Testosterone and Estradiol are Co-secreted Episodically by the Human Testis

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Abstract

In spite of a striking pulsatile pattern of luteinizing hormone (LH) secretion, testosterone (T) fluctuations in peripheral blood in normal adult men are irregular and of low amplitude. To determine whether T secretion by the human testis is episodic, T was measured in blood samples drawn at 15-min intervals for 4 h through a catheter placed in the testicular vein of six men with varicocele-associated infertility. Estradiol (E_2) concentrations were also determined in each sample. Each subject released testosterone in well-defined pulses. Gonadal vein T levels ranged from 1 to 1,540 ng/ml. Mean ($\pm$SE) pulse amplitude was 176$\pm$42 ng/ml, with a frequency of 4.0$\pm$0.3 pulses per 4 h. Testicular vein E_2 levels ranged from 0.01 to 6.8 ng/ml. E_2 secretory episodes were generally coincident with T pulses, and their amplitudes were highly positively correlated ($r = 0.90$, $P < 0.01$).

These results indicate that T secretion by the adult human testis is pulsatile, and suggest a functional relationship between intermittent LH secretion and normal testicular steroidogenesis in men. The failure to appreciate these fluctuations as hormone pulses in peripheral blood may relate to their absolute amplitude and frequency. The concordance between E_2 and T pulses suggests that the Leydig cell, under LH control, is the source of most of the E_2 secreted by the adult human testis.

Introduction

Luteinizing hormone (LH)[1] is secreted by the pituitary gland into the peripheral blood in distinct bursts (1) which have been shown to be a consequence of intermittent luteinizing hormone-releasing hormone (LRH) release by the hypothalamus (2). The gonadotroph requires this intermittent secretory mode for it to function normally (3). Temporal co-analysis of circulating LH and testosterone (T) levels in many male laboratory animals, including subhuman primates, reveals a tight coupling of LH pulses to subsequent T secretory episodes, which indicates a functional relationship between intermittent gonadotropin secretion and the steroidogenic function of the testis (4–7). This relationship has not previously been established in men.

T fluctuations in peripheral blood in men are of low amplitude and occur at irregular intervals (8–12). In one study, T increments in men were generally preceded by LH rises, but

only one of three LH pulses was followed by a rise in serum T levels (12). Some investigators have been unable to identify any convincing relationship between LH and T pulses (8, 10), whereas a statistically significant correlation between LH and T concentrations with a phase delay of 1–3 h has also been described (11). In an occasional subject, a large peak of LH bioactivity will be followed by a convincing rise in circulating T levels (13).

To determine whether T secretion by the human testis is episodic under physiologic conditions, T was measured in blood samples drawn at 15-min intervals for 4 h through a catheter placed in the gonadal vein of six men with varicocele-associated infertility who were undergoing gonadal venography. Estradiol (E_2) levels were also examined to compare the patterns of T and E_2 secretion in men.

Methods

Six men, ages 30–41 yr, with varicocele-associated infertility, volunteered for this study, which was approved by the Human Investigation Committee of Montefiore Hospital. Their clinical characteristics are summarized in Table I. Mean circulating LH, follicle-stimulating hormone (FSH), and T levels were normal for each man. Varicocele was visible and/or palpable when each patient assumed the upright posture. The internal spermatic vein (five patients on the left, one on the right) was catheterized using a femoral vein approach. Premedication with meperidine was avoided, since narcotic drugs inhibit gonadotropin secretion. The subjects were returned to a quiet room for the sampling study. Blood was sampled every 15 min for 4 h, allowed to clot at room temperature, and the serum was stored at −20°C for subsequent radioimmunoassay. All samples from an individual subject were analyzed in a single assay to eliminate the influence of interassay variation.

Serum concentrations of T and E_2 were determined in samples diluted 1:10 to 1:400 with phosphate-buffered saline, which were then ether extracted. The E_2 fraction was purified by chromatography on Sephadex LH-20 using chloroform-n-hepatane-methanol-water (200:200:30:1.2, vol/vol/vol/vol) as the solvent system. This step completely separated radiolabeled T from E_2. Separation of antibody-bound from free steroid was accomplished with dextran-charcoal. The assay sensitivities were <25 pg/tube for testosterone and 4 pg/tube for E_2. Anti-E_2 antiserum raised in our laboratory cross-reacted 0.03% with testosterone. The within assay coefficient of variation was 4.9% for testosterone and 6.3% for E_2. The level of T-binding globulin was measured by the method of Nisula et al. (14). Steroids were first removed from the samples by treatment overnight with dextran-charcoal at 2°C, and this was followed by retreatment for 1 h at 37°C. Serum LH and FSH concentrations were measured using double antibody radioimmunoassays (RIAs) using LER-907 as the reference standard.

A hormone pulse was defined as an increase above the preceding nadir value of at least twice the within-assay coefficient of variation (15). Correlation coefficient was calculated by a least-squares linear regression.

Results

The mean ($\pm$SE) gonadal vein T level was 306$\pm$142 ng/ml, ~50 times the T concentration in peripheral blood. Individual values varied from 1 to 1,540 ng/ml. Serial measurements in gonadal

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1. *Abbreviations used in this paper:* E_2, estradiol; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; LH, luteinizing hormone; LRH, luteinizing hormone–releasing hormone; T, testosterone.

Table I. Sperm Counts and Circulating Hormone Concentrations

Subject	Sperm density	FSH	LH	T	E_2
	million/ml	*ng/ml*	*ng/ml*	*ng/dl*	*pg/ml*
1	2.9	238	34	626	16
2	25.9	135	38	347	15
3	1.7	122	17	461	12
4	7.1	265	66	392	17
5	1.5	147	113	925	25
6	4.2	109	77	570	26
Mean±SE	7.2±3.8	169±27	58±14	554±86	18±2
Normal men	>20	<290	<150	300–1,100	<50

Figure 2. The correlation between the amplitude of each T secretory pulse with the concurrent rise in gonadal vein estradiol levels. Individual symbols represent the results from each of the six men.

vein serum revealed that T secretion in each subject was clearly pulsatile (Fig. 1). Sampling at 15-min intervals revealed hourly T pulses (4.0±0.3, range 3–5, pulses per 4 h). Mean pulse amplitude was 176±42 ng/ml, which represented an increase of 6.6±2.3-fold from the prepulse nadir to the peak. For each of the subjects, T pulse amplitude was highly variable.

Testicular vein E_2 concentrations were 1.35±0.83 ng/ml, ~75 times the levels in peripheral blood. Individual values were highly variable, ranging from 0.01 to 6.8 ng/ml. E_2 secretion in each of the six subjects was also strikingly pulsatile (Fig. 1). Mean pulse amplitude was 0.74±0.28 ng/ml, an increase of 4.7±1.2-fold from basal to peak. As for testosterone, E_2 pulse frequency was approximately one pulse per hour (3.5±0.4 pulses per 4 h).

Examination of T and E_2 secretory pulses revealed a striking concordance in both timing and amplitude (Fig. 1). Fig. 2 compares the absolute amplitudes of coincident T and E_2 pulses; they were highly positively correlated ($r = 0.90$, $P < 0.01$). Fig. 3 compares serial measurements of testosterone in gonadal and peripheral blood in a representative subject. The pulsatile mode of T secretion is not evident from the moment-to-moment changes in peripheral blood testosterone. Further, peaks of testosterone in peripheral blood are not uniformly associated with testosterone secretory pulses observed in gonadal vein blood.
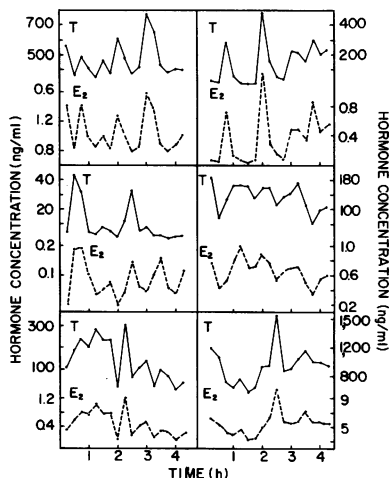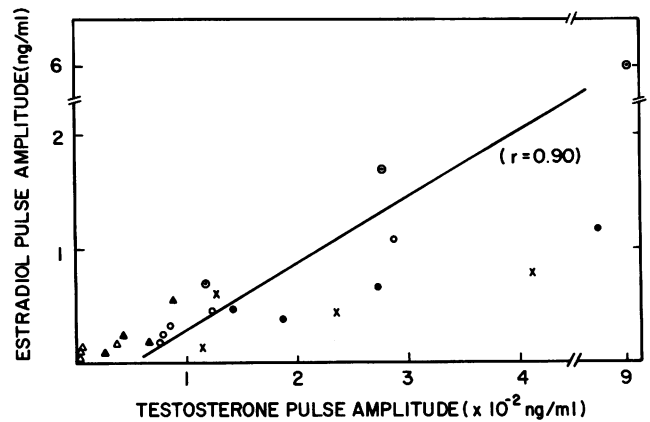
T-binding globulin concentrations were similar in peripheral (11.4±2.6 ng T bound/ml) and gonadal vein serum (9.3±3.2 ng T bound/ml). Therefore, ~97% of gonadal vein testosterone must be either free or albumin bound.

Discussion

The results of this study demonstrate that testosterone secretion in adult men occurs in discrete bursts that appear regular in frequency but vary in amplitude, and suggest a cause-effect relationship between pulsatile gonadotropin secretion and normal Leydig cell function in men. The tight coupling of T to E_2 pulses found in gonadal vein serum indicates that these fluctuations are not artifactual, but instead represent true biological signals. Although mean gonadal vein T levels are 200 times the E_2 concentrations, the use of a highly specific antiserum after complete chromatographic separation excluded radioimmunoassay cross-reactivity as an explanation for these concordant secretory episodes. Mean gonadal vein T and E_2 concentrations in this study are similar to values reported in previous studies in which single blood samples were taken (16–18). The variation in gonadal

Figure 1. Gonadal vein serum concentrations of testosterone and E_2 in six men with varicocele-associated infertility. Blood was sampled at 15-min intervals for 4 h.
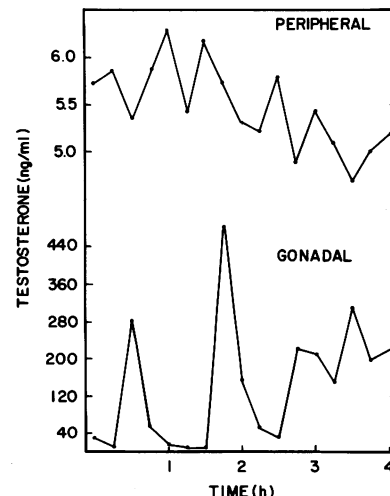

Figure 3. Profiles of T secretion observed by simultaneous peripheral and gonadal vein blood sampling in a representative subject. Blood was sampled at 15-min intervals for 4 h.

vein T and E_2 levels among the men studied may represent, in part, dilution of gonadal vein blood by the circulation of small collateral veins. Additionally, although circulating steroid and gonadotropin levels were within the range of normal in these subjects, testicular function and gonadal vein blood flow are not entirely normal in men with varicocele (19, 20).

Why are the T secretory episodes not clearly evident in the peripheral blood in man, whereas robust T pulses are observed in many experimental animals? To be detected, a hormonal pulse (a) must be of sufficient amplitude to be distinguishable from assay noise, (b) pulse frequency must not be so fast, nor metabolic clearance so prolonged, that the pulse is obscured, and (c) the sampling frequency and duration must be adequate to detect a periodic signal.

There is evidence to suggest that physiologic T secretion by the human testis is characterized by a state of hyporesponsiveness to gonadotropin stimulation compared with adult males of other species. T fluctuations in the peripheral blood of monkeys (6, 21), rams (4, 22), bulls (23), dogs (5), deer (24), rabbits (25), and mice (7) vary in amplitude from 2 to 30 ng/ml, compared with much smaller variations of <1–3 ng/ml in men (8–12). We are unaware of any other species in which moment-to-moment changes in testicular vein steroid levels have been studied. Mean T levels rise ~50% after acute human chorionic gonadotropin (hCG) stimulation in men (26), compared with 3–10-fold rises in experimental animals, including the rhesus monkey (23, 27, 28). Similarly, when LRH is administered to normal men, although pronounced increments in circulating LH levels occur, there are only slight increases in circulating T levels (11, 29). Again, T pulses accompany LH rises after acute LRH administration to lower animals, including monkeys (24, 30–33). The production of testosterone by human and rat Leydig cells after exposure to hCG in vitro has also been compared (34). In that study, hCG produced a 10-fold greater increase in T production by rat than by human Leydig cells. Both a smaller LH receptor concentration per Leydig cell and a lesser supply of pregnenolone to microsomal steroidogenic enzymes in the human testis appeared to play a role in this finding. Some caution in the interpretation of those data is needed, however, in that the testes of young adult rats were compared with those of elderly men undergoing orchiectomy as treatment for prostatic cancer. Previous work from our laboratory suggests that aging in men is characterized by a reduction in pregnenolone supply by mitochondria (35). Nevertheless, these results are consistent with the possibility that T pulses in young adult men are too small in amplitude to be detected in peripheral blood.

The lack of distinct T pulses in peripheral blood in men may also, in part, reflect a frequency-related summation of individual testicular secretory events. Spontaneous T pulses in gonadal vein blood occurred approximately hourly. Although this represents nearly twice most previous estimates of LH pulse frequency in normal men, recent studies in which alpha subunit pulses were used to track gonadotropin secretory episodes (36), or where LH was measured by RIA in peripheral blood samples drawn every 4 min (37), suggest that previous studies may have underestimated spontaneous gonadotropin pulse frequency. In comparison, the more striking circulating T fluctuations in lower species occur with a frequency that does not appear to exceed one pulse per 2 h. Further, slowing the gonadotropin pulse generator in normal men with fluoxymesterone allowed for the detection of individual T pulses in peripheral blood (38). The metabolic clearance of testosterone, when corrected for body surface area,

appears to be similar in humans (560 liters/m^2 per 24 h; reference 39) and in adult male rhesus monkeys (486 liters/m^2 per 24 h; reference 40). Therefore, there is no evidence suggesting a more prolonged half-life of circulating testosterone to explain the lack of detectable T pulses in men.

Both the duration and frequency of blood sampling are important in establishing the detailed picture of pulsatile hormone secretion. We limited our study to 4 h because of our concern for possible catheter complications with a longer study. It will be interesting to reexamine testicular steroid secretion with more frequent blood sampling, and to compare the findings with changes in gonadotropin levels. Neither intensive nor prolonged sampling of peripheral blood allows for the identification of convincing T secretory episodes, nor for their relationship to LH secretion.

The concordance of E_2 with T secretory episodes suggests that the Leydig cell, under LH control, is the source of most of the E_2 secreted by the adult human testis. This finding is consistent with the observation that aromatase activity is greater in whole testis homogenates than in isolated seminiferous tubules in men (41), that hCG produces a rise in gonadal vein E_2 levels (16), and with the results of studies in adult male rodents (42). However, additional factors may influence testicular E_2 secretion, since there is also a strongly positive correlation coefficient relating circulating FSH concentrations to intratesticular E_2 content among infertile men (43).

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