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

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ABSTRACT: The authors investigated immunoactive 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.

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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, 1988 a,b,c; 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 immunoactive (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 immunoactive 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 immunoactive FSH concentrations in young men (Santen, 1975; Urban et al, 1988a). Prolonged administration of testosterone in young men or women also decreases mean serum immunoactive FSH concentrations (Dahl and Matsumoto, 1989; Sheckter et al, 1989; Spinder et al, 1989). Blockade of endogenous androgen feedback actions using anti-androgens does not produce an increase in mean serum immunoactive FSH concentrations (Urban et al, 1988c),

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despite a reported increase in FSH pulse frequency in one study (Balzano 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 basal 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 I^{125} 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, diethylstilbestrol (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.

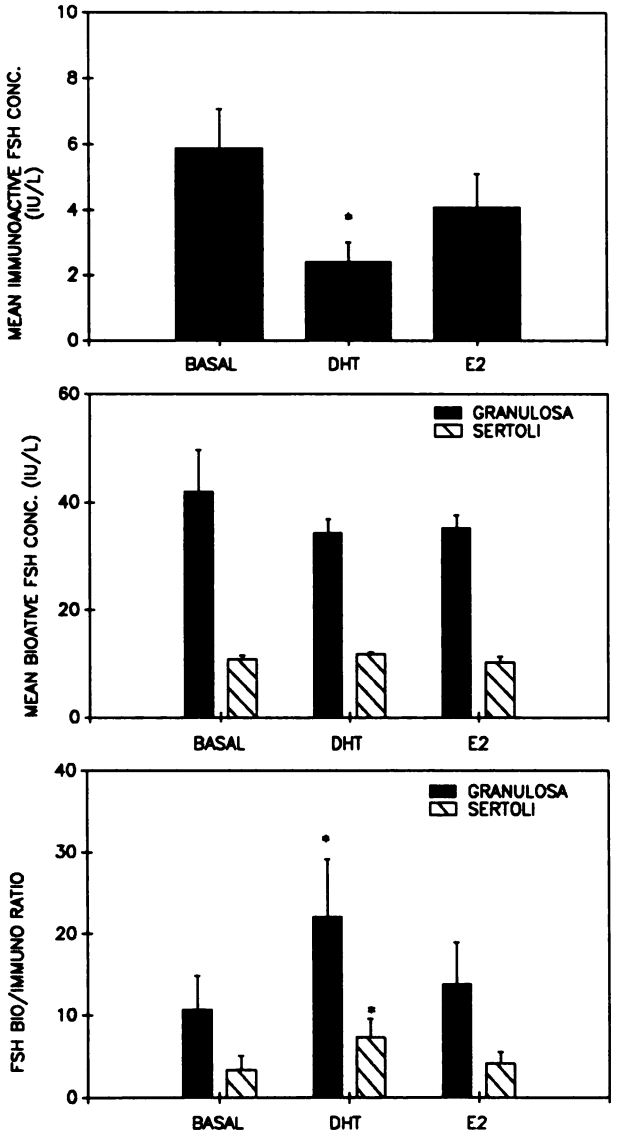

FIG. 1. Mean serum FSH concentrations in 24-hour time series of six men studied during hormonal infusions. Immunoradiometric (IRMA) values are shown in top panel, bioactive values from two separate *in vitro* FSH bioassays in the middle panel, and the FSH bio/IRMA ratio in the lower panel. An asterisk denotes a significant change from basal concentrations ($P \leq 0.05$). All values are presented as mean ± SEM. The two *in vitro* bioassays were conducted with different internal standards, but both values were converted to the second IRP–HMG standard for calculation of bio/immuno FSH ratios.

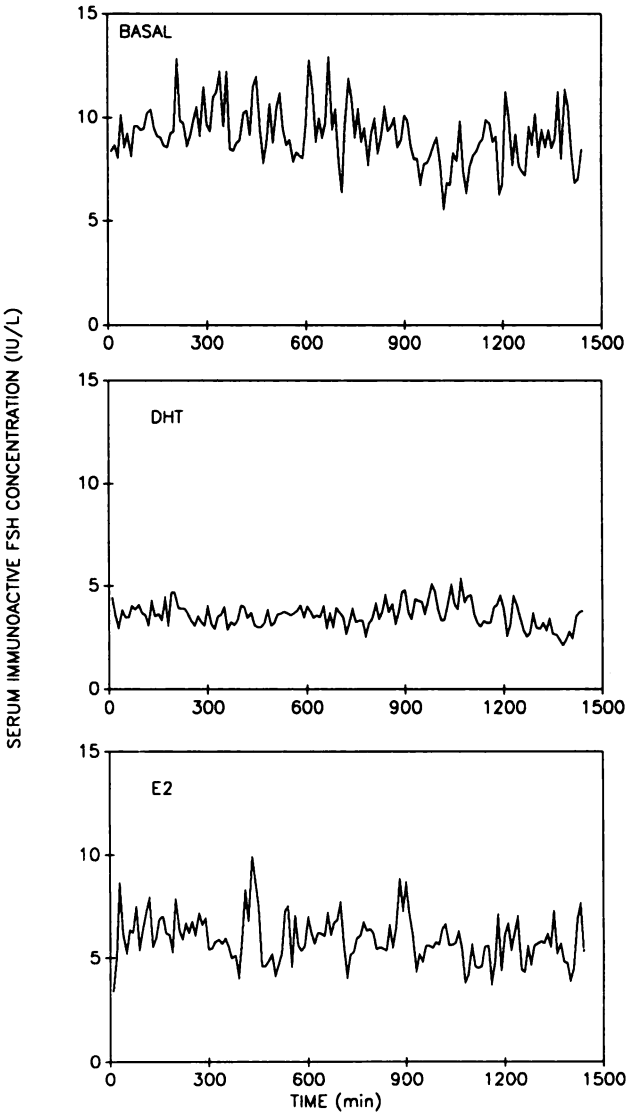

FIG. 2. Illustrative profiles of pulsatile serum immunoradiometric (IRMA) FSH concentrations comprising 24-hour time series from one of the six men studied basally and after DHT or E_2 infusion. The results are presented as sample mean ± SD

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 $P \leq 0.05$. 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).

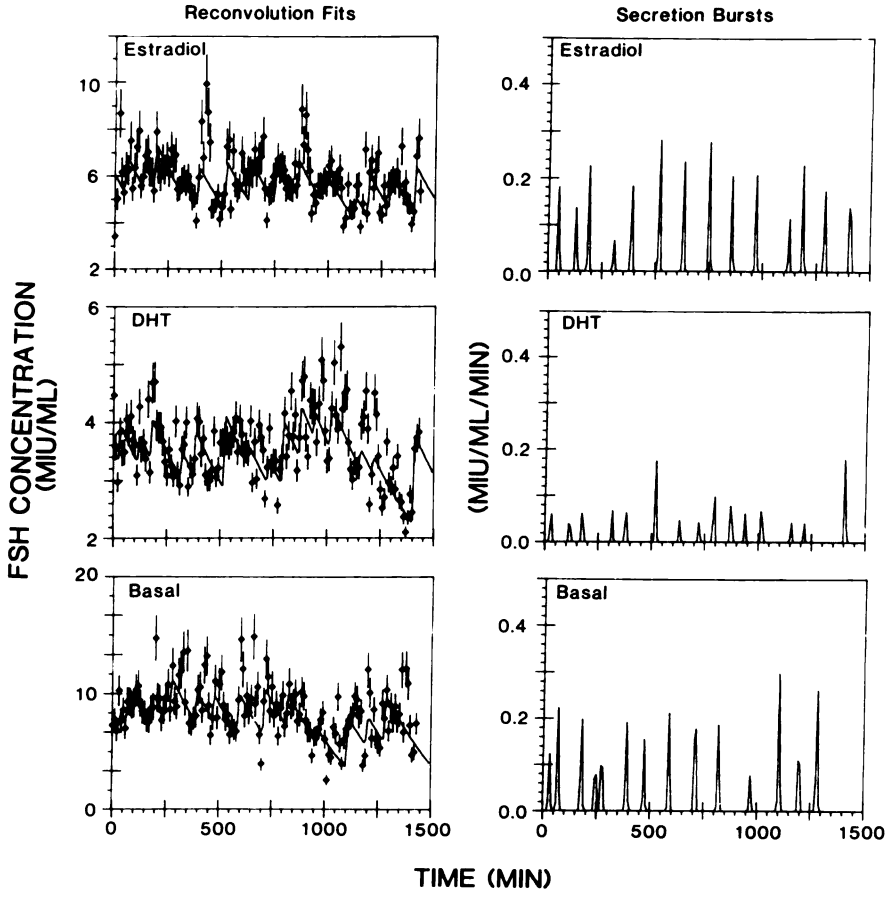

FIG. 3. Deconvolution-predicted 24-hour fits of serum FSH (IRMA) pulsatile profiles (left panel) and deconvolution-resolved FSH secretory bursts (right panel) in one representative man studied basally and after DHT and E_2 infusions (see Methods).

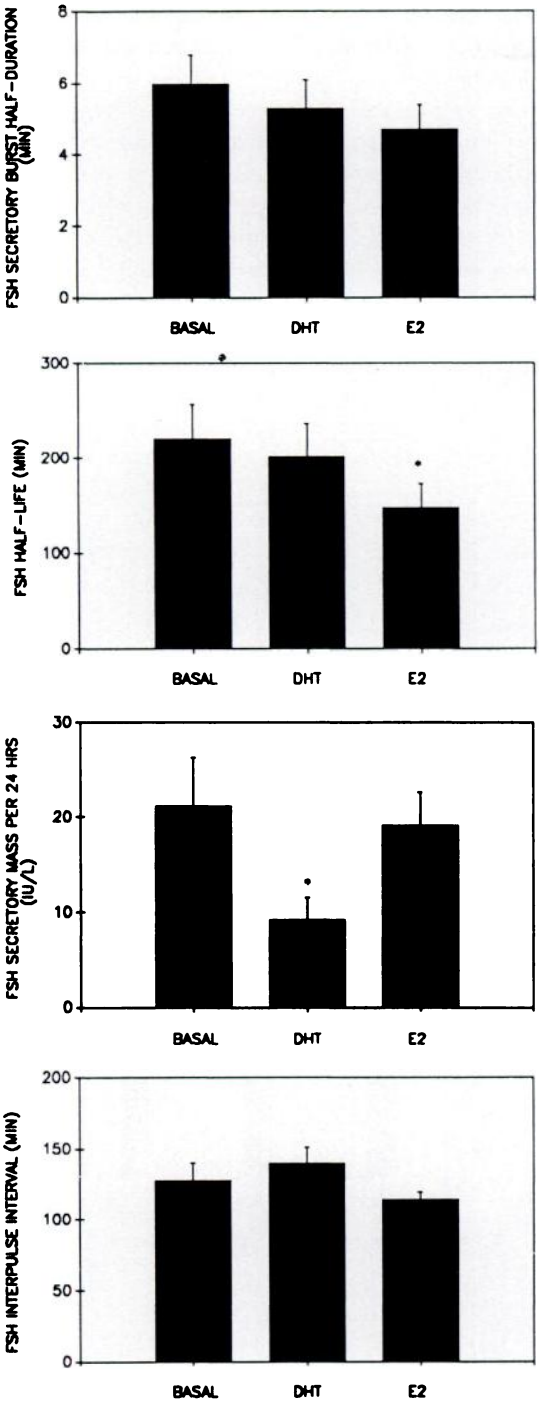

FIG. 4. Immunoradiometric FSH secretion and clearance parameters estimated by deconvolution analysis applied to each of the six individuals before and during steroid (E_2 or DHt) infusions. Data are presented as described in the legend of Fig 1.

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.

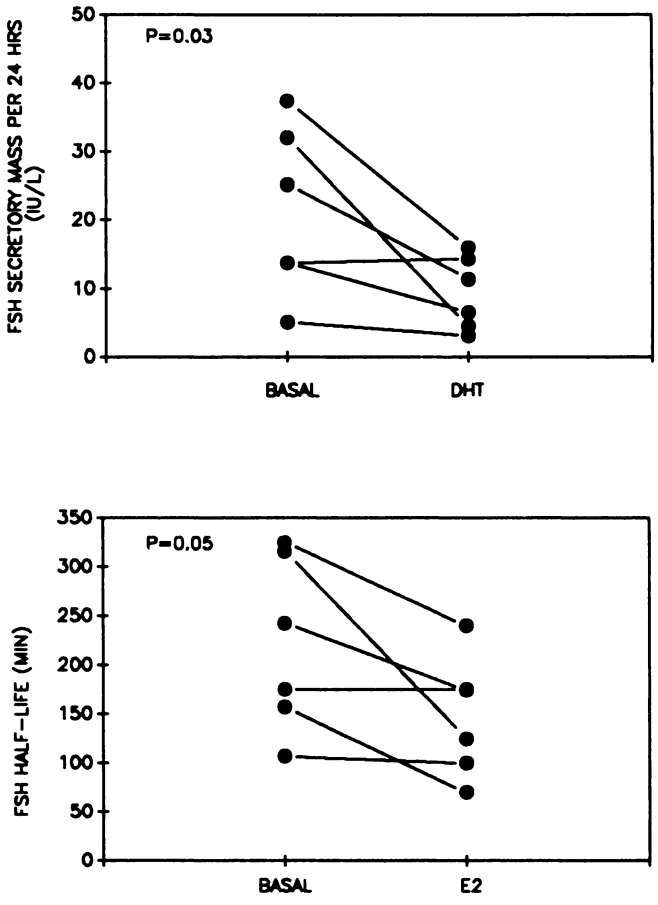

FIG. 5. Individual FSH (IRMA) secretion and clearance parameters altered by E_2 or DHT infusion in six normal men.

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 immunoactive (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 ($r = +0.94$, $P \leq 0.0001$) 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-

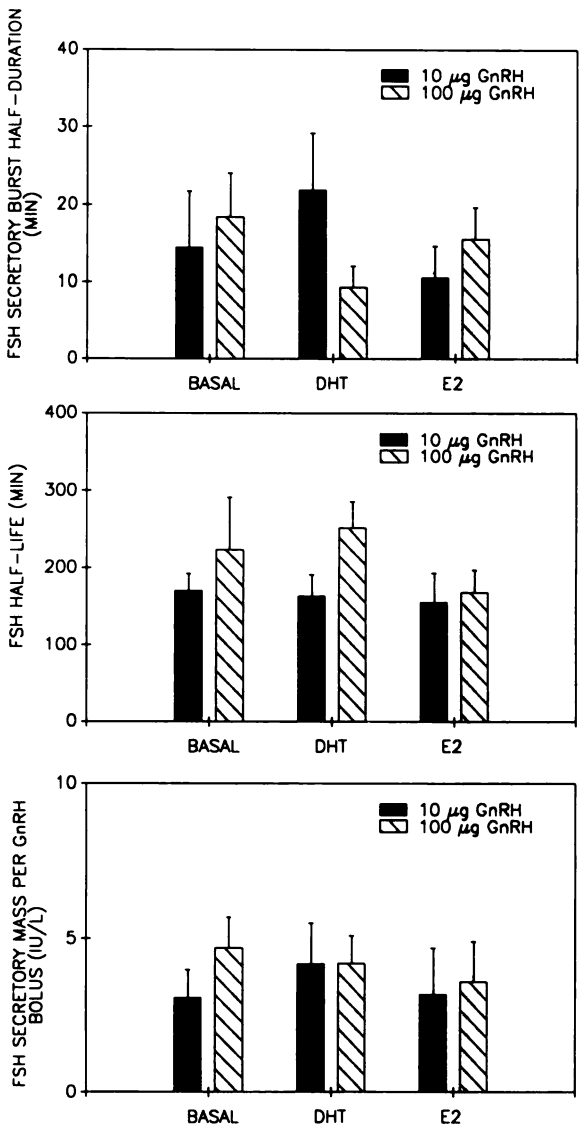

FIG. 6. Immunoradiometric FSH secretion and clearance parameters estimated by deconvolution of FSH release stimulated by IV bolus injections of exdogenous GnRH. Data are presented as described in the legend of Fig 1.

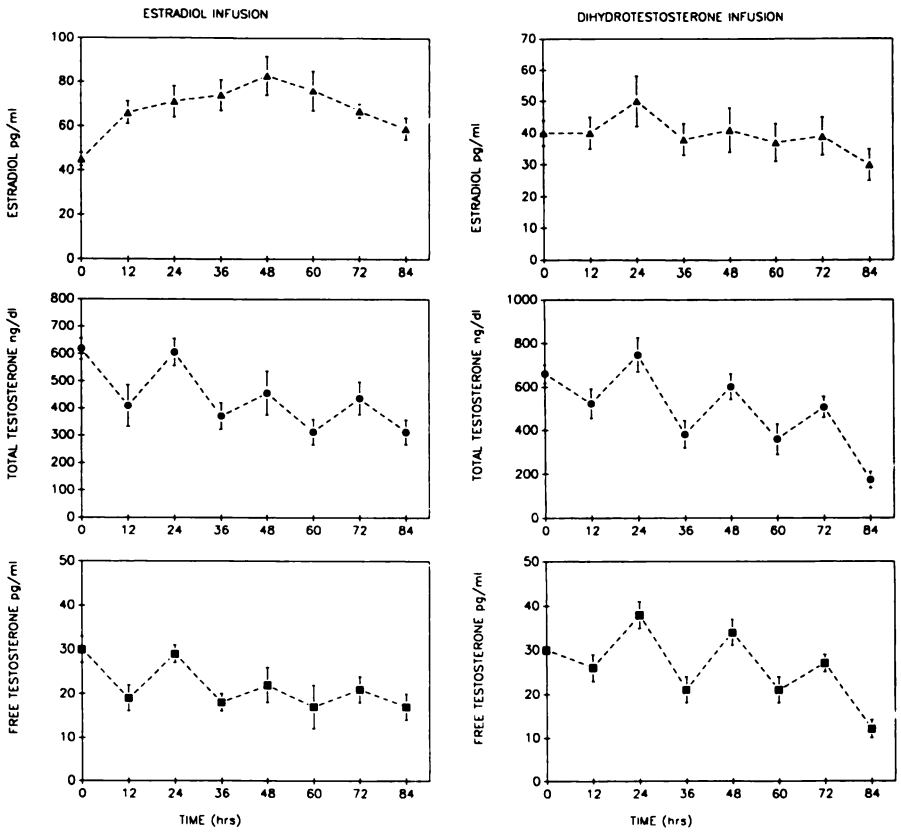

FIG. 7. Serum sex-steroid hormone concentrations (estradiol, total testosterone, and free testosterone) during DHT and E_2 infusions. Data are presented as mean ± SEM. Multiply estradiol concentrations by 3.671 to convert pg/ml to pmol/L, and free and total testosterone concentrations by 3.467 to convert ng/ml to nmol/L.

quency is in keeping with some previous findings showing dissociations between GnRH actions on LH and FSH release. 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 concentrations that are of similar magnitude, whereas pulsatile, but not continuous, administration of GnRH effectively increases 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). Moreover, administration of GnRH with increasing frequency in normal or hypogonadotropic men did not significantly alter serum FSH concentrations while increasing LH concentrations (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 consistent 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 (Metrodin) 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 indistinguishable from several other estimates in the literature (Kjeld et al, 1976; Yen et al, 1970). The range of endogenous 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 longitudinal 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 nyctohemeral variations in serum IRMA FSH concentrations is not known. In part, this decrease may reflect suppression of serum FSH concentrations that could make a residual circadian rhythm difficult to detect by cosinor analysis.

Immunoradiometric FSH secretion and clearance parameters 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 6). In addition, the IRMA FSH responses to exogenous GnRH were small and variable. Although previous studies have shown a serum immunoactive (RIA) FSH concentration dose–response to increasing amounts of GnRH (Besser et al, 1972; Synder et al, 1975), we were unable to detect a significant difference in immunoradiometric (IRMA) FSH release in response to 10 and 100 μg GnRH doses. A previous report found an increase in GnRH-stimulated RIA FSH release after shorter-term (48-hour) treatment with a higher dose (50 mg) of DHT (Ando et al, 1978). However, the latter results were not obtained during steady-state conditions, and FSH estimates were not carried out using an IRMA. The lack of androgenic regulation of GnRH-promoted FSH release is also suggested by the lack of differences in immunoactive FSH release in response to GnRH in young and older men who had significantly different total serum testosterone values (Winters and Troen, 1982). Although estradiol suppresses the GnRH-stimulated RIA FSH response in prepubertal girls and GnRH-deficient females (Marshall et al, 1983), we were unable to find any suppression of IRMA FSH release during estradiol infusion in normal men. However, hypogonadotropic men treated with physiologic GnRH doses showed diminished RIA LH and RIA FSH release in response to GnRH injections after administration of testosterone, indicating that testosterone or or its metabolites (estradiol or DHT) may have a direct effect on the pituitary gland (Sheckter et al, 1989). The current studies do not permit us to conclude categorically that specific steroidal infusions do not subtly affect the pituitary gland's GnRH-stimulated release of IRMA FSH, but no effects could be defined for the two GnRH doses tested. We did not evaluate GnRH-stimulated bioactive FSH release.

The steroid-associated changes in FSH secretory dynamics (ie, differential suppression of immunoactive and bioactive FSH concentrations and decreases in endogenous, calculated FSH secretory mass and half-life) indicate that the regulation of FSH secretion is complex and not necessarily frequency-modulated. Further studies using isoelectric or chromatofocusing to examine the dispersion of FSH isoforms under various sex-steroidal conditions may clarify the biochemical basis for regulated FSH secretion and clearance.

We conclude that sex-steroid regulation of IRMA FSH release involves changes in secretion, clearance, and circadian rhythmicity. Such regulation is distinct from the published effects of sex-steroid hormones on LH dynamics.

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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 spermatozoa.

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