Dynamic testosterone responses to near-physiological LH pulses are determined by the time pattern of prior intravenous LH infusion

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Veldhuis JD, Liu PY, Takahashi PY, Keenan DM. Dynamic testosterone responses to near-physiological LH pulses are determined by the time pattern of prior intravenous LH infusion. Am J Physiol Endocrinol Metab 303: E720–E728, 2012. First published July 17, 2012; doi:10.1152/ajpendo.00200.2012.—The long-lived glycoprotein hormone, human chorionic gonadotropin (hCG), downregulates testosterone (T) biosynthesis in vitro and in vivo in animals and humans. The degree to which short-lived pulses of pituitary luteinizing hormone (LH) do so, particularly at physiological concentrations, is not known. We test the hypothesis that continuous LH infusion compared with bolus injections of LH every 1 h or every 2 h overnight downregulates T secretion responses to a subsequent fixed template of three consecutive intravenous pulses of a physiological amount of recombinant human (rh) LH (triple stimulus). Nineteen healthy men ages 18–49 yr each underwent four separate randomly ordered overnight gonadotropin-releasing hormone-receptor antagonist treatments with superimposed intravenous infusions of saline or rhLH (1-h pulses, 2-h pulses, or continuously). Each 12-h infusion protocol was followed by the triple rhLH-pulse stimulus the next morning. During the triple stimulus, basal (nonpulsatile) as well as total (basal plus pulsatile) T secretion was higher after overnight 2- and 1-h rhLH pulses than after continuous rhLH or saline delivery. Approximate entropy, a probabilistic measure of feedforward-induced irregularity of T concentration time series, was higher after 1-h rhLH pulses than after continuous rhLH. Analytical estimation of pulsatile rhLH-T dose-response measures revealed higher T secretory sensitivity and greater rhLH potency (lower EC50) after exposure to 1-h than 2-h rhLH pulses. Collectively, these data indicate that in vivo dynamics of LH-stimulated T secretion under standardized conditions in men depend on the prior time mode of LH delivery in the bloodstream.

A consistent property of receptor-mediated processes is stimulus-response facilitation (upregulation or sensitization) or stimulus-response inhibition (downregulation or desensitization) (4). For example, short-term glucose exposure can potentiate pancreatic-islet insulin secretion (3, 28). Hypothalamic-pituitary hormones elicit receptor-dependent desensitization, as in the case of gonadotropin-releasing hormone (GnRH), thyrotropin (TSH), arginine vasopressin (AVP), corticotropin-releasing hormone, and melanocortin-1 receptors among others (5, 25, 48, 56, 57). Specific agonists also downregulate adrenergic and human chorionic gonadotropin (hCG)/luteinizing hormone (LH) receptors both in vitro and in vivo (15, 17, 52). Indeed, long-lived hCG exposure attenuates testis testosterone (T) biosynthesis at several mechanistic levels in healthy men (14, 15). Downregulation is alleviated by decreasing the dose and/or increasing the frequency of hCG injections (53). Conversely, a gene mutation that disables hCG receptor degradation results in constitutively elevated T biosynthesis and iso- sexual male precocious puberty (19). Such data suggest the clinical relevance of hCG/LH receptor downregulation in pathophysiology.

To date, most studies of hCG/LH receptor downregulation have been performed in vitro and in nonprimate species using hCG rather than LH (6, 18, 26, 49). This point is important, given the much longer in vivo half-life of hCG (~24 h) than LH (~1 h) and the much lower dissociation rate of hCG than LH from its receptor, which may heighten downregulation (29, 33, 58). Moreover, a recent clinical study comparing T responses with single injections of pharmacological doses of recombinant human (rh) LH and recombinant human CG demonstrated markedly different time delays to peak T concentrations, viz., 4 vs. 24 h, respectively (7). An even more rapid T response to an endogenous pulse of LH has been inferred by sampling LH and T or estradiol in human spermatic vein blood (16, 66). Because pulsatile LH rather than continuous hCG constitutes the dominant physiological lutotropic stimulus in healthy men (57), the question arises whether recurrent pulses of LH mediate Leydig cell T upregulation or downregulation. In principle, this basic issue could be studied by comparing T secretory responses with intravenous infusions of rhLH in pulsatile or as a continuous infusion of the same total amount.

An experimental paradigm for maintaining controlled LH pulses in healthy men was introduced recently (37, 64). The concept requires administering a potent selective GnRH-receptor antagonist subcutaneously overnight followed by intravenous injection of near-physiological doses of rhLH in frequent pulses, infrequent pulses, or continuously, as described recently. The paradigm is extended here by adding a 6-h standardized triple intravenous LH stimulus (3 consecutive rhLH pulses of fixed near-physiological dose) immediately thereafter to appraise testis downregulation. The present investigation tests the basic hypothesis that the time mode of LH delivery into the bloodstream determines testis responses to this glycoprotein hormone measured as T concentrations, T secretion, and T approximate entropy.

METHODS

Human subjects’ protocol. Nineteen healthy young/middle-aged (18–49 yr) men with body mass indexes of 18–31 kg/m2 were each studied four times prospectively in randomly assigned order double-
blind. This study was approved by the Institutional Review Board, and subjects provided written voluntary informed consent. Sessions were ≥10 days apart. The present analysis presents LH and T responses monitored over the last 6 h of a total 18-h study window. During the

Baseline and 6-h LH clamp data

<table>
<thead>
<tr>
<th>Measure</th>
<th>Baseline</th>
<th>Clamp</th>
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<tbody>
<tr>
<td>Total T, ng/dl</td>
<td>363 ± 17</td>
<td>351 ± 14</td>
</tr>
<tr>
<td>Free T</td>
<td>12 ± 0.92</td>
<td>11 ± 0.77</td>
</tr>
<tr>
<td>Bioavailable T</td>
<td>130 ± 11</td>
<td>124 ± 9.2</td>
</tr>
<tr>
<td>Mean LH, IU/l</td>
<td>4.7 ± 1.0</td>
<td>5.3 ± 0.8</td>
</tr>
<tr>
<td>Peak LH, IU/l</td>
<td>7.8 ± 1.3</td>
<td>8.0 ± 1.7</td>
</tr>
</tbody>
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Range

| Age, yr                  | 18–49    |
| SHBG, nmol/l             | 8.6–36   |
| Albumin, g/dl            | 3.5–4.2  |
| BMI, kg/m²               | 18–31    |
| Estradiol, pg/ml         | 8.1–28   |
| FSH, IU/l                | 1.3–8.0  |

Data are means ± SE; n = 19 experiments, except as noted (range, bottom). T, testosterone; LH, luteinizing hormone; SHBG, sex hormone-binding globulin; BMI, body mass index; FSH, follicle-stimulating hormone.

6 h, consecutive (standardized) rhLH pulses of 37.5 IU each were given by 6-min bolus intravenous injections every 2 h for a total of three pulses beginning immediately after each 12-h overnight intravenous saline/rhLH infusion (Fig. 1A). Blood was sampled every 10 min for 6 h concurrently during the interval 1100–1700. The question posed experimentally was: How do four separate categories of overnight exposure to saline/rhLH [saline alone, continuous rhLH infusion (112.5 U/12 h), six consecutive 6-min rhLH pulses (18.75 U every 2 h), and 12 consecutive rhLH pulses (1 pulse of 9.4 U every 2 h)] determine dynamic T responses to a subsequent regimen of three fixed rhLH pulses given as one pulse every 2 h? The template of three consecutive fixed-dose rhLH pulses is referred to here as a 6-h standardized triple rhLH-pulse stimulus. Each of the 19 subjects underwent this triple clamp four times, once after each of the four overnight saline/rhLH infusions. All 19 volunteers completed all four study sessions without ill effects. Breakfast and lunch were provided. Total blood loss was 380 ml across all four phases.

Experimental agents. rhLH was purchased from Emanuel Merck Darmstadt (Rockland, MA) under a U.S. Food and Drug Administration (FDA) investigator-initiated new drug (IND) file. Peptide was dissolved in 0.9% sodium chloride at a concentration of 3.75 IU/ml Serono (equaling 1.5 IU/ml by the Second International Reference Preparation) just before infusion. The total infusion volume was 30 ml overnight and 10 ml/bolus for each of the three standardized rhLH pulses thereafter. Pilot data were used to estimate the physiological LH pulse dose (37, 64).

Ganirelix was purchased from Organon (Oss, Netherlands) under an FDA-reviewed protocol-specific IND. A dose of 1.0 mg was injected subcutaneously at 1700 the evening of each study session.

Assays. LH was quantified in duplicate in two-site immunoenzymatic assay performed on the DXI 800 automated immunoassay system (Beckman Instruments, Chaska, MN). Intra-assay coefficients
ApEn. Approximate entropy, ApEn (1, 20%), was used as a scale- and model-independent regularity statistic to quantify the orderliness (regularity) of hormone release (43, 44). Higher ApEn denotes greater disorderliness (irregularity) of the secretion process. Cross-ApEn is the matching two-variable synchrony measure (46). Mathematical models and clinical experiments establish that greater irregularity signifies increased feedforward drive and decreased feedback control with high sensitivity and specificity (both >90%) (59, 63).

Deconvolution analysis. T concentration time series were subjected to deconvolution analysis exactly as described using a recently validated Matlab-automated algorithm (36). Biexponential T kinetics were used reflecting rapid (33%) and slow (67%) half-lives of 1.4 and 27 min, respectively (60). Primary output includes basal (nonpulsatile), pulsatile, and total (basal plus pulsatile) T secretion rates in units of nanograms per deciliter per 6 h.

LH-T dose-response analysis. A three-parameter logistic model was used to relate deconvolution-estimated sample T secretion rates to pulsatile LH concentrations over the last 6 h of sampling (triple rhLH-pulse stimulus), exactly as presented (30, 31). The model allows for possible sensitivity downregulation within coupled LH-T pulses. The output comprises maximum-likelihood estimates of LH-T dose-response characteristics, viz. sensitivity (slope), potency (ED50), and efficacy (maximal T secretion).
order of prior 2 h

There was broad visual separation of the curves, as defined by subjects completed all four sessions without adverse events. Unique (unshared) alphabetic superscripts denote significant interventional effect assessed by ANOVA. Data are otherwise presented as \( P \) (post hoc Tukey’s test). The boldface \( P \) value of \( 10^{-12} \) is the overall interventional effect assessed by ANOVA. Data are otherwise presented as noted in Fig. 3. Unique (unshared) alphabetic superscripts denote significant post hoc multicomparison differences.

**Statistical analysis.** Data are presented as means ± SD (\( n = 19 \) subjects), unless specified otherwise. ANOVA was applied to the incremental response (active LH or T response minus saline-associated LH or T response), thus yielding three independent factors adjusted for within-subject control (saline) values. Power was estimated as \( \geq 85\% \) for detecting a 30% effect of infusion type at \( P < 0.05 \) if 19 subjects each completed all four visits. Experimentwise \( P < 0.05 \) was considered significant. Post hoc testing was by Tukey’s honestly significantly different test (13).

**RESULTS**

Table 1 gives mean and peak serum LH concentrations, and mean measured total T and calculated free and bioavailable T concentrations at baseline (preintervention) and across the last 6 h of the infusions. Demographic data are included as range values.

Median LH and T concentrations attained by the 19 men across the overall 18-h protocol are shown in Fig. 1B. All subjects completed all four sessions without adverse events. There was broad visual separation of the curves, as defined by three distinct LH and T response strata in the descending rank order of prior 2 h = 1 h > continuous > saline infusion. Individual LH and T concentration profiles obtained during the 6-h triple rhLH-pulse stimulus after each of the four overnight (12-h) saline/rhLH interventions are depicted in Fig. 2. Data reflect sampling every 10 min over the morning time window 1100–1700 during the standardized triple rhLH-pulse stimulus (3 consecutive rhLH pulses, one every 2 h). Infused LH concentration pulses (Fig. 2A) are clearly visible in all subjects. Profiles illustrate intersubject and time variability of T concentration responses (Fig. 2B).

During the standardized triple rhLH-pulse stimulus, 6-h mean LH concentrations were lowest after overnight saline infusion, intermediate after overnight continuous rhLH stimulation, and significantly higher after overnight rhLH pulses of both 2- and 1-h frequencies than after either saline or continuous rhLH delivery (\( P < 10^{-4} \) (Fig. 3, top)). Mean 6-h T concentrations differed in the same ascending rank order: saline < continuous rhLH < 2- and 1-h rhLH pulses (\( P < 10^{-3} \) (Fig. 3, bottom). Post hoc Tukey’s test revealed no significant differences between the effects of overnight 2- and 1-h rhLH-pulse frequencies with respect to subsequent 6-h mean LH or mean T concentrations.

To compare effects of the morning 6-h and overnight 12-h rhLH/saline infusions, mean T concentrations over the last 4 h of the standardized triple rhLH-pulse stimulus were compared with those over the last 4 h of the overnight clamp (ending just before the triple rhLH stimuli) (Fig. 4). There were marked treatment effects, as assessed by ANOVA (\( P < 10^{-12} \)). Post hoc contrasts in 4-h mean T concentrations included: 1) three-fold stimulation by the last 4 h of the triple LH stimulus compared with the last 24 h of overnight saline (\( P < 10^{-9} \)) and 2) 1.3-fold stimulation by the last 4 h of the triple LH stimulus compared with the last 4 h of overnight continuous rhLH infusion (\( P < 10^{-3} \)). The triple LH pulse stimulus achieved comparable final 4-h mean T (and LH) concentrations after

**Fig. 4.** Mean T concentrations over the last 4 h of the overnight (12-h) fixed rhLH clamp (open bars) compared with the last 4 h of the (6-h) triple rhLH-pulse clamp (hatched bars). Delta nos. denote the 95% confidence intervals of significant paired differences defined by corresponding \( P \) values (post hoc Tukey’s test). The boldface \( P \) value of \( 10^{-12} \) is the overall interventional effect assessed by ANOVA. Data are otherwise presented as noted in Fig. 3. Unique (unshared) alphabetic superscripts denote significant post hoc multicomparison differences.

**Fig. 5.** Deconvolution-based estimates of mean ± SD basal (nonpulsatile), pulsatile, and total (basal plus pulsatile) T secretion over 6 h during the triple rhLH-pulse stimulus in healthy men. The overall ANOVA \( P \) value is in boldface. A priori paired \( t \)-test comparison \( P \) values are given between paired arrows between continuous rhLH and 2-h rhLH pulses. Data are presented otherwise as described in Fig. 3. Unique (unshared) alphabetic superscripts denote significant post hoc multicomparison differences.
morning vs. overnight administration of both 2- and 1-h rhLH pulses.

Deconvolution analysis was used to estimate basal (nonpulsatile), pulsatile, and total 6-h T secretion during triple rhLH-pulse stimulation after each of the four overnight saline/rhLH infusion types. Data are summarized in Fig. 5. Under the triple rhLH-pulse stimulus, basal T secretion rose by 1.5-fold more after overnight continuous rhLH exposure than saline infusion ($P < 0.001$); by 1.9-fold more after overnight 2-h rhLH pulses than saline; and by 2.0-fold more after overnight 1-h rhLH pulses than saline ($P < 10^{-6}$). The effects of 2- and 1-h pulses did not differ for any comparison (basal, pulsatile, or total T secretion). An a priori comparison between basal T secretory responses to the standardized triple rhLH-pulse stimulus after overnight 2-h rhLH pulses vs. continuous rhLH infusions was significant at $P = 0.016$. Unlike basal, 6-h pulsatile T secretion decreased after overnight exposure to 2- and 1-h rhLH pulses compared with overnight saline ($P < 10^{-3}$ overall) but not for the comparison of continuous vs. 2- and 1-h rhLH injections ($P = 0.22$). Total T secretion followed the same rank order as basal T secretion (overall $P < 10^{-3}$, a priori $P = 0.010$ for greater triple rhLH-pulse effect after overnight 2-h rhLH pulses than continuous rhLH). These outcomes suggest priming of basal and total T secretion and, conversely, blunting of pulsatile T secretion by overnight rhLH pulses compared with the saline control of ganirelix-mediated LH withdrawal.

To quantify pulsatile LH-T dose-response measures during the standardized triple rhLH-pulse stimulus after overnight 2-vs. 1-h pulsatile rhLH infusions, paired 6-h LH concentration and T secretion time series were subjected to nonlinear (3-parameter) logistic dose-response analysis with allowance for possible sensitivity downregulation. Salient findings are given in Fig. 6. The effects of prior infusions of 2- and 1-h rhLH pulses overnight differed by way of 1) greater sensitivity of testis T responses to 1- than 2-h pulses; 2) lower ED$_{50}$ (greater potency) of 1- than 2-h pulses; 3) less intrapulse downregulation of sensitivity after 1- than 2-h pulses; and 4) less intrapulse downregulation of potency (yielding lower ED$_{50}$) after 1- than 2-h rhLH pulses. Nonsignificant comparisons are given in Table 2, viz.: rhLH-pulse efficacy, pulsatile LH-T time lag to downregulation, and model error (all $P > 0.60$).

As objective measures of feedforward orderliness (regularity) and synchrony, T ApEn and LH-T cross-ApEn were calculated over the 6-h triple-stimulus window. Figure 7 shows that prior 2- and 1-h rhLH pulses evoked higher ApEn (Fig. 7, top) and cross-ApEn (Fig. 7, bottom) than prior saline infusion, signifying reduced T regularity and lower LH-T secretory synchrony ($P < 10^{-8}$). Continuous overnight rhLH stimulation yielded intermediate ApEn and cross-ApEn values that differed significantly from estimates during saline and 1-h rhLH-pulse infusions.

**DISCUSSION**

Pulsatility in many endocrine systems appears to confer optimal target-tissue responsiveness (57, 61, 62). This concept is well demonstrated for GnRH-driven LH secretion (2), growth hormone-induced muscle insulin-like factor type I gene expression (8, 10, 20, 27), and parathormone-stimulated bone growth (1, 39). The point is perhaps more debatable for glucagon, insulin, TSH, prolactin, adrenocorticotropin, follicle-stimulating hormone, oxytocin, and AVP action (22, 32, 35, 40 – 42, 50, 61, 62, 65). In the case of LH physiology, data in the male rat and sheep indicate that 2-h LH pulses are as stimulatory as continuous LH infusions in sustaining T secretion for up to 12 days (9, 21). However, LH pulses infused every 12 h over the same number of days were not effective. No comparable studies exist in the primate. The present investigation in 19 healthy men each studied under four randomly ordered infusions of saline, continuous rhLH, or pulsatile rhLH is unique in this respect. By applying deconvolution, ApEn, and LH-T dose-response analyses, the present experiments show that the time history of LH exposure (overnight) strongly determines (morning) basal, pulsatile, and total T secretion, T

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**Fig. 6. Analytical estimates of testis sensitivity (slope of T secretion on LH concentrations) and EC$_{50}$ (one-half-maximally stimulating concentration) of the LH-T dose-response logistic function. Data are the median and geometric mean ($n = 19$ men). P values were estimated by paired two-tailed t-tests. Dose-response sensitivity was quantified simultaneously during the rising initial phase of T secretion pulses (pulse onset, 2 plots on left) and during the falling phase of T secretion pulses (downregulated pulse offset, 2 plots on right). This allows for possible but not required intrapulse downregulation (METHODS).**
ApEn (regularity), LH-T cross-ApEn (synchrony), and LH-T dose-responsive sensitivity and potency (EC₅₀). In contrast, the pattern of intravenous LH delivery does not alter quantifiable LH efficacy or estimated LH-T coupling delay (Table 1). The collective data support the hypothesis that pulsatile LH delivery into blood achieves higher mean LH concentrations and greater stimulatory effects on total T secretion and total T concentrations than continuous LH delivery in healthy young and middle-aged men. Moreover, 2- and 1-h rhLH pulses, albeit yielding comparable mean LH concentrations, exert distinguishable effects on estimated LH-T dose-response potency and sensitivity, suggesting frequency dependence of the pulsatile LH-T feedforward process. The exact degree to which these inferences in the human apply to animal models cannot be ascertained fully due to differences in experimental design and analytical methods.

Downregulation of hCG action has been described extensively in vitro and in vivo (6, 12, 18, 49, 53, 55). At least under in vitro conditions, LH is also able to downregulate Leydig cell cAMP and T production (11, 23, 24). Exceptions include human fetal Leydig cells and precocious puberty due to an activating LH receptor mutation in which hCG-induced responses are downregulated in the opposite direction. Specifically, pulsatile T secretion was lower after overnight 2- or 1-h rhLH pulses than saline infusion. However, this was not the case, since (mean ± SD) pairwise incremental T concentration by >5- and 1.4-fold stimulation of basal T secretion by 1-h rhLH pulses compared with saline and continuous rhLH, respectively (P < 10⁻¹⁰). Higher basal and total (but not pulsatile) T secretion could indicate a more sustained induction of T biosynthetic enzymes and/or cholesterol uptake and delivery to mitochondrial enzymes by hourly rhLH pulses than by saline or continuous rhLH infusion (38). In this regard, nearly continuous patterns of T secretion have been observed after 24 h of intermittent (3-h) LH pulses in the juvenile rhesus monkey (47).

Univariate ApEn, a response regularity measure, and bivariate cross-ApEn, a model-free nonparametric measure of the synchrony of feedforward (LH-T) secretion patterns (45, 46, 61), were higher after overnight 1-h rhLH pulses than after continuous rhLH or saline infusions. Two-hour rhLH pulses produced intermediate T ApEn and LH-T cross-ApEn values, which were greater than that of saline infusion. Accordingly, pattern-sensitive regularity and synchrony analyses confirm that the time mode of overnight testis exposure to LH stimulation influences acute T secretory responses thereafter.

A caveat is that the 6-h triple rhLH-pulse stimulus itself may have produced downregulation. However, this was not the case, since (mean ± SD) pairwise incremental T concentration in the opposite direction. Specifically, pulsatile T secretion was lower after overnight 2- or 1-h rhLH pulses than saline infusion but not than overnight continuous rhLH. The contribution of lower pulsatile T secretion to total T secretion was countered by >5- and 1.4-fold stimulation of basal T secretion by 1-h rhLH pulses compared with saline and continuous rhLH, respectively (P < 10⁻¹⁰). Higher basal and total (but not pulsatile) T secretion could indicate a more sustained induction of T biosynthetic enzymes and/or cholesterol uptake and delivery to mitochondrial enzymes by hourly rhLH pulses than by saline or continuous rhLH infusion (38). In this regard, nearly continuous patterns of T secretion have been observed after 24 h of intermittent (3-h) LH pulses in the juvenile rhesus monkey (47).

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### Table 2. LH-T dose-response estimation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>2 h</th>
<th>1 h</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficacy, ng · dl⁻¹ · min⁻¹</td>
<td>16.9 (12.6, 57)</td>
<td>10.4 (8.2, 6.1)</td>
<td>0.64</td>
</tr>
<tr>
<td>Time lag (10-min samples)</td>
<td>2.6 (3.0, 1.8)</td>
<td>2.6 (3.0, 1.1)</td>
<td>0.94</td>
</tr>
<tr>
<td>Model error (SD)</td>
<td>3.0 (2.9, 1.0)</td>
<td>3.3 (3.4, 1.9)</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Nonlinear LH-T dose-response model estimates. The median and ±SD are in parentheses. P values are by paired two-tailed t-test. Parameters estimates are geometric means.

### Figure 7. Approximate entropy (ApEn) of T (top) and cross-ApEn of LH-T (bottom) in healthy men evaluated during a 6-h triple rhLH-pulse stimulus after overnight exposure to a 12-h saline/rhLH clamp. ApEn is a univariate regularity measure that increases under feedforward (stimulatory) inputs. Cross-ApEn is a bivariate synchrony measure that also rises under feedforward, here LH→T. Data are mean ± 95% confidence intervals.

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differences between the last and first 2-h blocks in the 6-h time window were saline (147 ± 56) > continuous rhLH (67 ± 72) > 2-h pulses (12 ± 44) and 1-h pulses (1.2 ± 53 ng/dl) (P < 0.001). Because LH exerts concentration-dependent effects, higher mean serum LH concentrations at the end of pulsatile than continuous rhLH infusions, as also reported for growth hormone injections (51), may explain the greater effects of pulsatile than constant rhLH stimulation. However, 1- and 2-h rhLH pulses achieved similar mean LH concentrations. By way of additional caveats, one cannot exclude the possibility that the difference between the LH concentrations attained during the first and third 2-h LH pulses contributed to Leydig cell stimulation. Alternatively, both reduced downregulation and increased upregulation of LH action might explain the rise in T secretion. At comparable LH levels, 1-h rhLH pulses yielded less quantifiable downregulation of the potency and sensitivity of analytical LH→T dose-response estimates than 2-h rhLH pulses (Fig. 6).

In conclusion, the present analyses indicate that, at comparable mean LH concentrations, 2- and 1-h rhLH pulses exert distinguishable T secretory effects as assessed by pulsatile LH-T dose-response analyses. The two pulse frequencies have similar short-term effects on mean T concentrations, T secretion rates, and T ApEn. Intermittent LH pulses also achieve higher mean LH and mean T concentrations and total T secretion rates than constant LH infusions. The combined results introduce the notion that, in healthy men, T production is significantly dependent on the temporal mode of LH’s delivery in the circulation. The extent to which the present inferences apply to long-term LH drive over days or weeks is not yet known.

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DISCLOSURES

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute on Aging or the National Institutes of Health. Matlab versions of ApEn and deconvolution methodology are available from veldhuis.johannes@mayo.edu.

AUTHOR CONTRIBUTIONS

Author contributions: J.D.V. and P.Y.L. conception and design of research; J.D.V., P.Y.T., and D.M.K. analyzed data; J.D.V., P.Y.L., and P.Y.T. interpreted results of experiments; J.D.V. prepared figures; J.D.V. drafted manuscript; J.D.V., P.Y.L., P.Y.T., and D.M.K. approved final version of manuscript; P.Y.L., P.Y.T., and D.M.K. edited and revised manuscript.

REFERENCES


