Specific Regulatory Actions of Dihydrotestosterone and Estradiol on the Dynamics of FSH Secretion and Clearance in Humans

RANDALL J. URBAN,* KRISTINE D. DAHL,† VASANTHA PADMANABHAN,‡ INESE Z. BEITINS,§ AND JOHANNES D. VELDHUIS*

From the *Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Virginia, Charlottesville, Virginia; the †Department of Medicine, Veterans Administration Medical Center, University of Washington, Seattle, Washington; and the ‡Department of Pediatrics, Division of Pediatric Endocrinology, University of Michigan, Ann Arbor, Michigan.

ABSTRACT: The authors investigated immunoreactive and bioactive follicle-stimulating hormone (FSH) secretion and clearance in six healthy young men during steady-state infusions of vehicle (basal, B, 28 hours), dihydrotestosterone (DHT, 4.5 days), or estradiol (4.5 days) accompanied by blood sampling at 10-minute intervals for 28 hours. Serum FSH concentrations were assayed by a two-site immunoradiometric assay (IRMA) and two separate in vitro bioassays (rat granulosa and Sertoli cell systems). FSH measurements included: 24-hour mean serum concentrations (IRMA and bioassay), multiple-parameter deconvolution of 24-hour pulsatile FSH time series and FSH release in response to exogenous gonadotropin-releasing hormone (GnRH) boluses (IRMA) to assess secretion and clearance, and circadian serum FSH concentration rhythms by cosinor analysis (IRMA). We found: 1) a significant decrease in 24-hour mean IRMA FSH concentrations during DHT infusion while both in vitro estimates of FSH bioactivity were unchanged; 2) significant decreases in the mass of IRMA FSH secreted per 24 hours during DHT infusion; 3) significant decreases in the IRMA FSH half-life during estradiol infusion without any change in FSH interpulse interval; 4) no steroidal effects on FSH secretory responses to exogenous GnRH; and 5) abolition of basal circadian FSH rhythms during sex-steroid infusions. Based on these findings, we conclude that steady-state sex-steroid hormone infusions selectively alter IRMA FSH secretion and clearance without affecting IRMA FSH pulse frequency or mean concentrations of bioactive FSH.

Key words: Sex steroids, pulsatile hormone, episodic, deconvolution, hormone infusion, IRMA.


The sex steroids, testosterone and estradiol, interact with the hypothalamic-pituitary-gonadal axis to regulate pulsatile secretion of the gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH; Jiz and Hsueh, 1985; Santen, 1975; Urban et al, 1985c; 1988; Veldhuis and Dufau, 1987; Veldhuis et al, 1984; Winters et al, 1984). In young men, continuous infusion of estradiol at its daily production rate decreased immunoreactive (RIA) LH pulse amplitude without affecting LH pulse frequency. However, continuous infusion of the unimpeded (nonaromatizable) androgen, 5-alpha dihydrotestosterone (DHT), decreased LH pulse frequency without altering LH pulse amplitude (Santen, 1975; Veldhuis et al, 1984; Winters et al, 1984). Conversely, blockade of endogenous estrogen receptors with nonsteroidal anti-estrogens augmented the pulsatile release of both bioactive and immunoactive LH in humans (Urban et al, 1988b; Veldhuis and Dufau, 1987). Blockade of endogenous androgen feedback actions using the anti-androgen, flutamide, also increased immunoreactive LH pulse frequency in young men (Balzano et al, 1987; Urban et al, 1988c).

Although far less is known about the regulation of pulsatile FSH release in humans, continuous infusions of estradiol and dihydrotestosterone can decrease mean serum immunoreactive FSH concentrations in young men (Santen, 1975; Urban et al, 1988a). Prolonged administration of testosterone in young men or women also decreases mean serum immunoreactive FSH concentrations (Dahl and Matsutomo, 1989; Shechter et al, 1989; Spinder et al, 1989). Blockade of endogenous androgen feedback actions using anti-androgens does not produce an increase in mean serum immunoreactive FSH concentrations (Urban et al, 1988c),...
Despite a reported increase in FSH pulse frequency in one study (Balzaro et al., 1987; Gooren et al., 1987). None of these studies employed a bioassay of FSH concentrations or a two-site immunoradiometric assay (IRMA) of FSH that should correlate with intact hormone; nor was deconvolution analysis used to estimate the extent to which steroids modify in vivo FSH secretion or clearance. To address these questions, our study investigated both immunoradiometric and bioactive FSH release in young men undergoing equilibrium infusions of specific steroid hormones.

Methods

Patient Characteristics

Six healthy male volunteers (aged 21 to 35 years) participated in the study, which was approved by the Human Investigation Committee of the University of Virginia. Each subject had normal basal serum concentrations of thyroxine, prolactin, LH, FSH, somatomedin C, free and total testosterone, and estradiol. Tests of hepatic and renal function and a complete blood count showed normal results. There was no history of any medical illness, medication or drug use, weight loss, or extreme stress or exertion in any of the participants. Results of physical examinations were normal.

Clinical Protocol

The subjects were admitted to the University of Virginia Clinical Research Center on three separate occasions. The first served as a baseline admission with blood sampling every 10 minutes for a period of 28 hours; 10 μg gonadotropin-releasing hormone (GnRH) and 100 μg GnRH were given intravenously at 24 and 26 hours, respectively. During this admission, 1 L of 5% dextrose in water was infused every 12 hours to simulate infusions of hormones that followed. An infusion pump maintained the rate, and Tygon tubing was used to minimize nonspecific steroid adsorption, which became relevant in the other two admissions (Veldhuis et al., 1984).

Approximately 1 month later, the men were admitted a second time and received a continuous intravenous infusion of 5-alpha-dihydrotestosterone at a dose of 7 mg/day for 4.5 days to produce steady-state DHT levels, as described previously (Santen, 1975; Veldhuis et al., 1984; Winters et al., 1984). Blood was sampled every 10 minutes (see above) during the final 28 hours of the admission.

The third admission, at least 1 month later, also entailed a continuous intravenous infusion of 17-beta-estradiol at 48 μg/day for 4.5 days as previously described (Santen, 1975; Veldhuis et al., 1984; Winters et al., 1984). To assess steady-state blood levels of endogenous sex-steroid hormones, blood was sampled every 12 hours (8:00 AM and 8:00 PM before and during the 4.5-day period of hormone infusion). All blood sampling was performed in the arm contralateral to the infusion.

Hormone Assays

Blood samples were allowed to clot at room temperature, and the sera were stored at −20°C. IRMA FSH concentrations were measured in duplicate using a two-site monoclonal assay (one of which was labeled with iodine-125) in an avidin-coated bead sandwich complex (Nichols Diagnostics, San Juan Capistrano, CA). The assay sensitivity was 0.2 IU/L (second International Reference Preparation, Human Menopausal Gonadotropin [IRP-HMG]) and showed negligible (<0.1%) cross-reactivity with human luteinizing hormone, human chorionic gonadotropin, human thyroid-stimulating hormone, and alpha and FSH beta subunits. The median intra-assay coefficient of variation in the current work was 6.7%. Serum concentrations of estradiol, total testosterone, and free testosterone (<10% cross-reactivity with DHT) were measured in duplicate by solid phase 125I radioimmunoassays (Coat-a-Count, Diagnostics Products Corp, Los Angeles, CA) as previously described (Urban et al., 1988c). Serum pools (20 μl/hour for 24 hours) obtained from each subject for each treatment condition were analyzed for bioactive FSH content using two separate in vitro FSH bioassays. One bioassay employed cultured, diethyl-stilbestrol (DES)-treated, rat granulosa cells with a sensitivity of 0.12 mIU/culture (LER 907) and an intra-assay coefficient of variation of 13% (Jiz and Hsueh, 1985). The other bioassay used cultured rat Sertoli cells with a sensitivity of 10 ng/L (hFSH-3) and an intra-assay coefficient of variation of 12.9% (Padmanabhan et al., 1987). Since the bioactive FSH values from the two in vitro bioassays were calculated using different standards, all bioactive FSH values are reported in terms of the second IRP-HMG standard (Nichols FSH IRMA) after conversion by the appropriate biopotency value.

Evaluating FSH Secretion and Clearance

The 24-hour serum FSH (IRMA) time series and the 4-hour FSH responses to two pulses of exogenous GnRH were analyzed by a multiple-parameter deconvolution model, as previously described in detail (Veldhuis et al., 1987a). In brief, this statistically based algorithm uses a convolution integral to define all serum hormone concentration values as a subject-specific and treatment-specific function of secretion and metabolic clearance (Veldhuis et al., 1987a). The program then finds the best estimate of fit (using multiple iterations) for secretion and clearance parameters.
Circadian FSH Rhythms

Assessment of FSH circadian periodicity was done by cosinor analysis as described previously (Veldhuis et al., 1989). In brief, we used simultaneous nonlinear, least-squares curve fitting of all serum FSH (IRMA) concentrations and their dose-dependent intrasample variances to compute the group mesor (mean), amplitude, and acrophase (time of maximal concentrations) with conjoint asymmetric statistical confidence limits for the 24-hour rhythm.

Statistical Analysis

For normally distributed measures (24-hour means), within-subject differences were sought by paired two-tailed Student’s t testing. Because of departures from normality, individual deconvolution parameters were compared after logarithmic transformation (Urban et al., 1988a). The results are expressed as the mean ± SEM. Statistical significance
was assigned to $P < 0.05$. One-way analysis of variance using Dunnett’s multiple comparison of treatments was used to assess differences in steroid hormone concentrations during infusions (compared to basal).

**Results**

**Mean Serum FSH Concentrations**

*FSH IRMA*—As shown in Figure 1 (top panel), there was a significant decrease in the 24-hour mean serum FSH concentration during DHT infusion compared to basal concentration. This decrease was not significant during estradiol infusion. The statistical power for the latter comparison was 90% for a 30% decrease in means.

*Bioactive FSH*—Measurement of bioactive FSH in two separate *in vitro* bioassays (granulosa and Sertoli cell systems; see Methods) showed no suppression of mean FSH concentrations during either hormone infusion (Fig 1, middle panel). Since the FSH IRMA levels fell, there was a significant increase in the FSH bio/IRMA ratio during DHT infusion in both FSH bioassays (Fig 1, lower panel). Note that differences in absolute bioactive FSH concentrations in the two *in vitro* assays is due to the different standards used for calculation (see Methods). All values have been converted in terms of second IRP-HMG for the calculation of bio/IRMA ratios. FSH IRMA measurements were done on the serum pools and gave results similar to the 24-hour mean from the samples taken every 10 minutes (Fig 1, top panel).

**Pulsatile FSH (IRMA) Time Series**

Figure 2 illustrates the 24-hour pulsatile IRMA FSH concentration time courses for one man, basally and during DHT and estradiol infusions. Using multiple parameter deconvolution, the FSH time series for all six men were assessed to quantitate individual (subject-specific and treatment-specific) secretion and clearance parameters (see Methods). Figure 3 shows the deconvolution-predicted fit of the FSH pulse profile (left panel) and the significant FSH secretory bursts (right panel) for one of the six men. Mean parameter results are summarized in Figure 4. There were significant decreases in FSH secretory mass per 24 hours during DHT infusion and decreases in FSH half-life during estradiol infusion (Fig 4, center panels). There was also a significant decrease in FSH secretory burst amplitude during DHT infusion (data not shown). Figure 5 shows the behavior of individual subjects’ values for FSH secretion and clearance. Note the wide range of endogenous IRMA FSH half-lives found in these six subjects (lower panel).

---

**FIG. 3.** Deconvolution-predicted 24-hour fits of serum FSH (IRMA) pulsatile profiles (left panel) and deconvolution-resolved FSH secretory bursts (right panel) in one representative man studied basally and after DHT and E$_2$ infusions (see Methods).

---

<table>
<thead>
<tr>
<th>Reconvolution Fits</th>
<th>Secretion Bursts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>Estradiol</td>
</tr>
<tr>
<td>DHT</td>
<td>DHT</td>
</tr>
<tr>
<td>Basal</td>
<td>Basal</td>
</tr>
</tbody>
</table>

**TIME (MIN)**

---

Journal of Andrology - January/February 1991
IRMA FSH release in response to the two bolus doses of GnRH (10 and 100 μg) was assessed using multiple-parameter deconvolution. Figure 6 summarizes the results from the three parameters studied. Although a GnRH-stimulated FSH response was seen under all study paradigms, there were no significant changes from basal concentration during infusion of either hormone, but the IRMA FSH responses to exogenous GnRH were variable.

Circadian FSH Rhythmicity

Cosinor analysis of the 24-hour IRMA FSH baselines from the three admissions demonstrated a significant FSH circadian rhythm basally with a mean maximal amplitude of 0.75 IU/L, mean acrophase of clock time 0655, and group mesor of 0.98 IU/L. However, sex-steroid infusion abolished this circadian rhythm despite a persistent circadian rhythm in total testosterone concentration, as illustrated in Figure 7.

As shown in Figure 7, at 24, 48, and 72 hours of estradiol infusion, estradiol levels were significantly elevated (P < 0.05) over those at the start of the infusion (time 0, 8:00 AM). There were no significant decreases in the serum concentrations of total testosterone or free testosterone during either infusion.

**Exogenous GnRH Stimuli**

IRMA FSH release in response to the two bolus doses of GnRH (10 and 100 μg) was assessed using multiple-parameter deconvolution. Figure 6 summarizes the results from the three parameters studied. Although a GnRH-stimulated FSH response was seen under all study paradigms, there were no significant changes from basal concentration during infusion of either hormone, but the IRMA FSH responses to exogenous GnRH were variable.

Circadian FSH Rhythmicity

Cosinor analysis of the 24-hour IRMA FSH baselines from the three admissions demonstrated a significant FSH circadian rhythm basally with a mean maximal amplitude of 0.75 IU/L, mean acrophase of clock time 0655, and group mesor of 0.98 IU/L. However, sex-steroid infusion abolished this circadian rhythm despite a persistent circadian rhythm in total testosterone concentration, as illustrated in Figure 7.

As shown in Figure 7, at 24, 48, and 72 hours of estradiol infusion, estradiol levels were significantly elevated (P < 0.05) over those at the start of the infusion (time 0, 8:00 AM). There were no significant decreases in the serum concentrations of total testosterone or free testosterone during either infusion.
Discussion

We used steady-state infusions of the sex steroids, estradiol and DHT, to examine specific steroidal mechanisms regulating both IRMA and bioactive FSH release in men. While the current studies used a putative, pharmacologic sex- steroid “clamp” consisting of the continuous infusion of a supraphysiologic dose of DHT, understanding the steady-state responses of the hypothalamic-pituitary-gonadal axis under these experimentally defined conditions offers important mechanistic insights into the regulatory behavior of this neuroendocrine unit.

The significant suppression of 24-hour mean FSH IRMA concentrations during DHT infusion without decreases in mean serum bioactive FSH values, as assessed in two separate in vitro bioassays, is consistent with preliminary findings in men receiving high-dose parenteral testosterone injections (Dahl and Matsumoto, 1989). The results of DHT infusion presumably reflect actions on the androgen receptor; however, the steroidal specificity of testosterone’s effects in vivo is not so well defined, since testosterone can act on either the androgen receptor or the estrogen receptor (after aromatization of testosterone). Interestingly, in relation to our findings of discordant suppression of bioactive and immunoradiometric FSH concentrations by steroids, a dichotomy in immunoactive and bioactive FSH release also has been found after administration of a potent GnRH antagonist. In the latter setting, however, serum bioactive FSH concentrations are preferentially suppressed over immunoactive values (Dahl et al, 1986). GnRH antagonists in both men and women also exert differential effects on the two gonadotropins by preferentially suppressing immunoactive LH, rather than FSH, values (Hall et al, 1988; Pavlou et al, 1986). A recent study has indicated that GnRH antagonists may result in the release of one or more FSH antagonists that may have immunologic activity but occupy the FSH receptor and inhibit FSH actions (Dahl et al, 1988).

Twenty-four hour mean serum FSH concentrations measured in the two-site IRMA and in two separate bioassays did not decrease during estradiol infusion. Previous studies using less intensive sampling but, in some cases, studying more subjects have reported a significant decrease in serum immunoreactive (RIA) FSH values during similar estradiol infusions (Santen, 1975; Winters et al, 1984). These studies differed in several respects, including the use of RIA determinations rather than IRMA and in vitro FSH bioassays (current work). However, the FSH IRMA is highly correlated ($r = +0.94, P = 0.0001$) with the FSH RIA when tested in a range of serum samples from different clinical conditions (Urban, Veldhuis, unpublished data). The current study design yielded a statistical power of 90% for detecting a 30% decrease in mean serum FSH concentrations, and permitted us to find a significant decrease in immunoactive FSH half-life during estradiol infusion. It is possible, however, that studying additional subjects would disclose smaller differences in FSH concentrations not presently discernible.

Deconvolution analysis revealed no changes in FSH secretory pulse frequency (or inter-burst intervals) during steroid-hormone infusions. Interestingly, at least three previous studies have indicated a decrease in GnRH pulse generator activity as assessed indirectly from LH pulse frequency estimates during otherwise similar or identical DHT infusions (Santen, 1975; Veldhuis et al, 1984; Winters et al, 1984). The inference of an altered endogenous GnRH pulse frequency that does not influence FSH pulse fre-

![FIG. 6. Immunoradiometric FSH secretion and clearance parameters estimated by deconvolution of FSH release stimulated by IV bolus injections of exogenous GnRH. Data are presented as described in the legend of Fig 1.](image-url)
frequency is in keeping with some previous findings showing
dissociations between GnRH actions on LH and FSH re-
lease. For example, a pulsatile mode of GnRH stimulation
of the pituitary gland is a more strikingly requisite for LH,
rather than FSH, release. Thus, administration of GnRH in
young men by either continuous infusion or intermittent
pulsatile injection will produce increases in FSH concentra-
tions that are of similar magnitude, whereas pulsatile, but
not continuous, administration of GnRH effectively in-
creases LH values (Fauser et al, 1983). Such observations
on the critical nature of the pulsatile GnRH signal for LH
(rather than FSH release) apply even when the dose of
GnRH administered is varied (Fauser et al, 1984). More-
over, administration of GnRH with increasing frequency in
normal or hypogonadotropic men did not significantly alter
serum FSH concentrations while increasing LH concentra-
tions (Sauder et al, 1988; Spratt et al, 1987). The current
observations that a dose of DHT that suppresses LH pulse
frequency does not decrease FSH pulse frequency are con-
sistent with the view that the dependencies of LH and FSH
release on the endogenous GnRH pulse signal are not equal.

We have given bolus injections of human FSH (Metro-
din) to hypogonadotropic men and measured FSH clearance
by this same IRMA (Beitins et al, 1990). The resultant
one-component clearance rate, or its commensurate half-
life, was 287 ± 13 minutes. This is equivalent to the mean
half-life of 221 ± 36 minutes calculated by deconvolution
from the 24-hour basal time series in this study and is in-
distinguishable from several other estimates in the literature
(Kjeld et al, 1976; Yen et al, 1970). The range of endoge-
nous FSH half-lives calculated by deconvolution analysis
(Fig 5) in normal men, if correct, indicates that intersubject
differences in FSH metabolic clearance can be substantial.
Such differences could significantly influence FSH pulse
detection by computer algorithms that assume a uniform
half-life for hormones among different individuals (Urban
et al, 1989; Veldhuis and Johnson, 1986). Further longitu-
dinal studies are needed among individuals to determine the
extent to which FSH half-lives are stable within the same
individual and variable among different individuals.

We were able to detect a circadian rhythm in serum
IRMA FSH concentrations as reported previously for FSH
measurements by RIA (Veldhuis et al, 1987b). This rhythm
was abolished during both hormonal infusions despite a
continued diurnal variation in total testosterone values (Fig
7). The basis for the loss in nychthemeral variations in
serum IRMA FSH concentrations is not known. In part, this
decrease may reflect suppression of serum FSH concentra-
tions that could make a residual circadian rhythm difficult to
detect by cosinor analysis.

Immunoradiometric FSH secretion and clearance param-
eters were also assessed after GnRH bolus injections (10 μg
and 100 μg) to test pituitary responsiveness. Such measures
of FSH release were unchanged during hormone infusions

FIG. 7. Serum sex-steroid hormone concentrations (estradiol, total testosterone, and free testosterone) during DHT
and E2 infusions. Data are presented as mean ± SEM. Multiply estradiol concentra-
tions by 3.671 to convert pg/ml to pmol/L, and free and total testosterone concentrations by 3.467 to convert ng/ml
to nmol/L.
References


Dahl KD, Matsumoto AM. High dose testosterone (T) administration increases bioactivity/immunoreactive (B/I) ratio and alters molecular heterogeneity of circulating FSH in normal men. 71st Annual Meeting of the Endocrine Society, 1989;166.


Snyder P, Reitano JF, Utiger RD. Serum LH and FSH response to syn-
thetic gonadotropin-releasing hormone in normal men. J Clin Endocri

nol Metab. 1975;41:938-945.


Veldhuis JD, Carlson ML, Johnson ML. The pituitary gland secretes in bursts: appraising the nature of the glandular secretory impulses by simultaneous multiple-parameter deconvolution of plasma hormone concentrations. Proc Natl Acad Sci USA. 1987a;84:7686-7690.


11th North American Testis Workshop
1991 Montreal, Canada—April 24-27

The 11th North American Testis Workshop entitled “The Male Germ Cell: Spermatogonium to Fertilization” will be held at the Holiday Inn Crowne Plaza, Montreal, Quebec, Canada, April 24-27, 1991. The Workshop will consist of an inaugural session on the evening of April 24th, followed by two and one half days of oral presentations by invited lecturers and poster presentations. The major topics to be covered include: genetic and hormonal control of spermatogenesis, specific gene expression during spermatogenesis, structure and function of specific organelles, and epididymal and post-epididymal changes in spermatooza.

For further information please contact:
Dr. Bernard Robaire, Department of Pharmacology, McGill University, 3655 Drummond Street, Montreal, Quebec H3G 1Y6 (Tel: 514/398-3630; Fax: 514/398-6690).