5α-Dihydrotestosterone Administration Converts Indolamine Metabolism and Porphyrin Content of the Female Syrian Hamster Harderian Gland to the Male Type (42978)

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Abstract. **The effects of ovariectomy and exogenous androgen administration on the indole and porphyrin metabolism of Syrian hamster Harderian glands were studied. Ovariectomy alone had no effect on any of the parameters analyzed. The administration of either testosterone or 5α-dihydrotestosterone increased the activity of *N*-acetyltransferase in the Harderian glands. However, androgen treatment failed to change the activity of hydroxyindole-*O*-methyltransferase. Melatonin content of the glands dropped 20 days after treatment with testosterone and 10 days after the administration of 5α-dihydrotestosterone. The porphyrin content of the Harderian glands was dramatically depressed after the administration of either androgen. It is concluded that the Harderian glands of Syrian hamsters are under an androgenic control involving 5α-dihydrotestosterone.** [P.S.E.B.M. 1989, Vol 192]

The Harderian glands are located in the posterior part of the orbital cavities of most of the mammals. Among other functions, the Harderian glands are reportedly involved in a retinal-pineal-gonadal axis (1). In recent years they have been increasingly studied as a model of indolamine and porphyrin synthesis.

In the Syrian hamster, the Harderian gland shows a remarkable sexual dimorphism. Female glands are pigmented, possess a single secretory cell type (type I), and contain large concentrations of porphyrins. Conversely, male glands are pale, possess two secretory cell types (types I and II), and a very low concentration of porphyrins (2). In addition, it has been recently reported that the Harderian glands of female hamsters show much higher concentrations of the main pineal hormone, melatonin, than those of males (3).

The metabolism of indolamines and porphyrins seems to be mainly controlled by means of an androgenic mechanism (2, 4–6), since castration of prepubertal males increases melatonin levels to those of female and gonadectomy of both young and adult males increases the porphyrin concentration of the glands. Testosterone replacement to castrated male hamsters prevents the feminization of the gland (4). Furthermore, androgen receptors have been described in the male hamster Harderian gland (7). In male hamsters, the enzymes involved in melatonin production, contrary to the pineal enzymes, are also affected by castration and subsequent testosterone replacement (5). On the other hand, ovariectomy has little effect on hamster Harderian gland indolamine and porphyrin metabolism (5, 8).

The purpose of this study was to elucidate the effect of exogenously administered androgen on indolamine and porphyrin metabolism in female Syrian hamsters. To avoid the influence of the estrous cycle (9), ovariectomized hamsters were used.

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Received February 23, 1989. [P.S.E.B.M. 1989, Vol 192]
Accepted June 30, 1989.

0037-9727/89/1922-0192$2.00/0

Materials and Methods

Seventy-two female Syrian hamsters (*Mesocricetus auratus*) ranging from 75 to 100 g were purchased from Sasco (Omaha, NE) and housed under a light:dark cycle of 14 hr:10 hr (lights on daily from 06:00 to 20:00 hr). Animals were housed four to five per cage; food and water were provided *ad libitum*.

Experiment 1. Thirty-two hamsters were bilaterally ovariectomized while under ether anesthesia. Sixteen of these animals received a subcutaneous implant of beeswax; another group of sixteen animals was implanted with a pellet of beeswax + testosterone (4 mg of testosterone/24 mg of beeswax). Ten days later, a second implant of either beeswax or beeswax + testosterone (same dose) was administered to the animals. Ten days after castration, roughly half of each group was sacrificed (at 15:00 hr) and the Harderian glands were collected; after an additional 10 days the remaining animals were sacrificed at the same hour.

Harderian glands were dissected, weighted, and immediately frozen on solid CO_2. Within 72 hr of tissue collection, glands were analyzed for *N*-acetyltransferase (NAT) and hydroxyindole-*O*-methyltransferase (HIOMT) activity using radioenzymatic methods (10). The melatonin content of the glands was determined according to Rollag and Niswender (11). Porphyrin content was measured following the method of Hoffman (2). Protein content of the samples was determined according to the method of Lowry *et al.* (12). The enzymatic activities and the immunoreactive melatonin were expressed as nanomolar (NAT), picomolar (HIOMT), and picograms (melatonin) per milligram of protein. Porphyrin content of the glands was expressed as micrograms of porphyrin per milligram of Harderian gland.

Experiment 2. In an attempt to determine whether the androgen 5α-dihydrotestosterone (5α-DHT) was able to produce effects similar to those of testosterone, another 16 female hamsters were ovariectomized. After castration, one group of eight animals received a subcutaneous implant of 5α-DHT (4 mg in 24 mg of beeswax). Ten days after castration, Harderian glands were collected at 15:00 hr from the following groups: eight intact, eight ovariectomized + beeswax, and eight ovariectomized + 5α-DHT-implanted. Tissues were processed as described in Experiment 1.

Data were analyzed using a one-way analysis of variance followed by the Student-Newman-Kuels test.

Results

Experiment 1. NAT activity of the intact female Harderian glands was 1.85 ± .09 n*M* product/mg protein. Ovariectomy, both after 10 and 20 days, had no effect on the NAT activity. However, NAT in the Harderian glands of castrated females with testosterone implants increased severalfold in comparison to either intact or ovariectomized animals. Similar results were obtained after 10 and 20 days of exogenous testosterone administration (Table I).

HIOMT activity in the Harderian glands of intact female Syrian hamsters was 241.0 ± 65.5 p*M* product/mg protein. Ovariectomy did not affect HIOMT activity. Testosterone administration to the castrated animals also did not change HIOMT activity levels (Fig. 1, left panel).

Melatonin concentration in the Harderian glands of intact female Syrian hamsters ranged from 175 to 200 pg/mg protein. Ovariectomy had no significant effect on the melatonin values after either 10 or 20 days. Testosterone administration for 10 days to ovariectomized animals failed to affect the melatonin content of the Harderian glands. However, 20 days of treatment with exogenous testosterone led to a significant ($P < 0.05$) drop in the melatonin content of the Harderian glands in comparison to both control and ovariectomized groups (Fig. 2, left and middle panels).

The Harderian gland porphyrin content of the intact female Syrian hamster Harderian glands ranged from 0.32 to 0.45 μg/mg of tissue. Ovariectomy, at least within the first 20 days, did not affect the porphyrin content. However, exogenous testosterone administration significantly decreased porphyrin levels both after 10 and 20 days of treatment ($P < 0.001$) (Fig. 3, left and middle panels).

Experiment 2. As in Experiment 1, ovariectomy had no effect on the Harderian NAT activity. Implants of 5α-DHT in ovariectomized animals for 10 days led to a significant increase of NAT activity ($P < 0.005$) (Table I). HIOMT activity was not affected by either ovariectomy or DHT implants (Fig. 1, right panel).

Ovariectomy had no effect on the Harderian gland melatonin content. However, 10 days of treatment with 5α-DHT were enough to produce a significant decrease of the melatonin content of the glands ($P < 0.005$) (Fig. 2, right panel).

The administration of 5α-DHT to ovariectomized female hamsters led to a significant drop of the Harderian porphyrin content ($P < 0.02$) (Fig. 3, right panel).

Discussion

This is the first study reporting a similar effect of 5α-DHT and testosterone on the indolamine and porphyrin metabolism in the female Syrian hamster Harderian glands. Harderian NAT has been shown to have a marked sexual dimorphism (5), being very sensitive to androgen levels. In castrated male hamsters, the exogenous administration of testosterone rapidly returns NAT activity levels to those of control animals (5). In this study, both testosterone and 5α-DHT administration to ovariectomized hamsters led to a rapid increase of NAT activity levels. The presence of a specific high-affinity intracellular androgen receptor in the male hamster Harderian gland has been recently

Table I. NAT Activity in the Harderian Glands of Female Syrian Hamsters (nmol Product/mg Protein)

	Treatment time (days)	
	10	20
Experiment 1		
Control	1.85 ± 0.09	2.13 ± 0.16
Ovariectomized	1.70 ± 0.12	1.96 ± 0.13
Ovariectomized + testosterone implants	7.27 ± 0.69[a]	8.05 ± 0.91[a]
Experiment 2		
Control	2.34 ± 0.19	
Ovariectomized	2.05 ± 0.13	
Ovariectomized + 5α-DHT implants	8.93 ± 0.94[a]	

[a] $P<0.005$ versus controls and ovariectomized groups.

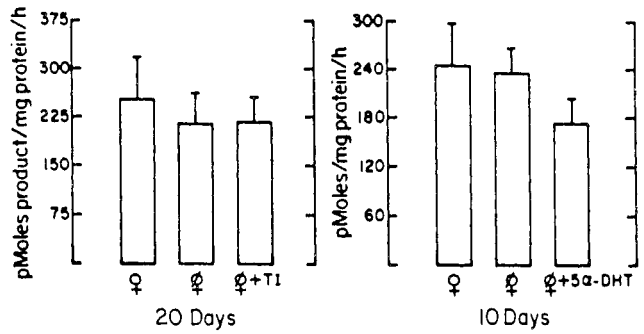

Figure 1. HIOMT activity in the Harderian glands of female Syrian hamsters in Experiment 1 (left panel) and Experiment 2 (right panel).

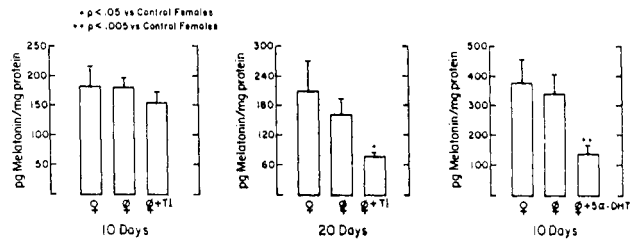

Figure 2. Melatonin content in the Harderian glands of female Syrian hamsters in Experiment 1 (left and middle panels) and Experiment 2 (right panel). Ovariectomy did not significantly change melatonin levels in comparison to intact control groups. The exogenous administration of testosterone (TI) and 5α-DHT resulted in a significant decrease ($P < 0.05$ and $P < 0.005$, respectively) in melatonin content from that of both intact and ovariectomized animals.

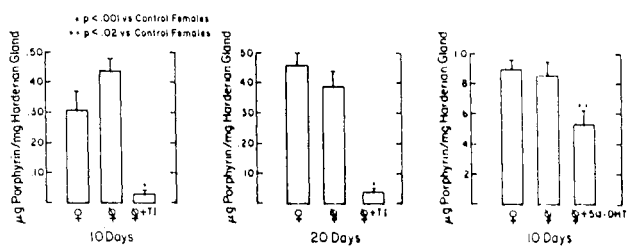

Figure 3. Porphyrin content of the Harderian glands of female Syrian hamsters in Experiment 1 (left and middle panels) and Experiment 2 (right panel). The animals treated with either testosterone (TI) or 5α-DHT showed a significant decrease in the porphyrin concentration ($P < 0.001$ and $P < 0.02$, respectively) in comparison to both intact and ovariectomized animals.

reported (7). However, the presence of androgen receptors in the Harderian glands of female hamsters has not been confirmed. The characteristics and regulation of the Harderian gland NAT seems to be different in comparison to those of the pineal gland acetylating enzyme. These differences have been reviewed by Menendez-Pelaez *et al.* (13).

HIOMT present in the Harderian gland of adult hamsters seems to be independent of androgen levels. Castration of adult male and female Syrian hamsters failed to change the activity of the methylating enzyme (5). However, in this same study, castration of prepubertal male hamsters produced an increase in HIOMT activity, with values reaching those present in the female glands. In this study, we were unable to modify HIOMT activity either after castration or after the exogenous administration of either testosterone or 5α-DHT to adult female Syrian hamsters. A possible explanation for these phenomena would be an inhibitory role of androgens over Harderian HIOMT activity only during the prepubertal stages. A study of the effects of androgen administration to prepubertal female hamsters on Harderian HIOMT will be necessary to define the role of androgens in the control of the methylating enzyme.

We have previously shown that melatonin is synthetized in the Harderian glands of female Syrian hamsters (14). In this study, melatonin levels do not precisely correlate with HIOMT activity levels. As shown previously, there are several HIOMTs involved in the metabolism of other indolic compounds (15). In male Syrian hamster Harderian glands, the production of methoxyindoles by different HIOMTs was investigated by Pevet *et al.* (16). The drop in melatonin content after testosterone and 5α-DHT administration may be a result of a change in the metabolism of this indole. We currently believe that melatonin may not be the most important indole in Harderian gland physiology. There are several indications that *N*-acetylserotonin, which is present in the Harderian glands (17), may be released to the circulation. It has been suggested that blood levels of *N*-acetylserotonin have an extrapineal origin (18). Thus, melatonin concentrations of the Harderian gland may not run completely parallel to the activity of the enzymes studied. Moreover, a possible uptake/release of melatonin from/into the circulation cannot be excluded in the Harderian glands of the Syrian hamster (3).

The effects of 5α-DHT on the porphyrin content of the female hamster Harderian gland have not been previously investigated. The existence of an androgenic control of male hamster Harderian porphyrin content is well established (2, 8, 9). In castrated male hamsters, 5α-DHT was as efficient as testosterone in maintaining the low porphyrin levels characteristic of male glands (4).

It has been shown that ovariectomy alone does not

produce masculinization of the female hamster Harderian gland (8). However, the administration of testosterone propionate to castrated female hamsters resulted in a masculinization of the gland (1) and in a significant decrease of the Harderian gland porphyrin content (8). In these previous studies, ovariectomy and androgen replacement were extended for at least 2 months. In our study, we have shown that 10 days of treatment only was sufficient to produce significant differences. Contrary to the situation for indolamine metabolism, testosterone was more potent than 5α-DHT for the androgenization of the porphyrin content in the Harderian glands.

The Harderian glands of Syrian hamsters seem to be controlled by an androgenic mechanism very similar to that of prostate and other accessory sex glands. The existence of an intracellular mechanism for converting testosterone to 5α-DHT in the Syrian hamster Harderian glands cannot be excluded.

This study was supported by NSF Grant DCB 8410592. A. M-P. was a postdoctoral fellow supported by the Spanish Ministery of Education and Science. A. G-B. was supported by a Caja de Ahorros de Canarias (Spain) postdoctoral fellowship.

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