumin and 750 μl of 10% trichloroacetic acid. Samples were centrifuged for 30 sec at 10,000 × *g* and 500 μl of supernatant were decanted onto a 0.5 ml column packet with Dowex-50W ion exchange resin and eluted with 500 μl of 10% acetic acid. Radioactivity in the eluate, corresponding to the ^{125}I released was counted in a gamma counter as an index of 5′-D activity. The recovery of ^{125}I in this process was higher than 95%. Specific enzymatic activity was determined by subtracting the control value, which usually amounted to less than 1% of the radioactivity added. 5′-D activity was expressed as fentomoles of ^{125}I released/mg protein/h. Protein content of the samples was measured by the method described by Lowry et al. [1951].

Total concentration of T_4 and T_3 in serum was estimated by radioimmunoassay (RIA) using an available commercial kit (Diagnostic Products, Los Angeles, CA). An inverse index of the fraction of T_3 and T_4 bound in plasma was determined from the proportional T_3 uptake (T_3; Diagnostic Products) and the free T_3 and the free T_4 indices (FT_3I, FT_4I) determined by multiplying the total T_3 and T_4 concentration by the T_3U. Plasma testosterone was measured using a commercial kit from Diagnostic Products (Los Angeles, CA). Serum melatonin levels were mea-

sured by means of radioimmunoassay as described previously [Champney et al., 1984].

Results are expressed as means ± mean standard errors (SEM). Data were statistically analyzed using either a cosinor analysis (for the significance of the rhythm) as previously described [Vaughan et al., 1994] or a one way ANOVA followed by a Student-Newman-Keuls multiple range test.

RESULTS

Nyctohemeral Profile of Harderian Glands 5′-D Activity in Male and Female Hamsters

The 24-h profiles of male and female Harderian glands 5′-D activity were studied. Animals maintained under the LD 14:10 cycle were killed either at 08.00, 14.00, 19.00, 23.00, or 04.00 h. As shown in Figure 1, 5′-D activity in male Harderian glands exhibited a nyctohemeral rhythm with maximal peak values at night (Table I), while in females no day/night rhythm was observed. However, the basal values obtained for the female Harderian 5′-D activity were always higher that those found in males (mean significantly different, $P < 0.001$).

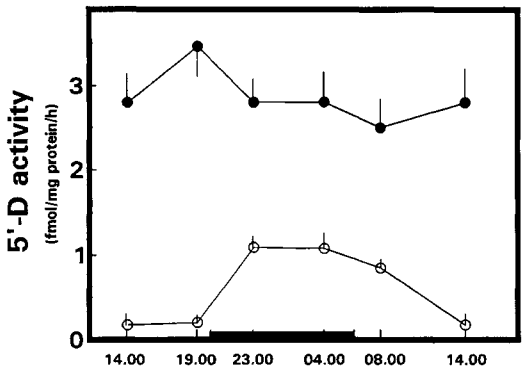

Fig. 1. Thyroxine 5′-D activity in the Harderian glands of male (○) and female (●) Syrian hamsters over a 24 h LD cycle. Animals were euthanized at the indicated times, and Harderian glands were immediately collected for 5′-D determination. Each value is the mean ± SEM of 8 animals. Cosinor analysis for the significance of the rhythm is shown in Table I.

TABLE I. Cossinor Analysis*

	Mean	Amplitude (AMP)	ACR
Male	0.758	0.542[a]	03.30h
Female	2.883*	—	—

*The significance of the rhythm (tested for 24h periodicity) is noted on the AMP. If entries are absent, a rhythm is not significant.
[a]$P < 0.01$.

Effect of Pinealectomy on Harderian 5′-D Activity in Male and Female Hamsters

The aim of this experiment was to study the relationship between the circulating melatonin rhythm and the 5′-D levels in male and female Harderian 5′-D activity. To test this, two groups of male, and two groups of female hamsters were surgically pinealectomized. Thereafter, both male and female hamsters (both pinealectomized and intact controls) were maintained under the LD 14:10 cycle for 10 days. On the day of the experiment, animals were killed either at 19.00 or 04.00h and Harderian glands were collected for 5′-D assay. We also collected serum of each animal to determine T_3 and T_4 levels as well as serum melatonin. As in the first study, we observed significant differences ($P < 0.05$) in male Harderian 5′-D activity between animals killed at 19.00 and 04.00 h (Fig. 2A, upper); these differences were prevented by pinealectomy. Thus, the 5′-D activity in the Harderian glands of pinealectomized males did not reach the 04.00h high levels as in the control animals. The day/night differences seen in the Harderian 5′-D activity correlated with the rhythm in serum melatonin (Fig. 2B, upper). Pinealectomy abolished the circadian rhythm of melatonin in both male and female hamsters (Fig. 2B). Lower panel of Figure 2A shows the 5′-D activity in the Harderian glands of female hamsters, with similar levels of the enzyme activity at the two time points studied; these values were not changed by pinealectomy. Serum levels of thyroid hormones either in both male and female hamsters exhibited a clear rhythm with highest values at 19.00 h ($P < 0.01$ for T_4 and $P < 0.05$ for T_3, Table II). These variations were abolished after pinealectomy.

Effect of Castration and Androgen Replacement in the Harderian 5′-D Activity in Male Syrian Hamsters

The effect of castration and subcutaneous implant of androgens on 5′-D activity in the Harderian glands of male hamsters was studied. Three groups of male hamsters were castrated. The animals of two groups were subcutaneously implanted immediately with 4 mg of either testosterone or 5α-dihydrotestosterone (DHT) in 24 mg of beewax. Animals were thereafter maintained for a period of 2 weeks under the usual

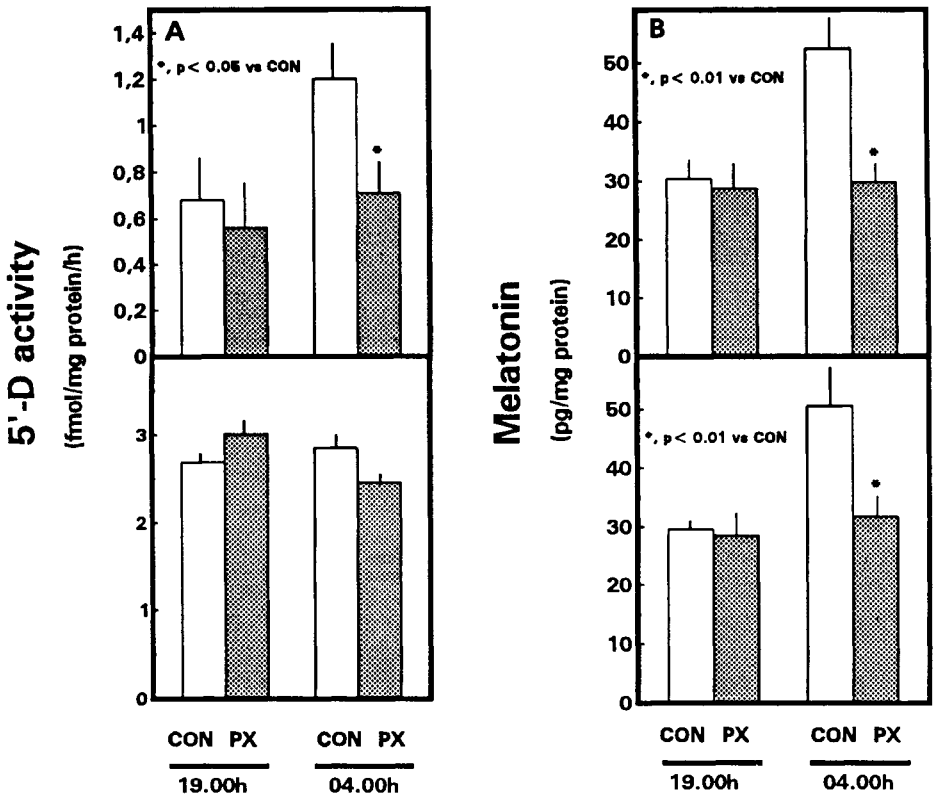

Fig. 2. Harderian 5'-D activity (A) and serum melatonin (B) in pinealectomized male and female hamsters. Two groups of male (*upper panel*) and two groups of female (*down*) hamsters were pinealectomized (PX) and maintained, as were the control groups (CON), under the LD 14:10 cycle for 10 days. Animals were killed either at 19.00 or 04.00h and Harderian glands were collected for 5'-D assay, and serum for melatonin determinations. Each value is the mean ± SEM of eight animals.

TABLE II. Serum Levels of T_4, T_3, FT_4I, and FT_3I in Pinealectomized Male and Female Hamsters*

	Time (hours)	Serum thyroid hormones			
		T_4	T_3	FT_4I	FT_3I
Male	19.00	7.25 ± 0.37[b]	78.53 ± 6.81[a]	4.05 ± 0.14[b]	43.77 ± 3.18[b]
	04.00	5.21 ± 0.46	52.02 ± 6.41	2.96 ± 0.61	18.09 ± 2.00
PX male	19.00	6.34 ± 0.48	70.16 ± 3.57	3.49 ± 0.28	36.68 ± 2.37
	04.00	5.80 ± 0.31	57.76 ± 2.35	3.35 ± 0.19	33.31 ± 1.47
Female	19.00	6.31 ± 0.59[b]	74.43 ± 3.36[a]	3.44 ± 0.36	40.52 ± 2.35[b]
	04.00	4.34 ± 0.23	45.64 ± 8.33	2.47 ± 0.20	26.09 ± 2.20
PX female	19.00	7.02 ± 0.98	57.94 ± 8.39	4.14 ± 0.56	34.17 ± 4.68
	04.00	5.45 ± 0.69	58.09 ± 2.37	3.12 ± 0.37	33.44 ± 1.03

*Two groups of animals of each sex were pinealectomized (PX) and maintained as well the control groups under the LD 14:10 cycle for 10 days. Animals were sacrificed at 19.00 and 04.00 h and serum was collected to measure thyroid hormones concentrations. Each value is the mean ± SEM of eight animals.

[a]$P < 0.05$ vs. control 04.00h.

[b]$P < 0.01$ vs. control 04.00h.

light-dark conditions (LD 14:10), and sacrificed at 16.00h. Harderian glands were collected and assayed for 5'-D activity; serum was used for testosterone determinations. Untreated control hamsters were maintained under the same light-dark conditions. Figure 3 shows that castration increased 5'-D activity ($P < 0.001$) with the levels reaching those found in previous experiments for females. Both androgens studied lowered 5'-D activity to that found in intact control hamsters. Table III shows that serum testosterone was only detectable in the control group and in castrated hamsters implanted with testosterone.

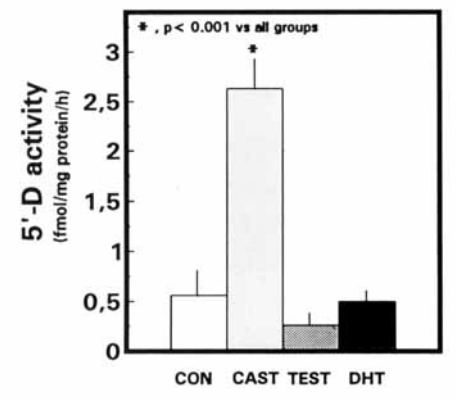

Fig. 3. Effect of androgen administration to castrated males Harderian 5′-D activity. Three groups of male hamsters were castrated (CAST); one of these groups was implanted subcutaneously with testosterone (TEST) and other group with 5α-dihydrotestosterone (DHT). These animals, along with a control group (CON), were maintained for 15 days under the LD 14:10 cycle. Hamsters were sacrificed at 16.00h and Harderian glands were collected for 5′-D assay. Each value is the mean ± SEM of eight animals.

TABLE III. Effect of Androgen Administration on Castrated Male Hamsters Testosterone Serum Levels*

	Serum testosterone (ng/ml)
Male (n = 8)	2.05 ± 0.48
CAS (n = 8)	ND
CAS + Test (n = 8)	0.95 ± 0.11[a]
CAS + DHT (n = 8)	ND

*Three groups of male hamsters were castrated (CAS); some of these received subcutaneous implants of either testosterone (CAS + TEST) or 5α-dihydrotestosterone (CAS + DHT). These animals and a control group (MALE) were maintained for 15 days under the LD 14:10 cycle. Hamsters were sacrificed at 16.00h and serum was collected for testosterone measurements. Each value is the mean ± SEM of eight animals.

[a]$P < 0.01$ vs. control group; ND = not detectable.

Effect of Testosterone and DHT Implants on Harderian 5′-D Activity of Female Hamsters

In this experiment we studied the effect of androgens on the female hamster Harderian gland 5′-D activity. Two groups of female hamsters were subcutaneous implanted with 4 mg of either testosterone or DHT in 24 mg of beewax. These two groups of hamsters and groups of control males and females were maintained under the same light-dark conditions (LD 14:10) for 2 weeks. Thereafter, animals were sacrificed at 16.00h. Harderian glands were collected and assayed for 5′-D activity; serum testosterone levels were also determined. It is clear from Figure 4 that the subcutaneous implants of both testosterone and DHT into females significantly inhibited 5′-D activity ($P < 0.01$). Again, 5′-D activity in intact males was very low, even lower that those measured in the androgen implanted females. Table IV shows levels of serum testosterone in female hamsters; the androgen was only detected in animals treated with the hormone.

Effect of the Antiandrogen Epitestosterone on Blocking the Androgen Induced Inhibition of Harderian 5′-D Activity of Female Hamsters

In this experiment we studied the effect of a specific testosterone receptor blocker on the action of the androgens on female Harderian 5′-D activity. Three groups of female hamsters were subcutaneous implanted with 15 mg of epitestosterone in 30 mg of beewax; two groups were also

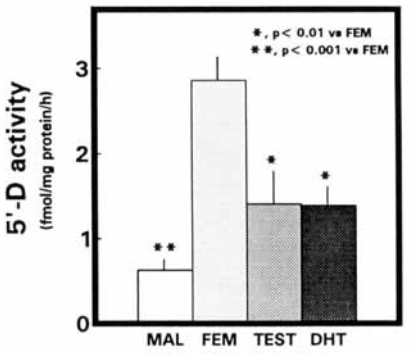

Fig. 4. Effect of androgen administration on female hamster Harderian 5′-D activity. Groups of female hamsters were implanted with either testosterone (TEST) or 5α-dihydrotestosterone (DHT). These two groups and group of control males (MAL) and females (FEM) were maintained under the LD 14:10 cycle for 15 days; animals were sacrificed at 16.00h and Harderian glands were collected for 5′-D assay. Each value is the mean ± SEM of eight animals.

TABLE IV. Effect of Androgen Administration on Female Hamster Serum Testosterone Levels*

	Serum testosterone (ng/ml)
Female (n = 8)	ND
Test (n = 8)	0.86 ± 0.24
DHT (n = 8)	ND

*Groups of female hamsters were subcutaneously implanted with either testosterone (TEST) or 5α-dihydrotestosterone (DHT). These animals and a control group (FEMALE) were maintained for 15 days under the LD 14:10 cycle. Hamsters were sacrificed at 16.00h and serum was collected for testosterone measurements. Each value is the mean ± SEM of eight animals (ND = no detectable).

implanted with 4 mg of either testosterone or DHT. These animals and a control group were maintained under the usual light-dark conditions (LD 14:10) for 2 weeks and thereafter they were sacrificed at 16.00h; Harderian glands were collected for 5′-D assay and serum for testosterone, T_3, and T_4 determinations. Figure 5 shows that epitestosterone by itself was without an effect on the enzyme activity. Epitestosterone by itself did not influence Harderian 5′-D activity but it did prevent the suppressive effect of testosterone but not of DHT on Harderian 5′-D activity ($P < 0.01$).

DISCUSSION

Rhythms in rodent pineal 5′-D activity have been described [Tanaka et al., 1986; Rubio et al., 1991a] with maximal levels occurring at night. In the pineal gland the peak is coincident with those of N-acetyltransferase (NAT) activity and melatonin [Guerrero et al., 1988a,b]. In the rat Harderian glands, where a rhythm of melatonin content similar to that described for the pineal gland has been found [Reiter 1983, 1986], a rhythm of activation of 5′-D also is reported [Osuna et al., 1992]. In male Syrian hamsters a similar rhythm, as reported herein, has been found with high values of 5′-D activity at night [Delgado et al., 1988; Guerrero et al., 1989]. An interaction between the Harderian glands and the thyroid axis has been demonstrated since the morphology in the Harderian glands are all known to be influenced by thyroid physiology [Smelser, 1943], porphyrin content [Hoffman, 1971], and indole metabolism [Hoffman et al., 1989b]. The circadian rhythm of 5′-D activity in Syrian hamsters suggests there may be an interaction between the pineal and Harderian glands in these animals. This suggestion is enhanced by the fact that removal of pineal gland caused the elimination of the day/night variations found at the time points studied in male of 5′-D activity in the Harderian tissue. This hormone may have a direct effect on the Harderian glands, or perhaps melatonin acts indirectly via the thyroid axis thereby altering 5′-D activity in the Harderian glands. When serum levels of thyroid hormones were measured, both male and female hamsters exhibited clear day/night differences; these rhythms have been previously reported. The present results shows that this normal daily variation has been disrupted after pinealectomy, as compared with intact animals. There is an established relationship between melatonin and thyroid metabolism. Thus, the exposure of hamsters to low intensity light, which reduced melatonin production, also decreases serum levels of T_4 followed by a decrease in T_3 and FT_3 [Hoffman et al., 1989c]. Intact animals of both sexes had highest T_3 and T_4 values during the day and basal ones at night. Melatonin is known to be related to thyroid hormones secretion via its depressant action on the release of thyroid stimulating hormone from the anterior pituitary gland [Vaughan et al., 1982].

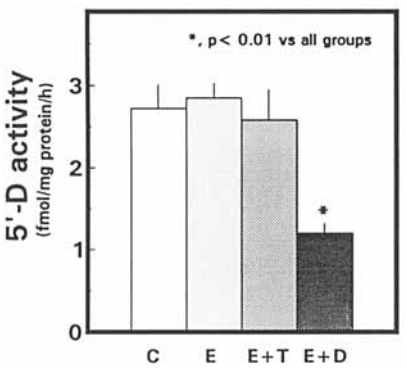

Fig. 5. Effect of epitestosterone on androgen action on Harderian 5′-D activity in female hamsters. One group of hamsters was implanted with epitestosterone (E), while others groups were implanted with either epitestosterone plus testosterone (E + T) or epitestosterone plus 5α-dihydrotestosterone (E + D). These animals along with a control group (C) were maintained under a LD 14:10 cycle for 15 days; after that were sacrificed at 16.00h and Harderian glands were collected for 5′-D assay.

The results also show that there are obvious differences between males and females with respect to basal levels of 5′-D activity in the Syriam hamster Harderian glands. This sexual difference is consistent with other gender related differences in these glands. Indeed, even cellular types in the gland are sexually dimorphic. Male glands have two secretory cell types and female only one type [Hoffman, 1971]. These sexual differences are regulated by androgens since castration in males leads to a feminization of the gland, while ovariectomy does not change Harderian glands morphology [McMasters, 1984]. Both females and castrated males Harderian glands, exhibit a male phenotype as a consequence of androgen administration [Clabough and Norvell, 1973; Payne et al., 1977; Hoffman, 1971; McMasters, 1984]. As seen in the third experiment, a similar pattern exists relative to 5′-D activity. Thus, enzyme levels increased in castrated males and levels fell when animals were implanted androgens. This situation was

reproduced using females. Thus, either testosterone or DHT inhibited 5′-D activity in female Harderian glands. The different values of the 5′-D activity were correlated with the testosterone levels since, when serum androgen levels were high, Harderian 5′-D activity was depressed. Both females and castrated males treated with DHT, also exhibited basal levels of the enzyme activity. It is well known that in some tissues testosterone is converted to DHT by 5α-reductase [Bardin and Mahoudeadu, 1970], and that DHT regulates Harderian NAT activity and porphyrin content [Marrufo et al., 1989; Menendez-Pelaez et al., 1991]. For testosterone to modify Harderian NAT activity it must be converted to DHT. This is consistent with the observation that inhibitors of 5α-reductase prevent the activation of the 5′-D activity normally induced by testosterone [Menendez-Pelaez et al., 1990]. The fourth experiment was performed to determine the nature of the inhibition of 5′-D activity by DHT. In this case we used the testosterone receptor blocker, epitestosterone, in both females and castrated males stimulated with either DHT or testosterone. Only the addition of DHT in the presence of epitestosterone was able to decrease 5′-D activity, indicating a probable direct effect of DHT by itself.

At the present time, the specific function of the Harderian glands relative to the thyroid axis remains unknown. It is clear however, that T_4 deiodination by 5′-D type II in the Harderian gland depends on the melatonin rhythm. Additional experiments are required to clarify the significance of 5′-D activity in the Harderian glands.

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