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# Androgenic Control of N-Acetyltransferase Activity in the Harderian Glands of the Syrian Hamster Is Mediated by 5α-Dihydrotestosterone

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N-acetyltransferase (NAT) activity in the Harderian glands of intact and gonadectomized male and female Syrian hamsters was evaluated. The exogenous administration of 5α-dihydrotestosterone (DHT) to castrated males and intact females produced an increase in NAT values, which reached the values present in the glands of intact males. The administration of a 5α-reductase inhibitor to intact males led to a decrease in NAT activity, suggesting that testosterone is converted in DHT within the glands. It is concluded that NAT activity in the Syrian hamster Harderian glands is under androgenic control, the active steroid being DHT.

**Key words: NAT, androgens, 5α-reductase, DHT, steroids**

The rodent Harderian glands are large, orbital, lacrimal-type glands that lubricate the cornea, produce pheromones, and seem to be involved in a pineal–pituitary–gonadal axis [1]. However, the precise and specific functions of these glands remain unknown. Androgenic control has been proposed for Syrian hamster Harderian gland metabolism [2–4]. Male glands have two secretory cell types and contain low concentrations of melatonin, porphyrins, and somatostatin [1,5,6]. On the other hand, female glands possess a single secretory cell type and higher levels of these substances. Castration in male hamsters leads to a feminization of the glands; ovariectomy per se has little effect on the Harderian gland morphology and metabolism [7]. Androgen replacement to castrated males has been shown to prevent the changes induced by castration [2,3,5]. Likewise, androgen administration to female hamsters can, under some circumstances, cause masculinization of the Harderian glands [7].

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Recently, we have examined the regulation of indolamine metabolism in the Syrian hamster Harderian glands [8–12]. These studies revealed a marked sexual dimorphism in the main enzymes involved in melatonin production [9]. Male glands exhibit high N-acetyltransferase (NAT; EC 2.3.1.5) activity and very low hydroxyindole-O-methyltransferase (HIOMT; EC 2.1.1.4) activity. Conversely, female glands show lower NAT but higher HIOMT activities. Castration of both prepuberal and adult male hamsters produces a rapid drop in NAT activity in the Harderian glands. Exogenous testosterone administration to either castrated male [9] or female hamsters [13] increases the activity of the acetylating enzyme to the levels of intact males.

It is well established that 5α-dihydrotestosterone (DHT) is the most potent androgenic factor in terms of the growth and metabolism of the reproductive tract and accessory sexual glands [14]. In these tissues, testosterone is converted to DHT by the action of the enzyme 5α-reductase. The objective of the present study was to determine the relative importance of DHT in the metabolism of the Harderian glands of Syrian hamsters.

## MATERIALS AND METHODS

### Animals

Animals used in this study were Syrian hamsters (*Mesocricetus auratus*) purchased from Sasco (Omaha, NE). Seventy-two animals (48 males and 24 females) ranging from 75 to 100 g in weight were housed under a light:dark cycle of 14:10 (lights being automatically turned on daily at 06.00 h). Animals were maintained at four per cage, with food and water provided ad libitum.

### Experimental Design

**Experiment 1.** Sixteen male and 16 female Syrian hamsters were bilaterally gonadectomized while under ether anesthesia. Immediately after gonadectomy, 16 of these animals (eight males and eight females) received a subcutaneous implant of DHT (4 mg DHT in 24 mg beewax). Ten days later, hamsters of the castrated group (males and females), castrated + DHT-implanted groups (males and females), and untreated control groups (eight males and eight females) were killed at 14:00 h. Harderian glands, accessory sexual glands (seminal vesicles and coagulating glands), and uteri were removed, weighed, and immediately frozen on $CO_2$.

**Experiment 2.** This experiment was designed to test whether testosterone is converted to DHT by 5α-reduction in the Syrian hamster Harderian gland. If so, inhibition of the enzyme 5α-reductase should duplicate the effects of castration in terms of the Harderian gland parameters measured. Twenty-four male hamsters were divided into three groups. Eight hamsters were bilaterally gonadectomized while under ether anesthesia. A second group was injected daily (at 14:00 h) with a 5α-reductase inhibitor [N,N-diethyl-4-methoxy-3-oxo-4-aza-5-androstane-17-carboxamida (4-MA), a gift from Merck, Sharp and Dohme]. Animals were injected subcutaneously with 6 mg 4-MA dissolved in a 20% solution of ethanol in peanut oil. Following 10 days of treatment, the gonadectomized, the 4-MA-injected, and intact control groups (eight hamsters) were killed at 10:00 h. Harderian glands, testes (when present), and accessory sexual glands (seminal vesicles + coagulating glands) were weighed to determine the state of androgen- and 5α-reductase-dependent tissues. Tissues were immediately frozen on solid $CO_2$.

### Assays

NAT activity was measured using the radioenzymatic method of Champney et al. [15]. NAT activity was expressed as nanomoles of product formed per milligram of protein per hour. Protein content of the samples was estimated using the method of Lowry et al. [16].

### Statistical Analysis

Data are expressed as mean ± SE and were analyzed using a one-way analysis of variance (ANOVA) and a Student-Neuman-Keuls test.

## RESULTS

### Experiment 1

Ten days after castration, the mean weight of the male accessory sex glands had decreased significantly ($P < 0.001$, Table I). The DHT implant prevented this weight reduction. Ovariectomy did not affect the weight of the uterus (Table I) within the 10 day experimental period.

NAT activity in the Harderian glands of the intact males was twice that of the experimental group 10 days after castration ($P < 0.001$; Fig. 1, left). The implantation of DHT into castrated males led to a recovery in the NAT activity, with values reaching levels comparable to those in the intact group.

NAT activity in the female Syrian hamster Harderian glands was much lower than in male glands (Fig. 1, right). Ovariectomy did not significantly modify Harderian NAT activity. The DHT implant in ovariectomized hamsters led to a significant increase in the Harderian NAT levels ($P < 0.001$, Fig. 1, right). These values were essentially similar to those in the intact male and castrated + DHT-implanted groups.

### Experiment 2

As in experiment 1, 10 days after castration the weight of the male accessory sex glands had decreased significantly ($P < 0.001$, Fig. 2, right). Likewise, the administration of 5$\alpha$-reductase inhibitor for 10 days to male hamsters led to a significant decrease in the weight of the accessory sexual organs ($P < 0.001$, Fig. 2, right).

The administration of a 5$\alpha$-reductase inhibitor for 10 days produced a significant reduction in Harderian NAT activity ($P < 0.032$, Fig. 2, left); castration produced a similar reduction ($P < 0.001$, Fig. 2, left). The weight of the Harderian glands did not differ among the three groups of hamsters.

**TABLE I. Weight of Accessory Sex Glands (mg/100 g bw) in Experiment 1 (means ± SE; n = 8 hamsters/group)**

| | Seminal vesicles + coagulating glands | Uterus |
|---|---|---|
| Intact males | 2.42 ± 0.13 | — |
| Castrated males | 1.06 ± 0.05* | — |
| Castrated males + DHT-implanted | 2.58 ± 0.14 | — |
| Intact females | — | 0.86 ± 0.07 |
| Castrated females | — | 0.80 ± 0.05 |
| Castrated females + DHT-implanted | — | 1.10 ± 0.12 |

*$P < 0.001$ vs. intact males.

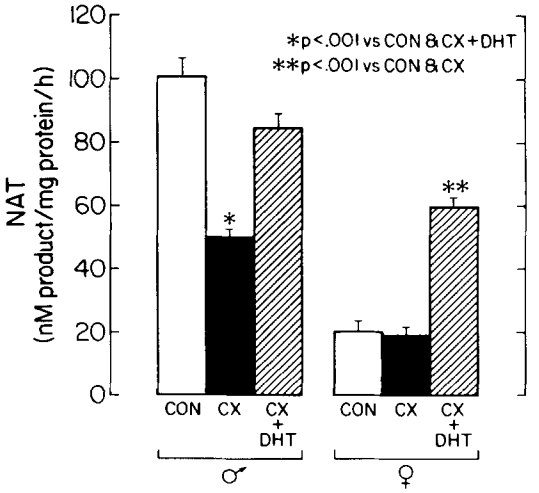


Fig. 1. Influence of either castration (CX) or castration + 5α-dyhydrotestosterone implants (CX + DHT) on Harderian gland NAT activity in male (**left**) and female (**right**) Syrian hamsters. Values are means ± standard errors, with eight animals/group.

## DISCUSSION

Previous studies have shown that orchidectomy in Syrian hamsters leads to a "female" NAT phenotype within the Harderian gland [9], whereas the implantation of testosterone into castrated male and female hamsters induces a "male" NAT phenotype in these same glands [9,13]. The purpose of the present study was to determine whether

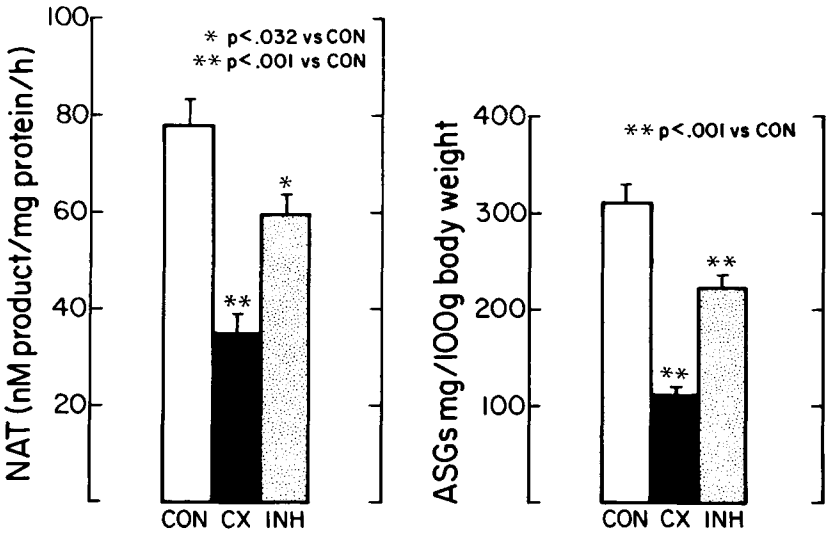


Fig. 2. Effects of castration (CX) or treatment with a 5α-reductase inhibitor (INH) on the Harderian NAT activity (**left**) and the weight of male accessory sex glands (**right**). Values are means ± standard errors, with eight animals/group.

testosterone or a metabolite of this steroid was important in determining NAT activity in the Harderian gland. The actions of testosterone have been shown to depend on either testosterone per se (as in muscle); on estradiol (as in the ovarian follicle and the brain), to which testosterone is aromatized; or on DHT (as in the accessory sex glands), a steroid that results from testosterone reduction by 5$\alpha$-reductase [17]. The present study illustrates that DHT is the compond essential in regulating NAT activity in the Harderian glands of castrated male hamsters (experiment 1). The effects of castration and DHT administration on NAT activity seem to correlate very precisely with the changes observed in the weight of male accessory glands. The apparent lack of effect of ovariectomy on uterine weights could be due to the fact that control animals were in the diestrous state when the organs were collected. The inhibition of 5$\alpha$-reductase activity led to a reduction in Harderian gland NAT activity (experiment 2), showing that the conversion of testosterone to DHT is essential in maintaining high NAT activity levels. In this experiment, castration was more effective in modifying NAT activity than was the administration of the 5$\alpha$-reductase inhibitor. This may relate either to an inadequate dose of the drug or perhaps to an insufficient length of treatment.

Androgen receptors have been identified in the Harderian glands of the Syrian hamster [18,19], suggesting that these steroids may have some activity in the metabolism of this gland. In contrast, NAT activity in the pineal gland seems to be relatively independent of testicular androgens [20], despite the fact that the pineal gland, like the Harderian glands, contains presumed androgen receptors [21].

The function of the Harderian glands remains unknown, although it has been speculated that they are physiologically related to thermoregulation [22], pheromone production [22], pineal gland function [11], and reproductive physiology [1]. The possibility exists that the Harderian glands and the reproductive system are reciprocally interrelated under certain conditions, although a definitive statement in this regard must await further experimentation.

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