Pergamon

0960-0760(94)00150-2

J. Steroid Biochem. Molec. Biol. Vol. 52, No. 1, pp. 105–112, 1995

0960-0760/95 $9.50 + 0.00

Feedback Regulation of Gonadotropins by Androgens in Rats: is 5α-Reduction Involved?

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The action of testosterone (T) on the sex accessory organs, such as ventral prostate (VP) and seminal vesicles (SV) is amplified by its 5α-reduction to dihydrotestosterone (DHT). This does not happen in the case of muscle (levator ani, LA) which contains little or no 5α-reductase activity. It has been suggested that the regulation of gonadotropins by T may also be mediated by its 5α-reduced metabolites. We investigated this question by utilizing two types of androgens: (1) T and 17α-methyl-testosterone (17MT), whose potency increases following 5α-reduction; and (2) 19-nortestosterone (NT) and 17α-methyl-19-nortestosterone (17MNT) whose potency decreases following 5α-reduction. Castrated rats were used to investigate the ability of these androgens to stimulate VP, and SV (androgenic action) and LA growth (anabolic action) and to suppress the post-castration rise in LH levels. In addition, modification of these actions by a 5α-reductase inhibitor (5α-RI) was studied. Compared to T, NT was approximately 5 times less potent in stimulating VP and SV. By contrast, it was twice as potent as T in stimulating LA growth. Similarly, 17MNT was 5 times less androgenic but twice as anabolic as 17MT. The antigonadotropic potency of both the 19-nor compounds was 2–3 times greater than that of their respective 19-methylated parent compounds. The similarity in their anabolic and antigonadotropic potency suggested that 5α-reduction is not a factor in their antigonadotropic action. This was confirmed by the use of the 5α-RI. Treatment of rats receiving the androgens with 5α-RI showed that it decreases the androgenic activity of T and 17MT while it increases the androgenic activity of NT and 17 MNT. In all cases the anabolic activity and the antigonadotropic potency remained unchanged. It is concluded that the regulation of pituitary gonadotropin secretion by T does not depend upon its 5α-reduction to DHT.

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INTRODUCTION

The multiple biological responses to testosterone (T) are manifested by the action of T *per se* or after its enzymatic conversion to either dihydrotestosterone (DHT) or estradiol (E_2). In the male sex accessory glands, pituitary, hypothalamus and skin, T undergoes 5α-reduction to DHT [1–3]. The 5α-reduction of T to DHT in the sex accessory glands is part of the metabolic pathway whereby the activity of T is amplified in these organs. Compared to T, DHT has higher binding affinity to androgen receptors (AR) [4]. Muscle on the other hand contains very little or no 5α-reductase and therefore, there is no amplification of T action. The presence of 5α-reductase in the pituitary and its ability to reduce T and progesterone has been reported [5–7]. It has been proposed that the action of T on the pituitary and hypothalamus may be mediated via its 5α-reduced metabolites [2]. The negative feedback effect of T on the secretion of gonadotropins seems to be partly at the pituitary level since it can inhibit GnRH-stimulated LH secretion both *in vivo* [8] and *in vitro* [9]. The action of T on the pituitary was shown to be mediated via AR [10]. What is not clear, or is rather controversial, is whether or not 5α-reduction of T to DHT is necessary for its regulation of gonadotropin secretion. Since DHT is more potent than T in inhibiting LH secretion *in vivo* [11, 12] and *in vitro* [13, 14], it has been suggested that 5α-reduction may be important for the negative feedback action of T [2, 15, 16]. However, more recent studies using a specific 5α-reductase inhibitor *in vivo* [17–19] and *in vitro* [20–22] do not lend support to this hypothesis.

In earlier studies, 19-nortestosterone (NT) was demonstrated to be more anabolic and less androgenic than testosterone. The binding affinity of NT to AR has been shown to be greater than that of T [23]. In contrast to the situation with T, where DHT has a

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Received 17 June 1994; accepted 26 Aug. 1994.

higher affinity for AR than T, the affinity of dihydronortestosterone (DHNT) to AR is lower than that of T and NT [24, 25]. Therefore, unlike in the case of T, 5α-reduction of NT results in a decrease of its stimulatory action on VP and SV. We hypothesized that if there is a significant reduction of T and NT to their 5α-reduced metabolites in the pituitary, then the relative antigonadotropic potency of NT on the pituitary should be lower than that of T, analogous to its action on sex accessory glands. Furthermore, the administration of a 5α-reductase inhibitor should decrease the antigonadotropic potency of T and have the opposite effect on the antigonadotropic activity of NT. Here we report the results of a comparative study of T, NT and their 17α-methylated analogs in castrated rats. In addition, the effect of a 5α-reductase inhibitor on the androgenic, anabolic and antigonadotropic activity of T and NT are reported.

MATERIALS AND METHODS

Reagents

Chemicals and solvents were reagent grade. Testosterone, 19-nortestosterone, 17α-methyl-testosterone (17MT) and 17α-methyl-19-nortestosterone (17MNT) were purchased from Steraloids Inc., Wilton, NH. The 5α-reductase inhibitor, *N*,*N*-diethyl-3-oxo-4-aza-5α-androst-1-ene-17β carboxamide (5α-RI), was kindly provided by Dr G. H. Rasmusson, Merck Sharp and Dohme Research Laboratories, Rahway, NJ. Molecusol (2-hydroxy-propyl-β cyclodextrin 45% aqueous solution w/v), used as vehicle for the administration of androgens, was purchased from Pharmatec Inc., Alachua, FL. Alzet osmotic pumps (models 2002 and 2001) were purchased from Alza Corp., Palo Alto, CA.

Animals and treatment

Male Sprague–Dawley rats (BW 225–250 g) were purchased from Charles River Laboratories, Kingston, NY and housed in accordance with the standards set forth in the NIH Guide for the care and use of Laboratory Animals. Rats were castrated through a scrotal incision and randomly distributed into treatment groups. Additional rats were used as intact controls. Androgen treatment was initiated immediately after castration via subcutaneously implanted Alzet osmotic pumps. At the end of the treatment, rats were exsanguinated and serum collected. The ventral prostate (VP), seminal vesicles (SV) and levator ani muscle (LA) (referred to as muscle) were removed, cleared of extraneous material and weighed. 5α-RI (4 mg/d) was administered s.c. once or twice daily in 0.2 ml cotton seed oil containing 5% ethanol.

Hormone assays

Serum levels of LH were determined by RIA using reagents provided by the National Hormone and Pituitary Program, Baltimore, MD. Serum LH values are expressed as nanograms per milliliter rLH-NIDDK-RP-3 reference standard.

Statistical methods

The dose–response effects of androgens were compared by the Allfit computer program which fits a family of sigmoidal dose–response curves using the four parameter logistic equation [26]. The bioassay data were evaluated by analysis of variance and the level of significance determined by Fisher's LSD test using BMDP software [27].

RESULTS

Differential effects of androgens

The stimulatory effect of T and NT on VP, SV, and LA is shown in Fig. 1. Based on the increase in weights of VP and SV, T was found to be more potent than NT. On the other hand, NT was more potent than T with respect to LA response. Using the Allfit computer

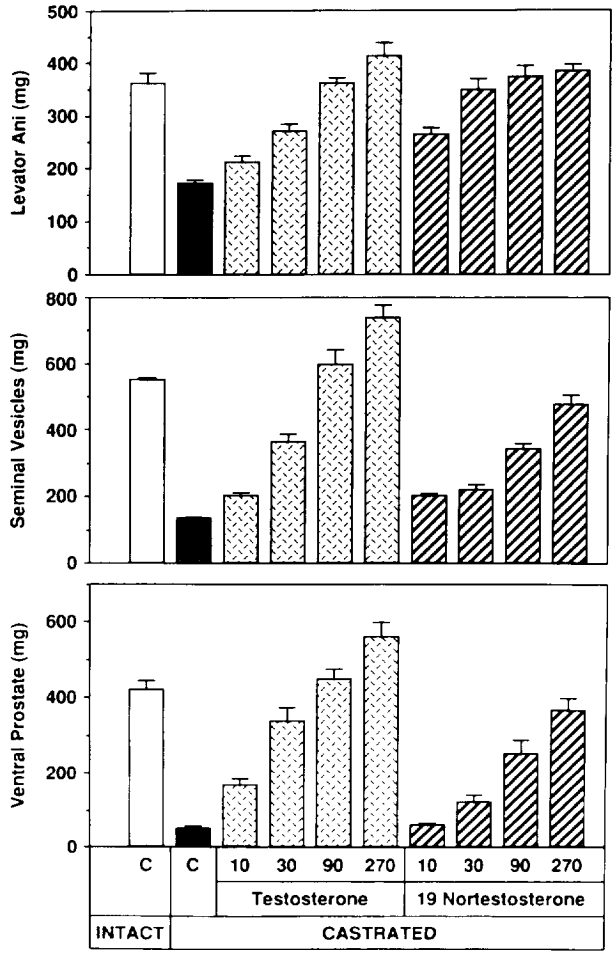

Fig. 1. Dose-dependent effects of androgen treatment on the weights of ventral prostate, seminal vesicles and levator ani in castrated rats. Testosterone and 19-nortestosterone were administered at doses of 10, 30, 90 and 270 μg/day via osmotic pumps for 14 days. Vertical bars represent SE of mean ($n = 6$ per group).

Table 1. Relative biopotency estimates of androgens in castrated rats

Steroid	Potency estimates based on weights of: Ventral prostate	Seminal vesicles	Levator ani
A. 1. Testosterone	1	1	1
2. 19-nortestosterone	0.2	0.2	2.4
B. 3. 17α-methyltestosterone	1	1	1
4. 17α-methyl-19-nortestosterone	0.2	0.3	2.3

Androgen treatment was started on the day of castration. The duration of treatment was 14 days. Potency estimated by "Allfit" computer program. Comparison of testosterone vs nortestosterone and methyl testosterone vs methyl-19-nortestosterone were done in separate experiments (see Figs 1 and 2 for details).

program, the dose–response (based on ED_{50}) of NT and T on VP, SV and LA were compared and the relative biopotencies calculated (Table 1). Based on the response of VP and SV the relative potency of NT was 5 times lower than that of T, while its relative potency based on LA response was twice that of T. The antigonadotropic potency, based on suppression of serum LH levels also showed NT to be more potent than T (Table 2). In a similar comparison, 17MT was found to be 4–5 times more potent than 17MNT on sex accessory glands whereas the latter compound was more anabolic (Fig. 2; Table 1). 17MNT was also more antigonadotropic than 17MT (Table 3). The antigonadotropic potency of 17MNT was similar to its anabolic activity. Unlike the dose-dependent effects of the androgens on tissue weights, their antigonadotropic effects occurred at a narrow range of doses.

Effects of the 5α-reductase inhibitor on androgen action

The effects of 5α-RI (4 mg/day s.c.) on the stimulatory effects of T and NT are shown in Table 4 and Fig. 3. In castrated rats receiving T, 5α-RI inhibited the stimulatory action of T (90 and 270 μg/d) on VP and SV but did not affect its action on LA. On the other hand, administration of 5α-RI to rats receiving NT

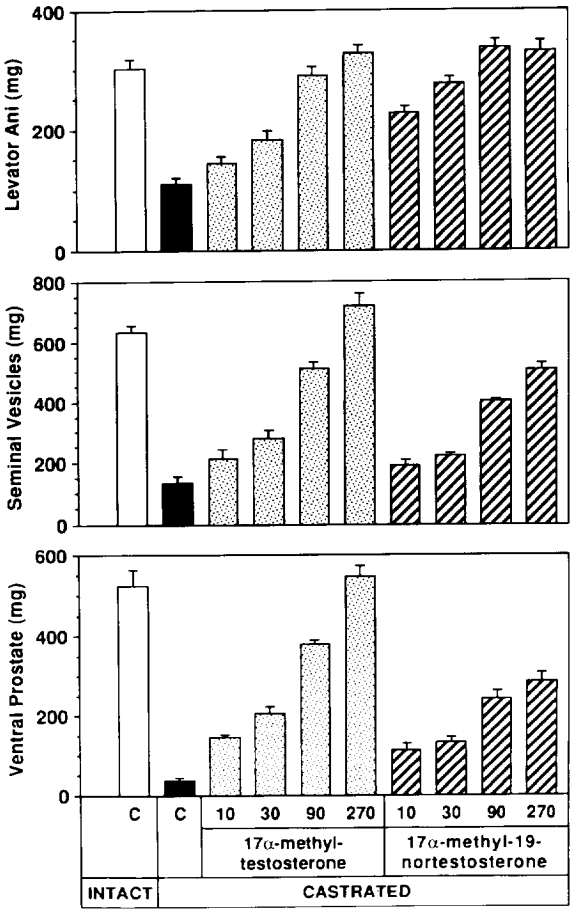

Fig. 2. Dose-dependent effects of 17α-methyl-testosterone and 17α-methyl-nortestosterone on the weights of ventral prostate, seminal vesicles and levator ani in castrated rats. Other details as per Fig. 1.

Table 2. Effect of testosterone and 19-nortestosterone on serum LH levels in castrated rats

Group/treatment	Serum LH (ng/ml)
1. Intact control	0.75 ± 0.15*
2. Castrated control	6.76 ± 1.27
3. Testosterone-10†	4.92 ± 0.77
4. Testosterone-30	6.86 ± 2.03
5. Testosterone-90	1.45 ± 0.86
6. Testosterone-270	0.79 ± 7.28
7. 19-nortestosterone-10	4.13 ± 1.46
8. 19-nortestosterone-30	0.12 ± 0.10
9. 19-nortestosterone-90	ND‡
10. 19-nortestosterone-270	ND

*Values are mean ± SE ($n = 6$ per group).
†Doses are μg/day. On the day of castration rats were implanted with osmotic pumps releasing the indicated doses of the steroid for 14 days (see Fig. 1 for details).
‡Nondetectable.

Table 3. Effect of 17α-methyl-testosterone and 17α-methyl-19-nortestosterone on serum LH levels in castrated rats

Group/treatment	Serum LH (ng/ml)
1. Intact control	1.59 ± 0.25*
2. Castrated control	7.34 ± 0.71
3. 17α-MT-10†	6.76 ± 0.63
4. 17α-MT-30	11.11 ± 3.99
5. 17α-MT-90	2.08 ± 0.59
6. 17α-MT-270	ND
7. 17α-MNT-10	6.57 ± 0.60
8. 17α-MNT-30	0.34 ± 0.25
9. 17α-MNT-90	ND‡
10. 17α-MNT-270	ND

*Values are mean ± SE, $n = 6$ per group.
†Doses are μg/day. On the day of castration rats were implanted with osmotic pumps releasing the indicated doses of the steroid for 14 days (see Fig. 2 for details).
‡Nondetectable.

Table 4. Effect of 5α-reductase inhibitor on the androgenic and antigonadotropic activity of testosterone

Treatment	Ventral prostate (mg)	Levator ani (mg)	Serum LH (ng/ml)
1. Intact control	462 ± 46*	319 ± 14	0.64 ± 0.12
2. Castrated (C) control	60 ± 5	160 ± 11	5.18 ± 0.78
3. C + T-90†	302 ± 17	242 ± 14	3.88 ± 0.56
4. C + T-90 + 5αRI	184 ± 16**	231 ± 11	3.99 ± 0.30

*Values are mean ± SE ($n = 6$).

†Doses are μg/day. Rats were castrated and implanted with osmotic pumps releasing the steroids for 7 days. 5αRI (4 mg in cottonseed oil) was injected s.c. once daily ($n = 5$ per group).

T, testosterone; 5αRI, 5α-reductase inhibitor.

**$P < 0.01$ compared to the androgen only group.

(270 μg/d) resulted in slightly, but not significantly heavier VP and SV as compared to rats receiving NT alone. Assuming that the dose of NT was too high, castrated rats were treated with lower doses of NT (90 or 180 μg/d) with 5α-RI (4 mg/d). As anticipated, the VP and SV weights were higher in rats receiving NT (90) and 5α-RI than those receiving NT alone (Fig. 4). As expected, 5α-RI did not alter the effect of any of the androgens on LA. The effect of 5α-RI was similar in rats receiving the methylated androgens (Fig. 5).

The serum levels of LH in T and NT treated castrated rats were similar to those of the rats that received 5α-RI in addition (Tables 4, 5 and 6). Thus the treatment with 5α-RI does not seem to alter the T or NT induced suppression of gonadotropins. Similarly, 17MT or 17MNT induced suppression of LH was not affected by the administration of 5α-RI (data not shown).

DISCUSSION

In this study, we extended the preliminary observations made in the previous study [19] regarding the role of 5α-reduction in the feedback regulation of gonadotropins by T. Based on the fact that 5α-reduction of T and NT results in differential effects on the sex accessory organs and muscle [23] we investigated whether these differences are also reflected in the regulation of gonadotropins.

Biopotency comparisons of T vs NT and 17MT vs 17MNT in castrated rats showed that the 19-nor derivatives were 5–6 times less potent than T and 17MT on VP and SV. On the other hand, their anabolic (LA response) and antigonadotropic (suppression of LH) potencies were 2–3 times higher than that of T and 17MT. The differences in the androgenic vs anabolic potency of T and NT are attributable to their 5α-reduction in the sex accessory glands but not in muscle. 5α-Reduction of NT in the VP and SV decreased its stimulatory action on these glands. This is because the 5α-reduced metabolite of NT, DHNT is a weak androgen *in vivo* [24]. Its binding affinity to androgen receptors is lower compared to NT or DHT [25]. The action of T in the muscle does not depend on its 5α-reduction since little or no 5α-reductase activity is detected in the muscle [28]. Pituitary, on the other hand does contain 5α-reductase and, therefore, if antigonadotropic action of T depended on its 5α-reduction, then T as well as NT should exert an antigonadotropic potency that reflects their biopotency on the sex accessory glands. However, a comparison of the antigonadotropic action of T and NT showed that NT, which was much less potent than T on sex accessory glands, was more potent than T as an antigonadotropic agent. Thus the antigonadotropic activities of T and NT do not suggest that 5α-reduction of these androgens played a role in their regulation of LH secretion.

The above conclusion was further supported in experiments that used a 5α-reductase inhibitor. 5α-RI must have inhibited the conversion of T to DHT and NT to DHNT in the sex accessory glands as reflected by decreased relative potency of T and increased

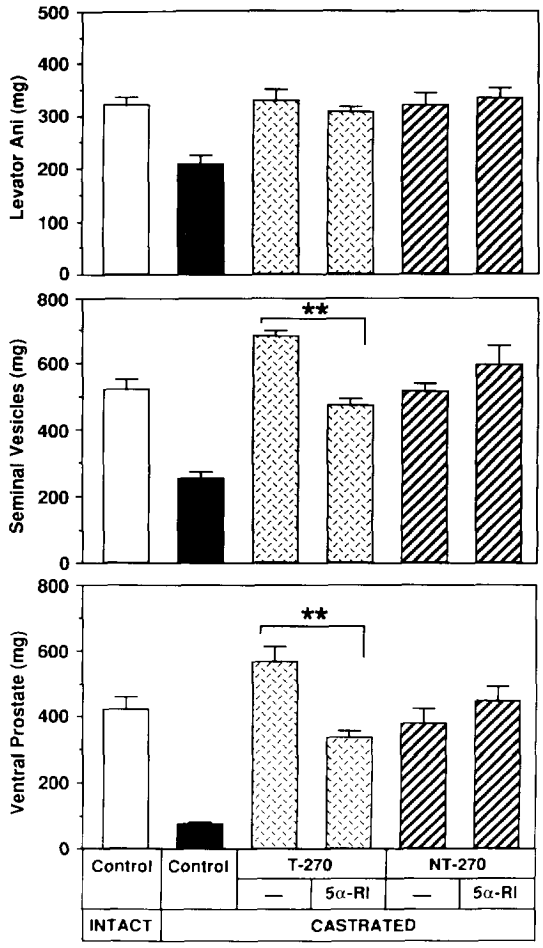

Fig. 3. Effects of 5α-reductase inhibitor (5α-RI) on the weights of ventral prostate, seminal veiscles, and levator ani in castrated rats treated with testosterone (270 μg/day) or 19-nortestosterone (270 μg/day) via Alzet osmotic pumps for 7 days. 5α-RI (4 mg/day) was injected s.c. daily ($n = 6$ per group). **$P < 0.01$.

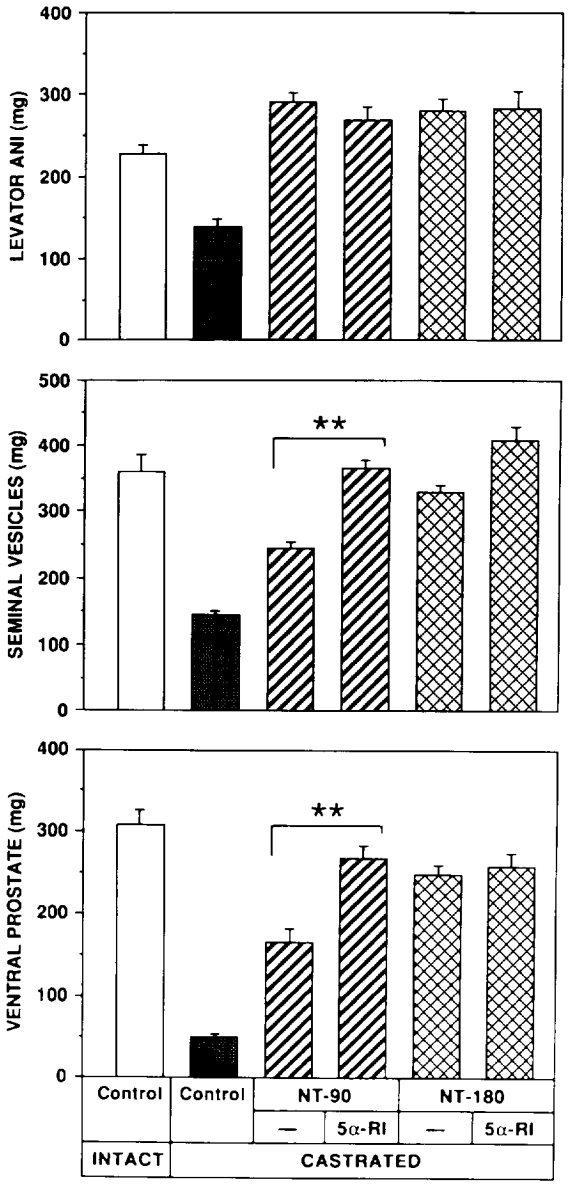

Fig. 4. Effect of 5α-reductase inhibitor on the bioactivity of 19-nortestosterone in castrated rats. On the day of castration 19-nortestosterone (90 or 180 μg/day) was administered by implanting Alzet osmotic pumps releasing the steroid for 7 days. 5α-RI (4 mg/day) was injected twice (2 mg/injection) daily ($n = 6$ per group). $^{**}P < 0.01$.

relative potency of NT, following 5α-RI treatment. Muscle, on the other hand, reflects the inherent activity of these androgens and thus concomitant treatment with 5α-RI did not alter their potency on muscle. The antigonadotropic activity of T and NT was also not altered by cotreatment with 5α-RI, suggesting that the antigonadotropic action of the androgens was not mediated by their 5α-reduced metabolites. In previous studies not reported here, 5α-RI did not affect the LH levels in intact or castrated animals. Others have reported that 5α-RI treatment of men led to a decrease in serum DHT levels without altering the gonadotropin levels. The lack of effect of 5α-RI on LH levels was particularly evident in rats receiving 90 μg of T where there was partial suppression of LH levels (Table 4).

The effect of androgens on the pituitary of the male rat does not seem to be mediated through its aromatization to estrogen since treatment with an aromatase inhibitor had no effect on its antigonadotropic activity (data not shown). Other studies using specific antiestrogen or aromatase inhibitor have also shown that aromatization of testosterone to estrogen does not play a role in gonadotropin secretion in male rats [29, 30]. However, this is not the case in monkeys and men [31, 32]. A recent study in men, with idiopathic hypothalamic hypogonadism given pulsatile GnRH and T, E_2 or DHT infusions for 72 h, suggested that the suppressive effects of T on gonadotropins are partly mediated by aromatization of T to E_2 but not by its

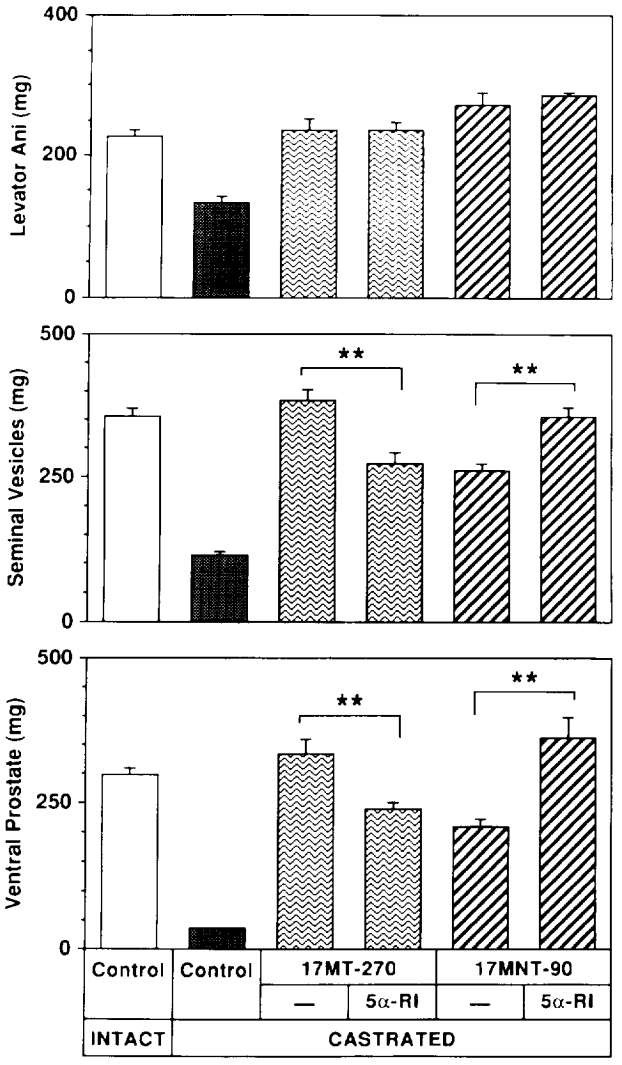

Fig. 5. Effect of 5α-reductase inhibitor on the bioactivity of 17α-methyl-testosterone (17MT) and 17α-methyl-19-nortestosterone (17MNT) in castrated rats. Details as per Fig. 4.

Table 5. Effect of 5α-reductase inhibitor on serum LH levels in castrated rats treated with T and NT

Group/treatment	LH (ng/ml)
1. Intact control	0.81 ± 0.17*
2. Castrated (C) control	6.01 ± 0.99
3. C + T-270†	0.24 ± 0.07
4. C + T-270 + 5α-RI	0.19 ± 0.07
5. C + NT-270	0.13 ± 0.02
6. C + NT-270 + 5α-RI	0.14 ± 0.02

*Values are mean ± SE ($n = 6$ per group).
†Doses are μg/day. Rats were castrated and implanted with osmotic pumps releasing the steroids for 7 days. 5α-RI (4 mg) was injected s.c. once daily.
NT, nortestosterone; T, testosterone; 5α-RI, 5α-reductase inhibitor.

5α-reduction to DHT [33]. However, the study does not rule out the possibility that intrapituitary conversion of T to DHT might have occurred and influenced gonadotropin secretion.

The importance of the 5α-reduction pathway in the pituitary is far from clear. It is possible that 5α-reduction is involved in the catabolism and clearance of T from the pituitary, as is the case in the liver. This is suggested by an indirect observation in which long term castration led to a significant increase in the 5α-reductase activity in the pituitary [34] and a decrease in the sensitivity of the pituitary to T feedback [19, 35, 36]. If 5α-reduction is involved in regulating the biological action of T, the increased 5α-reductase activity in the pituitary should enhance its inhibitory potency, which is not the case. In a previous study in castrated rats, it was shown that if T treatment was initiated 2 weeks after castration as opposed to immediately after castration, more T was required to overcome the post-castration rise in gonadotropin levels [19]. In contrast, the negative feedback effect of 7α-methyl-19-nortestosterone (MENT, a non-reducible androgen) was not altered significantly by delaying the initiation of treatment for 2 weeks.

Based on the above observations, it can be surmised that in the pituitary, 5α-reductase does not play a role similar to the one it does in sex accessory glands. It is possible that this is related to the differences in the specific site of location of the 5α-reductase enzyme in these tissues. It has been shown that 5α-reductase is predominantly localized on the nuclear envelope in the ventral prostate and seminal vesicles [37–39]. Thus it is possible that nuclear bound 5α-reductase in the sex accessory glands is responsible for the 5α-reduction of T and accumulation of DHT in the nuclei. The location of 5α-reductase in the pituitary is not clearly established. The subcellular distribution of 5α-reductase in the rat anterior pituitary was observed to be predominantly in the mitochondrial/microsomal fraction [40, 41]. Distribution studies of radioactive T in rats and monkeys shed some light on this point. Subcellular distribution of [^{3}H]T and its metabolites in the pituitary of intact rats after i.v. bolus, showed higher concentration of T rather than DHT in pituitary nuclei [42]. In contrast, another study in castrated and adrenalectomized rats found the pituitary nuclear concentration of [^{3}H]DHT to be twice that of [^{3}H]T but the authors cautioned that it may be because of the significant increase in 5α-reductase activity in pituitary after castration [43]. More recently it has been shown in adult male rhesus monkeys or fetuses that after the administration of [^{3}H]T most of the radioactivity extracted from the pituitary nuclei was in the form of [^{3}H]T and not [^{3}H]DHT, whereas administration of [^{3}H]DHT resulted in significant amounts of [^{3}H]DHT in the pituitary nuclei [44, 45]. Based on these observations it was concluded that the action of T in the pituitary does not involve its 5α-reduction. However, a recent study in long-term castrated male sheep showed that administration of 5α-RI could partially block the ability of T to inhibit LH release [46] suggesting possible species differences.

We utilized T and NT, whose 5α-reduced metabolites are more active and less active, respectively, to show that gonadotropin regulation does not involve the 5α-reduction of these androgens. Similar conclusions were reached through the use of a 5α-reductase inhibitor. In summary, our studies and published reports suggest that the feedback regulation of pituitary gonadotropin secretion in the rat is primarily a function of T and not DHT.

Table 6. Effect of 5α-reductase inhibitor (5αRI) on serum LH levels in castrated rats treated with 19-nortestosterone (NT)

Group/treatment	LH (ng/ml)
1. Intact control	0.48 ± 0.12
2. Castrated (C) control	5.11 ± 1.55
3. C + NT-90	0.08 ± 0.01
4. C + NT-90 + 5α-RI	0.12 ± 0.03
5. C + NT-180	0.05 ± 0.02
6. C + NT-180 + 5α-RI	0.10 ± 0.02

Details as per Table 5 except 5α-RI (4 mg/day) was injected twice daily.

Acknowledgements—This work was undertaken as part of the contraceptive development program sponsored and coordinated by the International Committee for Contraception Research of the Population Council, Inc., New York. Support for this work was provided through Cooperative Agreements No. DPE-3050-A-00-8059-00 from the U.S. Agency for International Development. Additional funding was provided through Grant No. U54HD29990 from the National Institute of Child Health and Human Development. We wish to thank Ms T. Arowosegbe, Mr L. Benalcazar and Mr W. DeJesus for technical assistance.

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