The Direct Pituitary Effect of Testosterone to Inhibit Gonadotropin Secretion in Men Is Partially Mediated by Aromatization to Estradiol

CARRIE J. BAGATELL, KRISTINE D. DAHL, AND WILLIAM J. BREMNER

From the Medical Service, Seattle Veterans Affairs Medical Center; and Department of Medicine and Population Center for Research in Reproduction, University of Washington School of Medicine, Seattle, Washington.

ABSTRACT: In men, administration of exogenous testosterone (T) exerts direct negative feedback effects at the pituitary as well as at the hypothalamic level. This study was undertaken to determine whether T itself causes the inhibitory effects on the pituitary, or whether conversion to estradiol (E₂) or dihydrotestosterone (DHT) is required. We assessed the biological activity of serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH), as well as immunoreactivity. Blood samples were drawn before, during, and after a continuous, 72-hour i.v. infusion of T (15 mg/day), E₂ (90 µg/day), or DHT (500 µg/day). Each of these doses is twice the daily production rate of the steroid. Each man received each of the three steroid infusions. We studied four men, ages 23–35, with idiopathic hypothalamic hypogonadism (IHH), who were treated with pulsatile gonadotropin releasing hormone (GnRH) until their gonadotropins reached the normal range. Serum levels of T, E₂, DHT, and levels of immunologically active and biologically active LH and FSH were measured. We found that administration of each steroid increased serum levels of the infused steroid to the upper physiologic range. Administration of T or E₂ resulted in decreased mean levels of biologically and immunologically active LH and FSH; administration of DHT did not alter gonadotropin secretion. These data suggest that some of the direct effect of T at the pituitary level in men is mediated by E₂, whereas peripherally formed DHT may not play an important role in this process.

Key words: LH, FSH, bioactivity, androgens, estrogens, DHT.

J Androl 1994;15:15–21

Secr __

Supported by NIH grants P50-HD-1262 and K0800890-01, research funds from the department of Veterans Affairs, and the Clinical Research Center of the University of Washington, supported by NIH grant RR-37.

Correspondence to: Dr. Carrie J. Bagatell, Endocrinology (111), VA Medical Center, 1660 S. Columbian Way, Seattle, Washington 98108.

Received for publication March 26, 1993; accepted for publication August 19, 1993.
the regulation of pituitary gonadotropin secretion in normal men are unclear. To examine this question, we studied men with IHH who were treated with pulsatile GnRH; while continuing GnRH therapy, we administered a 72-hour infusion of T, E₂, or DHT at twice the daily production rate of each hormone, in this way, we could compare the relative roles of E₂ and DHT in mediating the negative feedback effects of T directly at the pituitary level.

Materials and Methods

Subjects (Table 1)

We studied four men, ages 23–35, with IHH. All were otherwise healthy, as assessed by history and physical examination. None of the men took any medications at the time of entry into the study. Our diagnostic criteria for IHH were: 1) failure to undergo spontaneous puberty before age 18, 2) subnormal serum T (<8.7 nmol/L) and low or low-normal LH and follicle-stimulating hormone (FSH) (normal ranges: LH 8–50 μg/L, FSH 30–230 μg/L), and 3) absence of other hypothalamic or pituitary dysfunction as determined by endocrine evaluation and CT scanning of the sella turcica. Three of the men had scrotal testes at birth; the other man had a bilateral orchepaxy at age 6, leading to scrotal tests. At the time of entry into the study, testis volumes ranged from 2 to 15 ml. All of the men had received T injections in the past. Three men had previously received pulsatile GnRH, and two of them had fathered children on this regimen. Before enrolling in the study, all of the men signed a consent form approved by the University of Washington Human Subjects Review Committee, and all men discontinued T injections a minimum of 6 weeks before entry into the study.

Experimental Protocol

Pre-GnRH levels of T, E₂, LH, and FSH were measured in a blood sample obtained at least 6 weeks after the subject’s last T injection. The men were then begun on GnRH (Factrel, Wyeth-Ayerst, Philadelphia, Pennsylvania), 5 μg s.c. q 2 hours delivered by automatic infusion pump (Zyklomat, Ferring Laboratories, Suffern, New York) and followed until their gonadotropin levels reached the normal male range. Three of the subjects responded well to the 5-μg dose, and one man required a GnRH dose of 25 μg q 2 hours because of poor gonadotropin response to lower doses.

Once his gonadotropin levels normalized, each man continued pulsatile GnRH therapy and in addition received each of the following continuous i.v. infusions, in varied order, for 72 hours: 1) T enanthate, 15 mg/day; 2) 17-β estradiol (E₂), 90 μg/day; and 3) DHT, 500 μg/day. Each of these doses is twice the estimated daily production rate of that steroid in men (Santen, 1975). The studies were separated by 3–10 weeks. During each study, subjects were admitted to the Clinical Research Center of the University of Washington and remained there until the final blood samples were collected. Venous blood samples were collected every 10 minutes for 4 hours prior to the start of the infusion through an 18-gauge cannula placed in an arm vein. The cannula was kept patent with a continuous infusion of heparinized NaCl, 150 mmol/L. At the conclusion of the first sampling period, the cannula was heparin-locked; its patency was ensured by periodic flushes with heparinized saline. The steroid infusions were then begun through a separate i.v. line. From hours 68 to 72 of the infusion, samples were again collected every 10 minutes for 4 hours. All samples were stored at −20°C until analyzed. Subjects were free to ambulate throughout the University of Washington Medical Center except during frequent sampling periods, and meals were not restricted.

Steroid Preparation

Testosterone was obtained from the Upjohn Company, Kalamazoo, Michigan. 17-β Estradiol and DHT were obtained from Sigma Chemical Company, St. Louis, Missouri. The i.v. infusions were prepared by dissolving 7.5 mg, 45 μg, and 2.5 mg, respectively, of T, E₂, and DHT in 1 ml absolute ethanol. These solutions were then passed through a 0.2-μm filter and dissolved in 5% dextrose in water (DSW) to yield final steroid concentrations of 15 mg/L, 90 μg/L, and 500 μg/L, respectively. Each subject received 1 L of DSW solution per day. All steroid solutions were dispensed from glass containers and were administered to the subjects via IMED pump.

Hormone Assays

Immunologically active levels of serum LH and FSH (iLH and iFSH) were measured by radioimmunoassay (RIA) as previously described (Greenwood et al, 1963, Burger et al., 1972; Bremner et al., 1981), using reference standard LER 907 and reagents distributed by the National Pituitary Agency. The lower limit of detectability of the LH assay was 3.2 μg/L, and the intra-and inter-assay coefficients of variation were 5.5% and 8.4%, respectively. The lower limit of detectability of the FSH assay was 25 μg/L, and the intra-and inter-assay coefficients of variation (CVs) were 9.0% and 12.3%, respectively. Serum LH was mea-
Table 2. Mean (± SE) levels of biologically active and immunologically active LH and FSH and the bioactive/immunoactive (B/I) ratio in the four subjects prior to and during the last 4 hours of infusion of T, E₂, and DHT

<table>
<thead>
<tr>
<th></th>
<th>Pre-T</th>
<th>T infusion</th>
<th>Pre-E₂</th>
<th>E₂ infusion</th>
<th>Pre-DHT</th>
<th>DHT infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>bio-LH (μg/L)</td>
<td>160 ± 60</td>
<td>70 ± 20</td>
<td>390 ± 320</td>
<td>100 ± 50</td>
<td>370 ± 270</td>
<td>280 ± 180</td>
</tr>
<tr>
<td>iLH (μg/L)</td>
<td>33.1 ± 17.7</td>
<td>21.2 ± 8.6*</td>
<td>33.8 ± 7.3</td>
<td>11.6 ± 1.4</td>
<td>34.3 ± 9.6</td>
<td>34.0 ± 6.6</td>
</tr>
<tr>
<td>B/I</td>
<td>4.9 ± 2.2</td>
<td>4.2 ± 1.6</td>
<td>12.7 ± 10.3</td>
<td>12.0 ± 7.5</td>
<td>18.3 ± 15.3</td>
<td>12.4 ± 9.4</td>
</tr>
<tr>
<td>bio 7SH (μg/L)</td>
<td>1,010 ± 570</td>
<td>490 ± 320</td>
<td>570 ± 80</td>
<td>320 ± 160</td>
<td>750 ± 260</td>
<td>750 ± 270</td>
</tr>
<tr>
<td>iFSH (μg/L)</td>
<td>130 ± 70</td>
<td>72 ± 40</td>
<td>75 ± 14</td>
<td>34 ± 6*</td>
<td>127 ± 60</td>
<td>108 ± 40</td>
</tr>
<tr>
<td>B/I</td>
<td>7.5 ± 0.2</td>
<td>6.5 ± 1.1</td>
<td>7.7 ± 0.7</td>
<td>8.2 ± 2.7</td>
<td>6.7 ± 0.7</td>
<td>6.9 ± 1.7</td>
</tr>
</tbody>
</table>

Each LH value represents the mean of five representative samples assayed for both iLH and bio-LH. Each FSH value represents a pooled sample derived from equal aliquots of five samples collected during a 4-hour period.

* P < 0.05 compared to pre-infusion value.

Biologically active LH (bio-LH) was measured by a modification (Matsumoto et al., 1984) of the procedures described by Van Damme et al. (1974) and Dufau et al. (1977). In this assay, LH production was measured from dispersed Leydig cells isolated from adult Swiss-Webster mice. All samples were run in duplicate at a volume of 10 μl. The intra-and inter-assay CVs were 10.0% and 16.0%, respectively. Biologically active FSH (bio-FSH) was measured in vitro using a modification (Dahl et al., 1990) of the methods of Jia and Hseuh (1986). This assay measures FSH production from granulosa cells obtained from immature, diethylstilbestrol (DES)-treated Sprague-Dawley rats. The intra-and inter-assay CVs were 10.0% and 14.0%, respectively. Levels of bio-LH were measured in five representative samples from both the pre-infusion and end-infusion sampling periods. Levels of iLH were also measured on these five samples, the means of the five iLH values at each time point are presented in Table 2 and were used to determine the bioactive/immunoactive (B/I) ratio at each time point. There was inadequate volume of serum to allow measurement of bio-FSH on individual samples. Therefore, in each study, levels of bio-FSH in each man were measured on pooled samples prepared from equal aliquots of five pre-infusion and five end-infusion samples. In order to calculate B/I ratios, iFSH levels were also determined on these samples.

Equal aliquots of each of the pre-and end-infusion samples were combined to form a pool for the measurement of pre-and end-infusion levels of T, E₂, and DHT. Serum T was measured by RIA as previously reported (Matsumoto et al., 1983). Serum E₂ was measured by RIA using a kit obtained from ICN Biomedicals, Carson, California. The limits of detectability of T and E₂ were 0.3 nmol/L and 37 pmol/L, respectively. Serum DHT was measured by RIA using a kit obtained from Amersham Corporation, Arlington Heights, Illinois. In this assay, T and DHT are first separated from binding proteins by ether extraction. Testosterone is then oxidized, making it insoluble in ether and allowing for separation of T and DHT. DHT is then extracted with ether and is measured by RIA. The cross-reactivity of T in this DHT assay is less than 0.35% using T concentrations as high as 69.4 nmol/L (20 ng/mL), higher than any T level encountered in this study. The limit of detectability of DHT in the assay was 0.3 nmol/L.

Statistical Analysis

For each man, the mean of all iLH samples from each frequent sampling period was calculated. The group means for each sampling period were then calculated. The half-hourly iFSH samples and the bio-LH and bio-FSH samples were analyzed similarly. For each sample in which bio-LH or bio-FSH was measured, the B/I ratio was determined by dividing the bio-LH or bio-FSH value by the iLH or iFSH value on that same sample. For each infusion, the mean pre-infusion and end-infusion values of each hormone were compared using a paired Student’s t-test. Because of the large variance of the bio-LH and bio-FSH values, the pre-infusion and end-infusion levels of these hormones were compared using the Wilcoxon signed-rank test.

Incremental LH was defined as the absolute increase in serum LH frombasal to peak level. The incremental LH values of the two GnRH-induced LH pulses during each frequent sampling period were averaged, and the mean pre- and end-infusion values during each infusion were compared using a paired Student’s t-test.

The mean T, E₂, and DHT levels before and at the end of each infusion were compared using a paired Student’s t-test. P < 0.05 was considered significant. In the analysis of serum E₂ levels, some values were undetectable. For purposes of analysis, a value of the lower limit of detectability of the assay (37 pmol/L) was assigned to each of these samples.

Results

Pre-GnRH Hormone Levels

Before the men began pulsatile GnRH therapy, their mean serum T was 3.0 ± 1.4 nmol/L, and serum E₂ was undetectable. Mean serum iLH was 5.3 ± 1.0 μg/L, and serum iFSH was undetectable in all of the men.

Testosterone Infusion

During GnRH plus T administration, serum T levels increased significantly (Fig. 1). Mean E₂ was undetectable in all but one man prior to infusion, but rose to 108 ± 32 pmol/L at the end of infusion. Serum DHT increased significantly (P < 0.05) during T infusion.
During T infusion mean serum iLH decreased significantly, from 32.5 ± 8.3 μg/L to 21.0 ± 7.8 μg/L (60.3% of baseline, \( P < 0.05 \), Fig. 1). Mean serum iFSH prior to infusion was 118 ± 67 μg/L and decreased to 77 ± 44 μg/L during the last 4 hours of infusion (70% of baseline, \( P = 0.07 \), Fig. 3). Incremental LH decreased from 20.5 ± 3.5 μg/L to 10.1 ± 3.7 μg/L (\( P = 0.07 \)) during the infusion (Fig. 4).

Mean bio-LH levels decreased from 390 ± 320 μg/L to 100 ± 50 μg/L (\( P = 0.05 \)) during E\(_2\) infusion, and the mean B/I ratio did not change (Table 2). Mean bio-FSH decreased to 44% of baseline, resulting in a small but insignificant increase in the B/I ratio.

**DHT infusion**

During DHT infusion, serum DHT levels increased significantly (\( P < 0.01 \), Fig. 5), whereas the mean serum T declined from 34.5 ± 8.3 μg/L to 15.2 ± 5.1 μg/L (45.2% of baseline, \( P < 0.05 \), Fig. 3). Mean serum iFSH fell from a baseline of 126 ± 70 μg/L to 56 ± 25 μg/L during the last 4 hours of infusion (65% of baseline, \( P = 0.07 \), Fig. 3). Incremental LH decreased from 20.5 ± 3.5 μg/L to 10.1 ± 3.7 μg/L (\( P = 0.07 \)) during the infusion (Fig. 4).

Mean bio-LH levels decreased from 390 ± 320 μg/L to 100 ± 50 μg/L (\( P = 0.05 \)) during E\(_2\) infusion, and the mean B/I ratio did not change (Table 2). Mean bio-FSH decreased to 44% of baseline, resulting in a small but insignificant increase in the B/I ratio.

**Estradiol Infusion**

Serum E\(_2\) increased significantly during E\(_2\) infusion (\( P < 0.02 \), Fig. 3). Mean levels of T decreased during E\(_2\) infusion, although the degree of decrease did not reach statistical significance (\( P = 0.10 \)); mean serum DHT did not change.

After 72 hours of E\(_2\) infusion, mean serum iLH had declined from 34.5 ± 8.3 μg/L to 15.2 ± 5.1 μg/L (45.2% of baseline, \( P < 0.05 \)), Fig. 3). Mean serum iFSH fell from a baseline of 126 ± 70 μg/L to 56 ± 25 μg/L during the last 4 hours of infusion (65% of baseline, \( P = 0.07 \), Fig. 3). Incremental LH decreased from 20.5 ± 3.5 μg/L to 10.1 ± 3.7 μg/L (\( P = 0.07 \)) during the infusion (Fig. 4).

Mean bio-LH levels decreased from 390 ± 320 μg/L to 100 ± 50 μg/L (\( P = 0.05 \)) during E\(_2\) infusion, and the mean B/I ratio did not change (Table 2). Mean bio-FSH decreased to 44% of baseline, resulting in a small but insignificant increase in the B/I ratio.
levels did not change. Mean serum E₂ was 58.7 ± 13.6 pmol/L during this infusion (P = NS compared to the mean pre-infusion level). Neither serum LH nor iFSH levels changed in response to DHT infusion (Fig. 5), and there was no change in incremental LH (Fig. 6).

Mean bio-LH declined slightly at the end of infusion; the B/I ratio decreased slightly but not significantly (Table 2). Mean levels of bio-FSH and the B/I ratio did not change during DHT infusion.

**Discussion**

We treated men with IHH with pulsatile GnRH until their gonadotropin levels normalized; we then continued GnRH and administered continuous, 72-hour infusions of T, E₂, or DHT at twice the daily production rate of each steroid. T and E₂ administration resulted in decreased secretion of immunologically and biologically active LH and FSH in all of the men, whereas DHT infusion resulted in no change in mean levels of immunologically or biologically active gonadotropins. These data suggest that the suppressive effects of T exerted directly at the pituitary level are mediated largely by aromatization of T to E₂, whereas at the dosage of DHT we used, we were unable to demonstrate an important role of 5α-reduction to DHT. Our data also suggest that in this model, the effects of gonadal steroids on biologically active forms of both LH and FSH are similar to their effects on the immunologically active forms of these gonadotropins.

Our work confirms previous studies showing a direct suppressive effect of T administration on the pituitary gland in men (Valk et al., 1984; Scheckter et al., 1989; Finkelstein et al., 1991b). We have also demonstrated that this effect is similar whether gonadotropin output is measured in terms of immunologically active or biologically active product. In addition, our present work demonstrates a direct suppressive effect of E₂ on pituitary gonadotropin production. Previous studies of E₂ administration to normal men have shown that administration of low doses of E₂ for brief periods (i.e., 5 days or less) results in decreased mean levels of LH and FSH (Sherins et al., 1973; Stewart-Bentley et al., 1974; Santen, 1975; Winters et al., 1979; Veldhuis et al., 1984) and in decreased LH pulse amplitude (Veldhuis et al., 1984) and response to GnRH (Santen, 1975) with no change in pulse frequency (Veldhuis et al., 1984). These earlier studies could not distinguish between hypothalamic and pituitary effects of estradiol, because the decreased pituitary response to GnRH could have occurred as a result of decreased hypothalamic GnRH production. Finkelstein et al. (1991a) have recently reported immunoactive gonadotropin results similar to ours in IHH men treated with GnRH and E₂; bioactive gonadotropin levels were not presented in
that report. We have now demonstrated that levels of bioactive LH and FSH are both suppressed similarly to immunoreactive LH and FSH. Finkelstein et al. (1991a) have also shown that in IHH men treated with GnRH, the suppressive effect of T on serum LH and FSH is decreased by addition of aromatase inhibitor (testolactone), which inhibits conversion of T to E2. These data also support a major role of E2 in mediating the suppression of gonadotropin secretion resulting from T administration.

A direct pituitary effect of E2 is also supported by animal studies. Administration of E2 to hypothalamus-lesioned, GnRH-treated female monkeys results in an acute decrease in LH secretion (Nakai et al., 1978; Plant et al., 1978). Pau et al. (1988, 1990) have reported that a single dose of estradiol benzoate results in decreased LH secretion with no change in GnRH levels in gonadectomized male and female rhesus monkeys, suggesting that an acute decrease in LH secretion may be independent of E2 effects on GnRH and may also be independent of the sex of the animal.

We found that administration of DHT, 500 μg/day, to men with IHH under treatment with GnRH, did not suppress LH and FSH. Sherins and Loriaux (1973) found no change in gonadotropin levels when this amount of DHT was infused into normal men. When higher doses of DHT have been administered to healthy men, thereby causing supraphysiologic serum DHT levels, a variety of results have been observed. Schaison et al. (1980), who administered DHT percutaneously, and Gooren et al. (1984), who administered it orally, failed to find any change in LH or FSH levels after 6 weeks (Gooren et al., 1984) or in LH levels after 3 months (Schaison et al., 1980) of treatment. Other investigators, however (Stewart-Bentley et al., 1974; Santen, 1975; Loriaux et al., 1977; Kuhn et al., 1984; Veldhuis et al., 1984; Winters et al., 1984; Vermeulen and Deslypere, 1985; Urban et al., 1991) have found a decrease in mean LH levels after administration of up to 10 mg of DHT per day for periods of 6 hours (Santen, 1975) to 3 months (Vermeulen and Deslypere, 1985). In some of these studies, FSH levels were not suppressed or were not reported (Stewart-Bentley et al., 1974; Santen, 1975; Loriaux et al., 1977; Kuhn et al., 1984; Veldhuis et al., 1984); other investigators have reported suppression of FSH levels after high-dose DHT administration (Winters et al., 1984; Vermeulen and Deslypere, 1985; Urban et al., 1991). Additional evidence that peripheral levels of DHT do not play a major role in modulating serum gonadotropins in men comes from studies of the administration of blockers of the 5α reductase enzyme (Matzkin et al., 1992; Rittmaster et al., 1992). In these studies, administration of finasteride reduced DHT levels significantly, with no change in serum LH or FSH levels (Matzkin et al., 1992; Rittmaster et al., 1992).

In our studies, administration of DHT at a dose of 500 μg daily resulted in mean serum DHT levels in the upper male physiologic range. This level was not as high as the mean serum DHT levels achieved during T infusion. It is possible that had we achieved higher circulating DHT levels (i.e., of the same magnitude we observed when T was infused), we might have observed a decrease in mean LH and/or FSH levels. DHT administration has been reported to decrease LH pulse frequency, but not pulse amplitude (Veldhuis et al., 1984); in other studies, LH response to GnRH administration did not change (Gooren et al., 1984) or increased after DHT (Schaison et al., 1980). The predominance of evidence thus suggests a hypothalamic rather than a pituitary site of action for DHT.

In summary, we administered 72-hour infusions of T, E2, and DHT at twice their respective daily production rates to men with IHH who were being treated with pulsatile GnRH. We found that T and E2 infusions resulted in similar degrees of suppression of immunologically and biologically active gonadotropins, whereas DHT infusion had no effect on any gonadotropin measurements. These results suggest that much of the direct effect of T at the pituitary level in men is mediated by E2. Although we cannot rule out the possibility that intra-pituitary conversion of T to DHT is important in mediating the suppressive effect of T on gonadotropin secretion, it appears that peripherally formed DHT may not play an important role in this process.

Acknowledgments

We thank Ms. Dorothy McGuiness, Mr. Arlen Sarkissians, and Ms. Florida Flor for performing the hormone assays. We thank Ms. Elaine Rost for assistance with the illustrations and Ms. Liza Noonan for assistance with the statistical analysis.

References

Clarke IJ, Cummins JT. The temporal relationship between gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. Endocrinology 1982;113:1737–1741.
Finkelstein JS, O'Dea LS, Whitcomb RW, Crowley WF. Sex steroid control of gonadotropin secretion in the human male. II. Effects of


