# The Antigonadotropic Action of Testosterone but not 7α-Methyl-19-Nortestosterone Is Attenuated Through the 5α-Reductase Pathway in the Castrated Male Rat Pituitary Gland

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ABSTRACT: The enzyme 5α-reductase plays a significant role in the prostate to amplify the action of testosterone (T) by converting it to a more potent androgen, dihydrotestosterone (DHT). The role of 5α-reductase in the testosterone feedback inhibition of gonadotropin secretion from the pituitary has not been elucidated. Therefore, we investigated the role of 5α-reductase on T action in in vitro and in vivo models. Castration has been reported to increase the 5α-reductase activity in pituitary glands. Hence, the effect of castration duration on the conversion of T to DHT by pituitary homogenates and the responsiveness of pituitary monolayer cell cultures to gonadotropin-releasing hormone (GnRH) challenge exposure were investigated. Incubation of [$^3$H]-T with pituitary homogenates showed that the conversion of T to 5α-reduced metabolites was two- to threefold greater in pituitaries from rats who had been castrated for 14 days compared with those castrated for 1 day. In addition, the GnRH-stimulated release of LH from monolayer cell cultures of pituitaries from rats castrated for 1 day was twofold greater, whereas that from rats castrated for 2 weeks was six- to sevenfold greater compared with basal luteinizing hormone (LH) release. Hence we used rats castrated for 2 weeks to elucidate the role of 5α-reductase in T feedback inhibition. The inhibitory effects of the androgens T, 19-nortestosterone (19-NT), and 7α-methyl-19-nortestosterone (MENT) at 3 different concentrations ($10^{-9}$, $10^{-7}$, and $10^{-5}$ mol/L) on GnRH-stimulated LH release from monolayer cell cultures of pituitaries from rats castrated for 2 weeks were examined. All 3 androgens showed dose-dependent inhibition of LH release. MENT showed the greatest inhibition, followed by 19-NT and T. In the presence of finasteride (a 5α-reductase inhibitor), the inhibition of LH released by T and 19-NT were significantly greater. The inhibitory effect of MENT, which does not undergo 5α-reduction, was not altered by finasteride. In an in vivo study, rats castrated for 2 weeks received T with or without finasteride. There was a significantly greater suppression of serum LH in rats receiving T plus finasteride compared with those receiving T alone. These results suggested that 5α-reductase in the pituitary is not obligatory for the inhibitory action of T on gonadotropin secretion in the castrated rat. The action of MENT, a nonreducible androgen, on the pituitary is not affected by 5α-reductase.

Key words: Negative feedback, 5α-reduction.

**J Androl 2000;21:268–275**

Testosterone (T) exerts part of its physiologic action through its metabolic transformation to dihydrotestosterone (DHT) by 5α-reductase in the sex accessory glands and sexual skin, and to estradiol ($E_2$) by aromatase in the neuroendocrine and adipose tissues (Bardin and Catterall, 1981). The greater sensitivity of sex accessory glands to T is due to the accumulation of DHT, which has higher binding affinity to androgen receptors (AR) than does T (Bruchovsky and Wilson, 1968; Liao et al, 1973). In some other tissues that lack 5α-reductase, such as muscle, kidney, Wolffian ducts, and thymus, T acts intrinsically (Schultz and Wilson, 1974; Kumar et al, 1995a). The 5α-reduction of T also is involved in the formation of a male phenotype during embryogenesis and in the androgen-mediated growth of the sex accessory glands (Imperato-McGinley et al, 1974). Two distinct isoforms of 5α-reductase, type 1 and type 2, have been cloned (Andersson and Russell, 1990). Type 1 is predominantly expressed in liver and skin, whereas type 2 is mainly expressed in sex accessory glands and skin of the sex glands (Normington and Russell, 1992).

Testosterone regulates its own secretion via negative feedback by either inhibiting hypothalamic secretion of gonadotropin-releasing hormone (GnRH) or luteinizing hormone (LH) from the pituitary. In the male, T can be converted to $E_2$ and DHT in the brain and the pituitary; however, the relative importance of each in the negative feedback is not well established. Administration of $E_2$ to castrated male rats suppresses gonadotropin secretion, but the concentrations required are higher than those found in the systemic circulation of male rats (Swerdloff and Odell, 1968). The presence of 5α-reductase in the brain and pituitary is well documented (Jaffe, 1969; Massa et al, 1972; Noguchi, 1987), but its role in the feedback

inhibition of gonadotropin secretion by T has not been fully characterized (Martini, 1982; Kumar et al, 1995b).

Castration leads to an increase in serum levels of LH, whereas the pituitary sensitivity to feedback inhibition by T decreases (Damassa et al, 1976; Berger et al, 1984; Kumar et al, 1992). The reasons for the decrease in pituitary sensitivity to T are not clear. By binding assays, it was reported that castration led to a decrease in AR concentration in the pituitary (Thieulant and Pelletier, 1979, 1988). In contrast, others observed no change or increase in AR concentration and AR messenger RNA following castration (Schanbacher et al, 1984; Burgess and Handa, 1993; Choate and Resko, 1996). An increase in the 5α-reductase activity in the pituitary after castration and subsequent decrease by androgen administration have been reported (Denef et al, 1974; Vanderstichele et al, 1990). Because 5α-reductase is involved in the increased potency of T on prostate growth, a similar role of 5α-reductase could be expected in the pituitary. Such a role was suggested by the observation that the LH serum levels were higher in 5α-reductase deficient men (Imperato-McGinley et al, 1974; Canovatchel et al, 1994). Also, DHT was shown to be more potent than T in the inhibition of gonadotropins (Verjans and Eik-Nes, 1977; Liang et al, 1984). However, in vitro and in vivo studies in rats and men showed that finasteride (5α-reductase inhibitor) treatment did not alter the affect of T on gonadotropin secretion from the pituitary (Liang et al, 1984; Kamel and Krey, 1991; Matzkin et al, 1992; Lephart, 1995; Castro-Magna et al, 1996).

In the study reported here, we investigated the role of 5α-reductase on the action of T in the pituitary. Pituitary homogenates prepared from rats castrated for either 1 day or 2 weeks were used to compare the metabolism of [³H]-T. Inhibition of GnRH-stimulated LH release by T, 19-NT, and 7α-methyl-19-nortestosterone (MENT) in the presence of finasteride was examined by castrated rat pituitary monolayer cell cultures. In addition, the effect of finasteride on the suppression of serum LH by T in castrated male rats was investigated.

## Materials and Methods

### Chemicals

All solvents were of analytical reagent grade. Steroids were purchased from Steraloids Incorporated (Wilton, NH) or Sigma Chemical Company (St Louis, Mo). Finasteride was kindly provided by Dr GH Rasmusson, Merck Sharp and Dohme Research Laboratories (Rahway, NJ). MENT was supplied by Dr John Babcock, The Upjohn Company (Kalamazoo, Mich). GnRH was obtained from Dr M Karten (NICHD, Bethesda, Md). The radiolabeled steroids were purchased from NEN Research Products (Boston, Mass). The chemicals and media for cell culture were purchased from Life Technologies (Rockville, Md).

### 5α-Reductase Activity

Conversion of T to its 5α-reduced metabolites and androstenedione was examined by the method described by Noguchi (1987) with minor modifications. In brief, pituitaries and prostates from rats (n = 6) castrated for 1 day or 2 weeks were collected and homogenized in 17 mmol/L Tris-HCl buffer (pH, 7.4) containing 0.25 mol/L sucrose and 1.7 mmol/L $MgCl_2$. Aliquots (250 μL) of the homogenate in triplicate were incubated with 2 pmol/20 μL of [³H]-T (specific activity = 100 Ci/mmol), 50 μL of glucose-6-phosphate (G-6-P; 11.25 mg/mL), 50 μL G-6-P dehydrogenase (G-6-PDH; 0.5 U/tube), and 50 μL of NADPH (8 μmol/mL) at 37°C for 30 minutes. The reaction was stopped by the addition of 5 mL of ice-cold ethyl ether, and the contents were mixed vigorously on a vortex mixer. The ether phase was collected after freezing the aqueous phase in acetone dry ice mixture. The extraction was repeated, and the extracts were pooled. After evaporation of ether, the residues were dissolved in 100 μL of acetone and analyzed by thin-layer chromatographic (TLC) evaluation according to the method of Galbrath and Jellnick (1989). An aliquot (10 μL) of each sample was used for measurement of radioactivity. Eighty microliters of the solution was then spotted onto silica gel TLC plates (Brinkmann Instruments, Westbury, NY). The radiolabeled standards also were spotted in parallel lanes. The plates were then developed in a chamber containing chloroform:ethyl acetate (3:1 vol/vol) as the mobile phase. The plates were allowed to dry and scanned on a scanning radiometer (System 200/AC3000, Bioscan, Washington, DC). The percent conversion of T to 5α-reduced metabolites (DHT and 5α-androstane-3α, 17β-diol) and androstenedione was calculated in each lane from the area representing T or its metabolites corresponding to the position of the standards.

### Pituitary Cell Cultures

Male Sprague-Dawley rats (body weight, 225–250 g; Charles River Laboratories, Kingston, NY) were housed in accordance with standards set forth in the NIH Guide for the Care and Use of Laboratory Animals. Rats were bilaterally castrated through a scrotal incision. Pituitaries were collected from rats (n = 8–10) that had been castrated for either 1 day or 2 weeks. Pituitary cells were dispersed with trypsin digestion as described by Vale and Grant (1975). The cells were suspended in Dulbecco modified Eagle's medium (DMEM) containing 10% horse serum, 2.5% fetal calf serum, 0.1% glutamine, and 1% Gibco nonessential amino acids (DMEMS) and plated at a density of $10^5$ cells/50 μL in each well.

After a 1-hour incubation, 450 μL of DMEMS was added to each well and further incubated at 37°C in an atmosphere of 5% $CO_2$ and 95% air for 48 hours. The medium was then replaced with fresh DMEM without sera, containing T, NT, or MENT, at 3 dose levels ($10^{-9}$, $10^{-7}$, or $10^{-5}$ mol/L), in the presence or absence of $10^{-6}$ mol/L finasteride. Control pituitary cell cultures were treated with vehicle alone or with finasteride. The androgens were selected on the basis of marked differences in their ability to undergo 5α-reduction and the activity of their metabolites (Sundaram et al, 1995). T undergoes 5α-reduction to form DHT with increased androgenic activity, whereas 19-NT is 5α-reduced to form dihydro-19-NT with a loss in androgenic activity. MENT does not undergo 5α-reduction (Aggarwal and Mon-

der, 1988; Kumar et al 1995b). Following incubation with the androgens for 18 hours, fresh DMEM without sera, containing $10^{-6}$ mol/L GnRH was added and incubated further for 4 hours. The control pituitary cell cultures treated with vehicle or finasteride also received $10^{-6}$ mol/L GnRH. Cells for the basal release were not treated with GnRH. The media were harvested and stored at $-20°C$ until assayed. The LH levels in the media were measured by radioimmunoassay with reagents provided by the National Hormone and Pituitary Program (Baltimore, Md) and expressed as ng/mL by rLH-NIDDK-RP-3 as reference standard. The value of LH level stimulated by GnRH was considered as 100%, and the LH secreted in the presence of androgens was compared with this level. The percentage suppression of LH levels by the androgen was calculated as follows: (GnRH value $-$ treated value)/GnRH value.

### Role of 5α-Reduction in Castrated Rats

Effect of T on circulating serum LH levels in rats castrated for 2 weeks was evaluated. In a preliminary experiment, dose levels of T that partially suppressed serum LH levels in castrated rats were determined. Rats were injected SC for 5 days with different dosages of T (25, 50, 100, and 200 μg/d) dissolved in 0.2 mL of cotton seed oil containing 5% ethanol. The dosages of T that led to partial suppression of serum LH levels were used in combination with finasteride. In this experiment, castrated rats (n = 5/group) were administered finasteride dissolved in cottonseed oil containing 5% ethanol (4 mg/d), 30 minutes before starting T (25 or 50 μg/d) treatment. The animals were treated for 5 days and were euthanized 24 hours after the last injection for collection of trunk blood. Serum LH levels were measured by radioimmunoassay with reagents provided by the National Hormone and Pituitary Program and expressed as ng/mL rLH-NIDDK-RP-1 as reference standard.

### Statistical Methods

The effect of androgens on LH secretion in cell culture and in vivo experiments was evaluated by analysis of variance. Comparisons by *t* test were made only after treatment effects were demonstrated to be significant. The microcomputer version of Statistical Analysis System (1985) was used for all statistical analyses.

## Results

### 5α-Reductase Activity

The 5α-reductase activity in pituitary and prostate homogenates was estimated by measuring the conversion of [³H]-T to 5α-reduced metabolites. The pituitary and prostate homogenates were incubated for 30 minutes at 37°C with [³H]-T in the presence of NADPH. Homogenates of pituitaries from rats castrated for 1 day converted [³H]-T to androstanediol (9.1%), DHT (5.6%), and androstenedione (3.4%), with about 68% of T remaining unchanged (Figure 1). Pituitary homogenates from rats castrated for 2 weeks converted T to androstanediol (40%), DHT (7.5%), and androstenedione (2%) with 36% of T re-
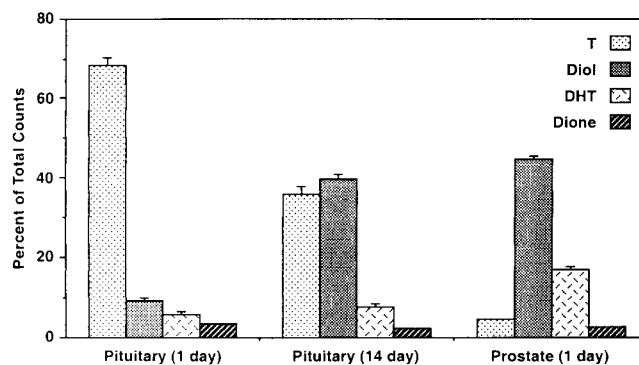


Figure 1. The effect of castration (1 day or 14 days postsurgery) on the biotransformation of [³H]-T by anterior pituitary homogenates in the male rats. Ventral portion of prostate from rats castrated for 1 day was used for reference. Radiolabelled testosterone (T) and metabolites formed and were separated by thin-layer chromatography (TLC) and scanned on a Bioscan. The percent conversion of T to metabolites (dihydrotestosterone [DHT], Diol, 5α-androstane-3α, 17β-diol, and Dione, androst-4-ene-3, 17-dione) was calculated in each lane from the area representing unconverted T or its metabolites corresponding to the mobility (Rf) of standards. Values are mean $\pm$ SE (n = 3).

maining unchanged (Figure 1). Pituitaries from intact sham control rats also were used for comparison. The incubation of [³H]-T with the control pituitary homogenates resulted in the formation of androstanediol (17%), DHT (7%), and androstenedione (5%), whereas the rest of the T remained unconverted (52%). The results were comparable to the metabolism of [³H]-T by the pituitary homogenates from rats castrated for 1 day. Homogenates from the ventral portion of the prostate of rats castrated for 1 day showed significant accumulation of DHT in addition to androstanediol (Figure 1). Addition of finasteride to pituitary and prostate homogenate incubation mixtures led to significant inhibition of conversion of [³H]-T to DHT and androstanediol. In the prostate homogenate, 10% of DHT was formed even in the presence of finasteride (Table 1).

### Effect of Androgens on LH Secretion by Pituitary Cells in Culture

The GnRH-induced LH release from monolayer cell cultures of pituitaries obtained from rats castrated for 1 day was compared to the response of pituitary cells from rats castrated for 2 weeks. GnRH ($10^{-6}$ mol/L) caused a six- to sevenfold increase in LH release over basal LH release from pituitary cells from rats castrated for 2 weeks, as compared with only a twofold increase over basal LH release from rats castrated for 1 day (Table 2). Pituitary cells from rats castrated for 2 weeks were used for further investigation. Dose-dependent suppression of LH secretion was observed with T, 19-NT, and MENT. The LH suppression by the androgens was statistically compared by *t* test. MENT at doses of $10^{-5}$ and $10^{-7}$ mol/L, showed significantly greater suppression of LH than T at the same

Table 1. *Effect of finasteride on testosterone metabolism in the pituitary and prostate of castrated male rats in vitro*

| Incubation Mixture† | Testosterone and Its Metabolites (CPM)* | | | |
|---|---|---|---|---|
| | T | Diol | DHT | Dione |
| Pituitary homogenate | | | | |
| PH + NADPH | 315 (16)‡ | 1169 (58) | . . . | 60 (3) |
| PH + NADPH + finasteride | 1418 (70) | 82 (4) | . . . | . . . |
| Prostate homogenate | | | | |
| PrH + NADPH | 70 (4) | 668 (33) | 692 (34) | 96 (5) |
| PrH + NADPH + finasteride | 1350 (67) | . . . | 199 (10) | 84 (4) |

* CPM indicates counts per minute; T, testosterone; diol, 5α-androstane-3α, 17β diol; DHT, dihydrotestosterone; and dione, androstenedione.
† PH indicates pituitary homogenate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; and PrH, prostate homogenate.
‡ Numbers in parentheses are percentages of total CPM. The percentage does not add up to 100 because minor peaks are not included.

doses. Similarly, 19-NT ($10^{-7}$ and $10^{-9}$ mol/L) was a more potent suppressor of LH than T. MENT at a dose of $10^{-5}$ mol/L also showed greater suppression than 19-NT. Thus MENT was the most potent, followed by 19-NT and T, in suppressing LH secretion from pituitary cells in culture (Figure 2). The effect of finasteride on the inhibitory effect of androgens was compared. In the presence of finasteride, the LH suppression by T and 19-NT was significantly greater over that seen with the androgens alone. Finasteride had no effect on MENT's ability to suppress LH (Figure 2). Finasteride alone had no effect on GnRH-induced LH release. In pituitaries of rats castrated for 1 day, there was some suppression of LH levels by T with and without finasteride, but the suppression was not dose dependent (Table 3).

### Role of 5α-Reductase in Castrated Rats

In the first experiment, castrated rats treated with T at different dosage levels showed that T at dosages of 25 to 50 μg/d caused partial suppression of LH levels. Higher dosages of T (100 and 200 μg/d) suppressed LH levels to intact levels (data not shown). In the second experiment, T was administered to the group of rats, 2 weeks after castration, at a dosage of 25 or 50 μg/d with or

without finasteride (4 mg/d) for 5 days. A dose-dependent suppression of serum LH levels was observed with T (Table 4). In the presence of finasteride, both doses of T showed significantly greater suppression of LH compared with T alone. Finasteride alone had no effect on serum LH (Table 4).

## Discussion

In sex accessory glands, 5α-reduction of T is obligatory in amplifying its androgenic potency (Bardin and Catterall, 1981). The role of 5α-reductase in the negative feedback of T on pituitary LH secretion is not established so far. This study showed that 5α-reductase in the pituitary does not amplify the LH inhibitory action of T in cas-

Table 2. *Effect of GnRH on LH release from cultured pituitary cells obtained from rats castrated 1 day or 14 days earlier**

| Treatment (n = 6 wells/treatment) | LH Concentration in Medium (Mean ± SE ng/mL) | Fold Increase Over Basal LH Release |
|---|---|---|
| 1-day castrated rats—Basal | 3.43 ± 0.40 | . . . |
| 1-day castrated rats—GnRH $10^{-6}$ M | 7.47 ± 0.70 | 2 |
| 14-day castrated rats—Basal | 2.23 ± 0.22 | . . . |
| 14-day castrated rats—GnRH $10^{-6}$ M | 15.35 ± 1.93 | 7 |

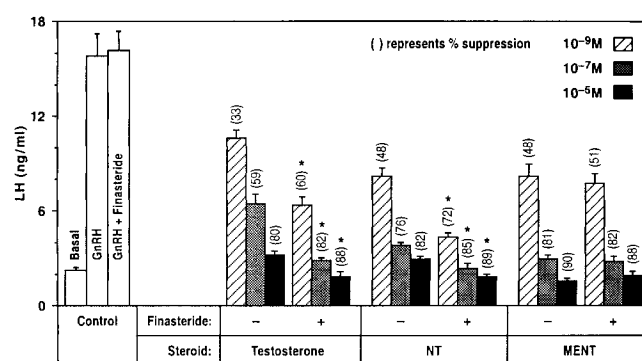* GnRH indicates gonadotropin-releasing hormone; LH, luteinizing hormone.



Figure 2. Dose-related effect of T, 19-nortestosterone (19-NT), and 7α-methyl-19-nortestosterone (MENT) on gonadotropin-releasing hormone (GnRH)-stimulated luteinizing hormone (LH) release from pituitary cells in culture. Pituitary cells prepared from rats castrated for 2 weeks were cultured for 48 hours and then treated with 3 different doses ($10^{-9}$, $10^{-7}$, and $10^{-5}$ mol/L) of T, 19-NT, and MENT for 18 hours. The cells were then treated with $10^{-6}$ mol/L GnRH. Levels of LH released into the media were measured by radioimmunoassay. The values are mean ± SE (ng/mL; n = 6–8 wells). Numbers in parentheses show percentage inhibition of GnRH-stimulated LH release. The LH suppression by T, 19-NT, and MENT was statistically compared by *t* test. MENT > 19-NT ($10^{-5}$ mol/L; $P < .05$), MENT > T ($10^{-5}$ and $10^{-7}$ mol/L; $P < .05$), and 19-NT > T ($10^{-7}$ and $10^{-9}$ mol/L; $P < .05$). *Significant difference ($P < .05$) in the LH suppression by T or 19-NT in the presence of finasteride compared with T or 19-NT treatment.

Table 3. *Effect of testosterone on GnRH-stimulated LH release from dispersed pituitary cells obtained from rats castrated for 1 day*

| Treatment (n = 4–6 wells/treatment) | Dose of Testosterone | LH in Medium (Mean ± SE ng/mL) | % Suppression |
|---|---|---|---|
| Basal | ... | 3.43 ± 0.40 | ... |
| GnRH ($10^{-6}$ M) | ... | 7.47 ± 0.70 | ... |
| GnRH + finasteride ($10^{-6}$ M) | ... | 6.25 ± 0.76 | ... |
| GnRH + testosterone | $10^{-9}$ | 4.17 ± 0.75 | 44 |
| | $10^{-7}$ | 4.04 ± 1.01 | 46 |
| | $10^{-5}$ | 3.95 ± 1.03 | 47 |
| GnRH + testosterone + finasteride ($10^{-6}$ M) | $10^{-9}$ | 3.62 ± 0.16 | 52 |
| | $10^{-7}$ | 3.62 ± 0.63 | 52 |
| | $10^{-5}$ | 2.69 ± 0.95 | 64 |

Table 4. *Effect of finasteride treatment on testosterone-induced LH suppression in rats castrated for 2 weeks\**

| Treatment† (n = 5) | Serum LH (Mean ± SE ng/ml) |
|---|---|
| Castrated (C) | 37.6 ± 1.4 |
| C + F (4 mg/day) | 38.7 ± 2.1 |
| C + T (25 μg/day)‡ | 30.4 ± 2.0 |
| C + T (25 μg/day) + F (4 mg/day) | 23.2 ± 0.9 |
| C + T (50 μg/day)§ | 22.1 ± 0.6 |
| C + T (50 μ/day) + F (4 mg/day) | 15.7 ± 0.9 |

\* LH indicates luteinizing hormone.
† C indicates control; F, finasteride; and T, testosterone.
‡ T versus T + F: $P < .05$.
§ T versus T + F: $P < .05$.

trated rats. In contrast, the action of T on the pituitary to inhibit LH release in vitro and in vivo was enhanced in the presence of 5α-reductase inhibitor whereas the effect of MENT was not altered. This suggests that 5α-reductase in the pituitary may not be involved in the negative-feedback effect of T on LH secretion in castrated rats.

In the pituitary, T is transformed to DHT by the action of 5α-reductase, which is considered the key enzyme in T metabolism (Samperez and Jouan, 1979). DHT is further metabolized to 5α-androstane-3α,17β-diol by 3α-hydroxysteroid dehydrogenase. Orchidectomy induces increased metabolism of T to its reduced metabolites (Massa et al, 1972). A comparison of the metabolism of T by pituitary homogenates showed greater metabolism of T to 5α-reduced metabolites by the pituitaries obtained from rats castrated for 2 weeks. The major metabolite formed by the pituitary homogenate was 5α-androstane-3α, 17β-diol, whereas prostate homogenate yielded DHT and diol in equal proportion. Treatment with finasteride, a 5α-reductase inhibitor, was able to inhibit the reductive metabolism of T by the pituitary and prostate homogenates. The predominant isozyme of 5α-reductase in the rat brain and pituitary appears to be the type I enzyme (Lephart, 1993; Yokoi et al, 1996). Finasteride is known to specifically inhibit the activity of human 5α-reductase type II isoform. Human 5α-reductase type-1 isozyme is insensitive to finasteride inhibition (Andersson et al, 1991). In contrast, rat 5α-reductase type I and type II isozymes are sensitive to finasteride inhibition (Anderson and Russell, 1990; Jenkins et al, 1992). The sensitivity of the rat 5α-reductase type 1 to finasteride has been shown to be a consequence of the tetrapeptide sequence: Val-Ser-Ile-Val in the amino terminal region of the protein that forms a part of the substrate-binding domain (Thigpen and Russell, 1992).

To elucidate the role of 5α-reductase on the action of T in the pituitary, we studied the effect of finasteride on the androgen-induced inhibition of LH secretion by pi-

tuitary cell cultures. Pituitaries obtained from rats castrated for 2 weeks were used, because their 5α-reductase activity based on their metabolizing capacity in vitro was higher compared with pituitaries from rats castrated for 1 day. Furthermore, GnRH elicited greater LH release from pituitary cells obtained from rats castrated for 2 weeks than that from rats castrated for 1 day. These observations confirmed the earlier reports of increased 5α-reductase activity and increased responsiveness of the pituitary to GnRH after castration (Denef et al, 1974; Krieg et al, 1990; Gratton et al, 1995).

Testosterone, 19-NT, and MENT were the androgens selected for investigation because they exhibit differential growth–promoting effects on the ventral portion of the prostate and levator ani (muscle), based on their ability to undergo 5α-reduction (Kumar et al, 1992, 1995b). Androgenic potency of MENT, based on stimulation of prostate growth, was three- to fourfold higher than that of T, whereas 19-NT possessed only one fifth of the potency of T. The binding affinity of MENT to AR was greatest, followed by that of 19-NT and T (Liao et al, 1973; Celotti and Negri Cesi, 1992). The reason for the decreased androgenic potency of 19-NT relative to T in vivo was due to its metabolic transformation in the sex accessory glands to a 5α-reduced metabolite (dihydro-19-NT), which has a binding affinity to AR that is lower than that of T (Drill and Riegel, 1958; Bergink et al, 1985). Therefore, unlike T, 5α-reduction of 19-NT results in decreased androgenic activity. Assuming a similar role for 5α-reductase in the action of 19-NT in the pituitary, a decrease in the antigonadotropic potency would have been expected. However, the antigonadotropic effect of 19-NT was greater than that of T in castrated rats (Kumar et al, 1995b). In the present in vitro study, MENT was most potent, followed by 19-NT and T, in inhibiting GnRH-stimulated LH release from pituitary cells. However, the reason for the greater suppression of LH by low concentrations of T or 19-NT in the presence of finasteride compared with the same dosage levels of MENT is not clear at present. The addition of finasteride inhibits the reduc-

tive metabolism thereby possibly increasing the uptake of T and 19-NT by the pituitary cells. MENT is not 5α-reduced; however, it may be metabolized via other pathways (Aggarwal and Monder, 1988). The metabolic clearance rate of MENT in men was shown to be greater than that of T (Kumar et al, 1997).

The possibility that aromatization of MENT to estrogens may be responsible for its potent antigonadotropic activity was ruled out because the serum LH levels were not altered in intact male rats treated with T or MENT in the presence of aromatase inhibitor (data not shown). In addition, Bhatnagar et al (1992) have also shown that treatment of castrated male rats with T and aromatase inhibitor did not block feedback inhibition of gonadotropins by T. However the aromatase inhibitor significantly attenuated T-induced gonadotropin suppression in men (Bhatnagar et al, 1992; Bagatell et al, 1994). This suggests that neither 5α-reduction nor aromatization pathways in the rat pituitary may be obligatory in the inhibition of gonadotropin secretion by T. The role of 5α-reductase in the pituitary was further investigated in vitro by treating pituitary monolayer cell cultures with androgens in the presence or absence of finasteride. The results showed that finasteride did not block the inhibitory effect of T on LH secretion. Instead, it enhanced the LH suppressive effect of T. A similar effect was observed with 19-NT. As expected, finasteride did not modify the effect of MENT on LH secretion because MENT does not undergo 5α-reduction (Aggarwal and Monder, 1988). Because castration significantly increases pituitary 5α-reductase activity, which may lead to higher conversion of T to DHT, the inhibitory action of T in the presence of finasteride should have been decreased. On the other hand, finasteride enhanced the inhibitory effect of T on LH release from pituitary cells. Furthermore, in vivo treatment with finasteride increased the inhibitory effect of T on LH secretion in castrated rats. This could possibly be explained by the decreased metabolism of T to diol through reductive pathway and increase in T uptake by the pituitary. Thus, 5α-reduction may be involved in the metabolism of T to more polar and inactive metabolites, without significant accumulation of DHT in the pituitary, similar to the metabolism of T in the liver (Gower and Monour, 1975; Sundaram and Kumar, 1996). This suggests that 5α-reduction may be more important for the androgenic activity of T in the prostate than for its LH-inhibitory activity on the pituitary.

The role of 5α-reductase in the pituitary has been investigated earlier by in vitro and in vivo methods. The in vitro studies, with pituitary monolayer cells, demonstrated that treatment with 5α-reductase inhibitor suppressed the conversion of T to DHT without altering the ability of T to inhibit gonadotropins (Kao and Weisz, 1979; Kamel and Krey, 1991). Infusion of [³H]-T into adult castrated rats and monkeys led to the accumulation of [³H]-T, and not [³H]-DHT, as the major radioactive steroid in the nuclei of gonadotrophs (Rezek, 1977; Bonsall and Michael, 1989). In addition, administration of 5α-reductase inhibitor (testosterone-17β-carboxylic acid) also resulted in an increased feedback inhibition of LH by T in castrated rats, although this effect could be demonstrated only with the administration of low doses of T in combination with 5α-reductase inhibitor (Kao and Weisz, 1979). Similarly, in our in vivo study, the treatment of castrated rats with finasteride and T resulted in significantly greater LH suppression compared with the effect of T alone. These observations supported the conclusion that 5α-reduction of T to DHT may not be obligatory for feedback inhibition of gonadotropins. The significant role of 5α-reductase in the negative feedback regulation of gonadotropins by T was, however, demonstrated in vitro with rat pituitary monolayer cells (Nagamoto et al, 1994). The GnRH-induced release of follicle-stimulating hormone and LH was significantly inhibited by the treatment of pituitary cells with T or DHT, with DHT being more potent than T. In addition, treatment with 5α-reductase inhibitor decreased the effect of T but not DHT on the inhibition of gonadotropin release from GnRH-treated pituitary cells. These results suggested that 5α-reductase may play a significant role in T feedback inhibition of gonadotropins. Nagamoto et al (1996) used the cells from pituitaries of intact adult male rats, which resulted in only a marginal increase in the GnRH-induced release of gonadotropins over the basal release. In contrast, in examining pituitaries from rats castrated for 2 weeks, we obtained a six- to sevenfold increase in LH release over the basal LH levels compared with only a twofold increase over the basal LH release from pituitary cells from rats castrated for 1 day. This could also be the reason for the lack of dose-dependent inhibition of LH secretion by androgens from pituitary cells of rats castrated for 1 day in our study.

In conclusion, this study demonstrated that the conversion of T to DHT in the pituitary may not be obligatory for the negative feedback inhibition of gonadotropins by T in the castrated rats.

## *Acknowledgments*

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