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Testosterone Administration Inhibits Gonadotropin Secretion by an Effect Directly on the Human Pituitary*

CAROL B. SHECKTER, ALVIN M. MATSUMOTO, AND WILLIAM J. BREMNER

Medical Service and Gerontology Research, Education, and Clinical Center, Veterans Administration Medical Center; Population Center for Research in Reproduction; and the Divisions of Endocrinology and Gerontology, Department of Medicine, University of Washington School of Medicine, Seattle, Washington 98108

ABSTRACT. Testosterone (T) administration slows LH pulse frequency in man, presumably by an effect on the hypothalamic GnRH pulse generator, but it also may have a direct action on the pituitary. To determine if T does indeed affect gonadotropin secretion by acting directly on the pituitary, we studied the effect of T on GnRH-stimulated gonadotropin secretion. Six men with hypogonadotropic hypogonadism were treated with physiological doses of GnRH (5 μg every 2 h, sc by automatic infusion pump) for 6 weeks. Once their gonadotropin levels were normal, the men received a supraphysiological dosage of T enanthate (200 mg, im, weekly for 8 weeks) in addition to GnRH. They then received GnRH alone for a final 8-week period. Blood sampling was performed every 10 min for 8 h at the end of each of the three study periods. T administration suppressed the mean serum LH level to about 50% of the value during GnRH alone [18 ± 2 (±SE) *vs.* 37 ± 2 μg/L; $P < 0.05$] and suppressed the mean serum FSH level to about 30% of the value during GnRH alone (39 ± 6 *vs.* 128 ± 28 μg/L; $P < 0.05$). Eight weeks after stopping T, while continuing GnRH alone, serum LH and FSH levels were similar to those at the end of the first period of GnRH administration. The mean LH response to GnRH was reduced during T administration (17 ± 3 μg/L) compared to that during the initial period of GnRH alone (31 ± 4 μg/L; $P < 0.05$). Serum T and estradiol levels were in the low normal range after GnRH alone before T administration (11 ± 2 nmol/L and 105 ± 17 pmol/L, respectively) and increased to just above the normal adult ranges after 8 weeks of T administration (36 ± 5 nmol/L and 264 ± 49 pmol/L, respectively). These results demonstrate that T and/or its metabolites inhibit LH and FSH secretion by a GnRH-independent mechanism, probably directly on the pituitary gland, in man. (*J Clin Endocrinol Metab* **68:** 397, 1989)

THE REGULATION of gonadotropin secretion from the pituitary is mediated in part by negative feedback effects of sex steroids from the testes. Whether negative feedback is exerted at the level of the pituitary, in the brain, or both is not clear. In several mammalian species, including rats (1), sheep (2), and monkeys (3), castration results in increased LH pulse frequency, while testosterone (T) replacement returns the LH pulse frequency toward the normal range. In men who have primary hypogonadism LH pulses occur at a higher frequency than in normal men, and the frequency can be decreased by T replacement (4). Similarly, in normal men the administration of androgens slows pulsatile LH secretion (5, 6). Because LH pulse frequency directly reflects the frequency of GnRH secretion from the hypothalamus (7), it is thought that T-induced slowing of LH pulses is an effect of T negative feedback at the level of the brain.

Whether T has a direct effect on the pituitary to regulate gonadotropin secretion and, if so, whether the effect is stimulatory or inhibitory are still not clear, despite a number of animal studies *in vitro* (8–10) and *in vivo* (11–13). Most such studies indicate that T directly regulates gonadotropin secretion. Its effects may be stimulatory or inhibitory, depending on a number of variables, including the type and length of androgen exposure and the dose of GnRH used to stimulate pituitary secretion. In this study, we assessed whether T administration regulates gonadotropin secretion directly at the level of the pituitary in man. To do so, we studied men with idiopathic hypogonadotropic hypogonadism (IHH), since they are known to have absent or markedly diminished hypothalamic GnRH secretion (14). These men were given a physiological dosage of GnRH using a pulsatile infusion pump set to administer a constant amplitude of GnRH at a constant frequency. After several weeks of GnRH administration, which normalized their serum gonadotropin levels, the men received T along with GnRH, and the effect on gonadotropin secretion was determined. Because the regimen of GnRH stimulation

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Address requests for reprints to: William J. Bremner, M.D., Ph.D., Veterans Administration Medical Center (111), 1660 South Columbian Way, Seattle, Washington 98108.
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to the pituitary did not change throughout the study, the alterations in gonadotropin secretion that occurred during the period of T administration were presumed to be the result of steroid action directly at the level of the pituitary.

Materials and Methods

Subjects

Six men with IHH, aged 21–33 yr, were studied. All were otherwise healthy, as determined by history and physical examinations, and none was taking any medications at the time of entry into the study. The diagnostic criteria for IHH included failure to undergo spontaneous puberty before age 18 yr, subnormal serum T levels (<9.7 nmol/L), low or low normal serum gonadotropin levels (normal adult range, 8–50 μg/L for LH; 30–230 μg/L for FSH), absence of other hypothalamic or pituitary pathology based on endocrine testing (serum T_4, PRL, and cortisol levels and adrenal response to ACTH stimulation or insulin-induced hypoglycemia), and computerized axial tomography of the sella turcica. All men had scrotal testes at birth, and their testicular volumes ranged from 3–12 mL. In the past, five men had received T injections. Three of these five men had received hCG for 1–5 yr, and one of them had also received pulsatile GnRH therapy. None had received FSH. All hormonal therapy was stopped at least 6 weeks before this study.

Experimental protocol

The men gave informed consent to the study in accordance with procedures established by the University of Washington Human Subjects Review Committee. Serum LH, FSH, T, and estradiol (E_2) levels were measured initially in single blood samples obtained at least 6 weeks after hormone therapy was discontinued. The men then were given a physiological dose of GnRH (5 μg every 2 h), delivered sc by automatic infusion pump (Zyklomat, Ferring Laboratories, Suffern, NY) for approximately 22 weeks. The pre-T GnRH alone period lasted approximately 6 weeks, until all men had serum gonadotropin levels within the normal range. Then, all men were given T enanthate (200 mg, im, weekly) for 8 weeks in addition to pulsatile GnRH (GnRH + T period). The men then received GnRH alone for 8 weeks (post-T GnRH alone period).

Venous blood sampling was done every 10 min for 8 h at the end of each study period: 1) pre-T GnRH alone period, just before starting T injections, *i.e.* after about 6 weeks of pulsatile GnRH administration alone; 2) GnRH + T period, 7 days after the last T injection, *i.e.* after 8 weeks of simultaneous pulsatile GnRH and weekly T administration; and 3) post-T GnRH alone period, after the final 8 weeks of GnRH administration alone. The men were admitted to the Clinical Research Center of the University of Washington Hospital for the frequent sampling studies. Beginning at 0700–0900 h, blood was sampled through an indwelling 18-gauge polyethylene cannula placed in an arm vein. The men continued to receive GnRH during the blood sampling; the initial dose was given at the start of the frequent sampling study. Activity was unrestricted, and meals were given *ad libitum*. The patency of the iv line was maintained with a continuous infusion of heparinized 150 nmol/L NaCl. Before taking each 4-mL blood sample, the iv line was cleared by withdrawing 6 mL blood. After the 4-mL blood sample was obtained, the 6 mL blood mixed with heparinized NaCl were reinfused, and the line was flushed with heparinized NaCl. The serum samples were stored at −20 C until analyzed.

Hormone assays

Serum LH and FSH concentrations were measured by double antibody RIA, as previously described (15), using reagents distributed by the National Pituitary Agency. The reference preparation was LER 907. The sensitivity of the LH assay was 6 μg/L, and the intra- and interassay coefficients of variation (CVs) were 5.5% and 8.4%, respectively. The sensitivity of the FSH assay was 25 μg/L, and the intra- and interassay CVs were 7.3% and 9.7%, respectively. LH was measured in every sample; FSH was measured hourly. For each man, all samples obtained during the the pre-T GnRH alone and GnRH + T periods were analyzed in the same assay, and the post-T GnRH alone period samples were analyzed in another assay.

Serum T and E_2 concentrations were measured by RIA, as previously described (4, 16). The assay sensitivity was 0.35 nmol/L for T and 44 pmol/L for E_2. The intra- and interassay CVs were 5.1% and 9.8%, respectively, for T and 8.2% and 8.8% for E_2. Serum T and E_2 were measured in the first sample obtained on each study day.

Statistical analysis

For each man, the mean of the 49 LH measurements from each study was calculated. Then, group means and SEs were calculated for each study period. The serum FSH values in the nine hourly samples and the single serum T and E_2 values were analyzed in a similar fashion. The group means from the different periods were compared by the Wilcoxon signed rank test.

The increments (defined as the absolute increase in serum LH level from basal to peak) of all GnRH-induced pulses on each study day for each man were averaged, and the mean values for the different periods were compared by the Wilcoxon signed rank test. $P < 0.05$ was considered significant.

Results

Serum LH

Before GnRH administration, serum LH levels in the six men were low, averaging 8 ± 2 (±SE) μg/L (range, 5–15 μg/L). After 6 weeks of GnRH administration, the mean serum LH level was in the normal adult range (Table 1). In all men a distinct LH pulse was detected after each exogenous GnRH pulse (Fig. 1). No LH pulses were detected other than those generated by exogenous GnRH. After 8 weeks of T and pulsatile GnRH administration, the pattern of pulsatile LH secretion was similar. However, the mean serum LH levels was about 50% of that during the pre-T GnRH alone period [18 ± 2 (± SE) *vs.* 37 ± 2 μg/L; $P < 0.05$; Table 1]. The mean

TABLE 1. Serum LH, FSH, T, and E_2 levels before and during the GnRH and GnRH + T study periods in six men with IHH

	Pre-T		Post-T	
	Basal[a]	GnRH alone[b]	GnRH + T[b]	GnRH alone[b]
Serum LH (μg/L; range, 8–50)[c]	8 ± 2	37 ± 2	18 ± 2[d]	46 ± 5
Serum FSH (μg/L; range, 30–230)	43 ± 6	128 ± 18	39 ± 6[d]	133 ± 16
Serum T (nmol/L; range, 10–30)	1.0 ± 0.4	11 ± 2	36 ± 5[d]	11 ± 2
Serum E_2 (pmol/L; range, 48–206)	68 ± 40	105 ± 17	264 ± 49[d]	109 ± 11

[a] Mean ± SE (n = 6) of single samples drawn before GnRH administration.

[b] Mean ± SE (n = 6) of samples obtained every 10 min for 8 h for LH, samples obtained hourly for 8 h for FSH, and single samples for T and E_2.

[c] The numbers in parentheses are the normal adult ranges for each hormone. To convert T concentrations to ng/mL, divide by 3.467. To convert E_2 concentrations to pg/mL, divide by 3.671.

[d] $P < 0.05$ compared to the pre-T GnRH alone period.

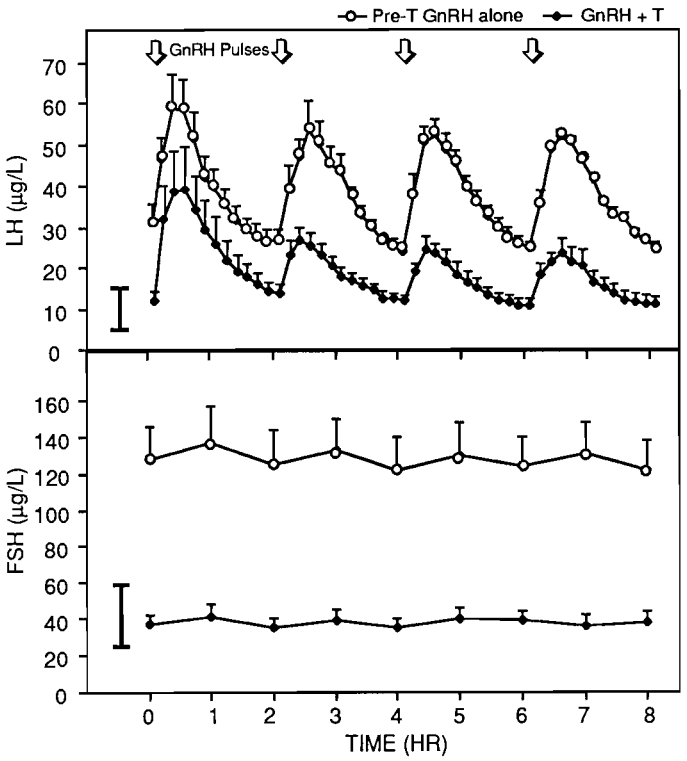

FIG. 1. Mean (±SE) serum LH (*upper panel*) and FSH (*lower panel*) levels in six men with IHH during 8 h of frequent blood sampling at the end of the pre-T GnRH alone (○) and GnRH + T (●) periods. The *arrows* indicate the times when the sc GnRH pulses were given. The *horizontal bars* indicate the range of serum LH and FSH concentrations in these men before GnRH administration. Note the suppression of the mean serum LH and FSH levels and the LH increments in response to GnRH induced by T administration.

increment in serum LH in response to the GnRH pulses was lower during the GnRH + T period (17 ± 3 μg/L) compared with the pre-T GnRH alone period (31 ± 4 μg/L; $P < 0.05$; Fig. 1). The mean serum LH level on the post-T GnRH alone sampling day (46 ± 4 μg/L) was similar to that on the pre-T GnRH alone day (37 ± 2 μg/L; Table 1).

Serum FSH

Before GnRH administration, the serum basal FSH values were low or low normal, averaging 43 ± 6 μg/L (range, 25–60 μg/L). During GnRH administration the mean serum FSH level increased into the normal range (128 ± 18 μg/L; Table 1). During the GnRH + T period, the mean serum FSH level declined to 39 ± 6 μg/L, a value similar to that before GnRH administration and significantly lower ($P < 0.05$) than that during the pre-T GnRH alone period (Table 1 and Fig. 1). During the post-T GnRH alone period, the mean serum FSH level rose to approximately the value during the pre-T GnRH alone period.

Serum T and E_2

The mean serum T and E_2 levels increased from very low basal values during the pre-T GnRH alone period into the low normal range during GnRH alone (Table 1). During the GnRH + T period, the mean serum T and E_2 levels increased to just above their respective normal adult ranges. During the post-T GnRH alone period, the serum T and E_2 levels fell to values similar to those during the pre-T GnRH alone period.

Discussion

In this study, men with IHH were given small doses of GnRH intermittently to raise gonadotropin levels into the normal range. Administration of T along with the same dose of GnRH resulted in marked reductions in mean serum LH and FSH concentrations and in the incremental LH response to each GnRH pulse. T administration did not alter the timing of LH pulses that followed each exogenous GnRH pulse. Thus, T and/or its metabolites, albeit in supraphysiological doses, exert suppressive effects on gonadotropin secretion in man by a process that is independent of its (their) effects on GnRH secretion. Our results support the hypothesis that part of the suppressive effect of T on LH and FSH secretion is mediated directly at the level of the pituitary.

Previous studies which suggested that T directly alters gonadotropin secretion were inconclusive; both positive and negative feedback effects were found. In cultured rat pituitary cells T augmented (9), suppressed (10), or initially augmented and then suppressed (9) hypothalamic

extract-stimulated or GnRH-stimulated LH release; T augmented basal FSH secretion (8, 10) and suppressed hypothalamic extract-stimulated FSH release (9); and T increased basal FSH/LH ratios (8). Androgens implanted into rat hemipituitaries increased tissue FSH/LH ratios (12). In castrate male rats with pituitary stalk sections, T both stimulated and inhibited GnRH-induced LH secretion, depending on the dose of GnRH and T used (11). Finally, in castrate hypothalamus-lesioned male rats, exposure to physiological T levels acutely augmented gonadotropin responsiveness to GnRH (13). In primates, Plant and Dubey (17) found that orchidectomy in arcuate nucleus-lesioned rhesus monkeys replaced with pulsatile GnRH led to a minimal increase in serum LH and a more substantial rise in FSH. Further work, however, suggested that the effect on FSH was more likely due to nonsteroidal testicular factors (18, 19). In humans, an inhibitory effect of T on LH (20, 21) and FSH (20) secretion, exerted directly at the pituitary level, is suggested by two short term (4- to 5-day) studies of GnRH-treated men with IHH.

Whether the feedback effects on gonadotropin secretion that we measured were mediated by T itself, by a metabolite of T such as E_2 or dihydrotestosterone (DHT), or by a combined effect of these hormones is not known. Both serum T and E_2 levels were just above their physiological ranges when measured 7 days after the preceding T dose and were undoubtedly higher during the time between the dose and the study; the E_2 increase was presumably due to peripheral aromatization from T. Estrogen receptors have been found in both the hypothalamus (22) and anterior pituitary (23), and there is evidence that E_2 inhibits gonadotropin secretion at both hypothalamic (24) and pituitary levels (5, 25). The nonaromatizable androgen DHT, could also be mediating the effects on gonadotropins, as DHT also inhibits LH secretion (5, 6). A hypothalamic site of action for DHT is suggested by the finding that DHT decreases LH pulse frequency in normal men (6). *In vitro* work suggests that DHT might also regulate gonadotropin secretion directly at the level of the pituitary (26).

The GnRH clamp model used in this study eliminated possible gonadal steroid feedback effects on hypothalamic GnRH secretion. Therefore, the suppressive effects on gonadotropins found during T administration were exerted at the level of the pituitary. We cannot, however, exclude the possibility that the effects of T on gonadotropin secretion were mediated by a hypothalamic hormone other than GnRH which is capable of altering LH and FSH secretion at the level of the pituitary. Such an effect could theoretically be mediated by endogenous opioid peptides, which can inhibit LH release and whose secretion is regulated by gonadal steroids (27–29). Opioids also may directly inhibit pituitary gonadotropin secretion (30–33). However, opiate receptors are scarce in the anterior pituitary (34, 35), and most evidence suggests that opioids inhibit gonadotropin secretion primarily by suppression of hypothalamic GnRH release (36, 37).

In summary, T in supraphysiological dosages inhibits gonadotropin secretion by a GnRH-independent mechanism, probably by direct action on the pituitary gland, in man. Whether this effect is mediated by T itself or by one of its metabolites is not known. Whether more physiological dosages of T would have suppressed gonadotropin secretion is not known. Finally, the GnRH clamp model used in this study appears to be a useful approach for studying the direct effects of hormones on the pituitary gland.

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