

# Twenty-Four-Hour Rhythms in Plasma Concentrations of Adenohypophyseal Hormones Are Generated by Distinct Amplitude and/or Frequency Modulation of Underlying Pituitary Secretory Bursts\*

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**ABSTRACT.** To evaluate the nature of anterior pituitary secretory events *in vivo*, we have applied a novel waveform-independent deconvolution technique that dissects the underlying secretory behavior of endocrine glands quantitatively from available serial plasma hormone concentration measurements assuming one- or two-compartment elimination kinetics. We used this new tool to ask the following physiological questions. 1) Does the pituitary gland secrete exclusively in randomly dispersed bursts, and/or does a tonic (constitutive) mode of interburst hormone secretion exist? 2) What secretory mechanisms generate the nyctohemeral rhythms in plasma hormone concentrations? Analysis of 24-h plasma concentration profiles of GH, LH, FSH, PRL, TSH, ACTH, and  $\beta$ -endorphin (n = 6–8 men/group) revealed that 1) pituitary secretion *in vivo* occurs in an exclusively burst-like mode for all hormones except TSH and PRL (for the latter two, a mixed burst and constitutive mode pertained); 2) significant nyctohemeral regulation of se-

cretory burst frequency alone was not demonstrated for any hormone; 3) prominent 24-h variations in secretory burst amplitude alone were delineated for ACTH and LH; 4) TSH, GH, and  $\beta$ -endorphin were both frequency and amplitude controlled; 5) no significant diurnal variations in FSH secretory parameters occurred; and 6) a fixed hormone half-life yielded fits of the 24-h data series with a normalized residual variance of less than 8%.

We conclude that the normal human anterior pituitary gland releases its multiple (glyco)protein hormones via punctuated secretory episodes unassociated with tonic basal (constitutive) hormone secretion, except in the case of TSH and PRL. Hormone-specific amplitude and/or frequency control of secretory burst activity over 24 h provides the mechanistic basis for the classically recognized 24-h rhythms in plasma concentrations of adenohypophyseal hormones in men. (*J Clin Endocrinol Metab* 71: 1616–1623, 1990)

PLASMA concentrations of adenohypophyseal hormones are known to fluctuate in a diurnal manner (1–3). The exact mechanisms by which such 24-h rhythms in plasma hormone concentrations are generated *in vivo* have not been defined in detail. However, at least four specific models can be considered: 1) nyctohemeral variations in tonic (basal or constitutive) secre-

tion of a hormone with superimposed secretory bursts, 2) diurnal modulation of the amplitude of hormone secretory bursts, 3) 24-h variations in secretory burst frequency, and 4) combinations of the three preceding basic mechanisms.

In relation to such possible mechanisms, recent studies of episodic cortisol release in normal men have demonstrated that combined amplitude and frequency modulation of otherwise randomly dispersed cortisol secretory bursts gives rise to the daily glucocorticoid rhythm, and that there is no need to postulate the presence of or any 24-h variation in interpulse tonic (basal or constitutive) cortisol secretion (4). This concept of adrenal secretory activity was inferred by applying model-based deconvolution analysis, in which the number and amplitude of hormone secretory bursts as well as subject-specific hormone half-life can be calculated from the nyctohemeral

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plasma concentration profiles (5). In its simplest form, such a deconvolution technique assumes waveform-specific (*e.g.* Gaussian) secretory events without interpulse secretory activity (5).

In contrast, a more recently implemented deconvolution procedure is waveform independent and permits calculation of sample hormone secretion rates and their statistical confidence limits without assuming that secretion occurs as discrete burst-like events and/or in a tonic pattern (6). Accordingly, we have applied this more general technique to evaluate hormone secretion as a function of time of day and thereby test the preceding three hypotheses regarding specific *in vivo* neuroendocrine mechanisms that generate the 24-h rhythms in plasma hormone concentrations.

Our analyses have revealed that nyctohemeral rhythms in the plasma concentrations of individual pituitary hormones in man are specified by corresponding distinct secretory mechanisms.

## Materials and Methods

### *Clinical protocols*

After providing written informed consent approved by the University of Virginia School of Medicine, normal male volunteers, aged 21–43 yr, were admitted to the Clinical Research Center and underwent blood sampling at 10-min intervals for 24 h. Subjects were admitted the evening before study and had normal baseline biochemical measures of hepatic, renal, metabolic, and hematological function. No subjects were receiving medications, had undertaken recent transmeridian travel, or had underlying acute or chronic illness. Morning serum concentrations of immunoactive LH, FSH, GH, PRL, TSH, T<sub>4</sub>, testosterone, and estradiol were normal. Subjects were allowed to ambulate and received meals at 0830, 1230, and 1700 h. Vigorous exercise was not permitted during the sampling protocol.

### *Assays*

LH and FSH were measured by RIA, using the dual label procedure described previously, and with median intra- and interassay coefficients of variation of 6.5% and 10% (7). Serum concentrations of PRL, TSH, GH, ACTH, and  $\beta$ -endorphin were determined in exactly the manner described previously by two-site immunoradiometric assays, with median intraassay coefficients of variation ranging between 3.5–8.1% and interassay coefficients of variation of 8–10.2% (7–11). All samples from an individual volunteer ( $n = 145$  samples/24 h) were evaluated in duplicate in the same assay in order to avoid interassay variations.

### *Waveform-independent deconvolution technique*

Deconvolution analysis was used to calculate all individual sample secretion rates (and their variances), given an estimated hormone half-life and its associated variance. Specifically, the

concentration of a hormone at any particular time was defined as the sum of the amount of hormone secreted in each previous sample corrected for the amount of hormone eliminated after it was secreted. This methodology requires that one specify values for the elimination rate constants or half-lives in order to evaluate the sample secretory rates. Elimination kinetics were described by a monoexponential system (TSH, PRL, and  $\beta$ -endorphin) or a biexponential function (other hormones; see below). The latter is defined by two half-lives and a fraction denoting their relative contributions (6). By summing all the appropriate prior sample secretion and elimination terms in a convolution product, one can describe the concentration of a hormone at any time  $t$ . The values of all  $n$  observed plasma hormone concentrations are given by a family of  $n$  such equations. All of the  $n$  sample secretory rates of interest (one for each data point) are estimated simultaneously. The initial estimates for the  $n$  secretory rates are found using Gold's ratio method, followed by a steepest descent, least-squares procedure that computes the logarithm of the secretory rate at each data point. (The logarithm of the secretory rate is used to guarantee positive secretory estimates.) The SDs of the calculated secretory rates and their first derivatives are evaluated as the root-mean-square sum of the deviations that are introduced by 1) varying the assumed elimination half-lives and repeating the deconvolution process, and 2) point by point perturbation of the data set and repeating the deconvolution procedure (6, 12). Assuming Gaussian-distributed experimental uncertainty in the dependent variable (secretory rate), the above procedure yields the maximum likelihood sample secretory rates and their SDs. The algorithm does not assume the absence or presence of tonic release or a shape for the secretory events.

To identify distinct secretory pulses or peaks in the calculated sample secretion profiles, we require that the candidate secretory rates exhibit 1) a significant first derivative and 2) a significant (nonzero) value, based on a 95% probability level for a one-tailed test of the null hypothesis of a zero first derivative value or zero secretion. Nadirs were defined as all regions (sample secretory rate values) between significant secretory peaks (6). A pooled  $z$  score was used to test for significant basal interpeak hormone secretion. The  $z$  score was derived from the combined variances of the between-sample (within-nadir) and the between-nadir secretory rates.

A smoothing component was used by convolving the elimination term with a Gaussian function that had a SD equal to half the sampling interval. The amount of smoothing is, therefore, based on the properties of the unit normal distribution.

The half-lives chosen from the literature as nominal values for the various hormones were as follows: LH: first component, 18 min; second component half-life, 90 min; with a fractional amplitude of the second component of 0.37 (13); FSH: 102 and 398 min; fraction, 0.48 (14); GH: 3.5 and 21 min; fraction, 0.63 (15); ACTH: 3.5 and 14 min; fraction, 0.33 (16); and monocomponent half-lives for TSH, PRL, and  $\beta$ -endorphin of 35, 25, and 45 min, respectively (17).

### *Cosinor analysis of 24-h rhythms in hormone secretory features*

To evaluate 24-h rhythms in hormone secretory features, we determined 1) whether secretory burst amplitudes in the group

of men as a whole exhibited a significant 24-h rhythm; 2) whether interburst nadir secretory rates (an estimate of tonic or constitutive hormone secretion) manifested a significant 24-h rhythmicity; and 3) whether secretory burst frequency (or its reciprocal, intersecretory burst interval) maintained a significant 24-h rhythm. Accordingly, we applied statistically based cosinor analysis to each of these deconvolution-resolved secretory features. Cosinor analysis was carried out exactly as described previously (4). Statistical confidence limits were defined for the mesor (mean value about which the 24-h rhythm oscillates), amplitude (half the difference between nadir and zenith), and acrophase (time of maximal value) using nonlinear least-squares methods of error propagation assuming highly correlated, joint asymmetric variance spaces (4).

## Results

As summarized in Table 1, the waveform-independent deconvolution procedure allowed us to estimate pituitary hormone secretory burst frequency and interpulse intervals in normal men. We observed a range of secretory burst frequencies of 13–24 events/24 h for LH, FSH, PRL, TSH, ACTH, and  $\beta$ -endorphin and a lower frequency of detectable secretory bursts of  $10 \pm 0.72$  ( $\pm$  SEM) peaks/24 h for GH. The corresponding intersecretory burst intervals varied between 60–129 min (lowest value for TSH and highest for GH). Illustrative profiles for the 6 anterior pituitary hormones studied here (LH, TSH, FSH, PRL, GH, and  $\beta$ -endorphin) are given in Fig. 1. Note that the predominant mode of hormone secretion is that of individually distinct bursts occurring throughout the 24 h with or without low interburst (valley) secretory rates (below). The error bars shown on the secretory profiles give a measure of how significantly the secretion rates differ from zero between bursts as well as within bursts.

The 24-h variations in secretory burst amplitude, interburst nadir secretory rate, and interburst intervals are illustrated for several hormones in Fig. 2. For each hormone, all data from the entire group of six to eight men are displayed in the same panel with the fitted cosine

TABLE 1. Deconvolution-based estimates of pituitary hormone secretory burst frequencies and interpulse intervals in normal men

Hormone	Secretory burst frequency (no. of bursts/24 h)	Intersecretory burst interval (min)
LH	$23 \pm 1.0$ (8)	$61 \pm 3.4$
FSH	$19 \pm 1.4$ (8)	$75 \pm 5.2$
PRL	$24 \pm 0.69$ (6)	$57 \pm 1.8$
TSH	$24 \pm 0.93$ (6)	$60 \pm 3.3$
GH	$10 \pm 0.72$ (7) <sup>a</sup>	$130 \pm 13$
ACTH	$18 \pm 0.87$ (8)	$76 \pm 4.1$
$\beta$ -Endorphin	$13 \pm 1.1$ (8)	$110 \pm 9.9$

Data are the mean  $\pm$  SEM. Numbers in parentheses denote how many men were studied.

<sup>a</sup> Minimum number of secretory bursts, since undetectable GH concentrations may contain additional pulses (see *Discussion*).

curve of periodicity 24 h. Quantitative data for all seven hormones are summarized in Tables 2 and 3. For each significant 24-h rhythm in a specific secretory feature, we determined the acrophase (clocktime at which the maximal values occurred; Table 2); the amplitude (half the difference between the zenith and the nadir), and the mesor (mean value about which the sinusoidal rhythm oscillates; Table 3).

We observed that 24-h variations in specific secretory features fell into one of several mechanistically distinct categories: 1) no hormone demonstrated isolated frequency modulation of secretory bursts over 24 h, 2) LH and ACTH secretion were subject only to 24-h amplitude control, 3)  $\beta$ -endorphin, GH, and TSH exhibited both amplitude and frequency regulation, 4) FSH exhibited no significant 24-h rhythms in any secretory parameter, and 5) PRL manifested a significant 24-h variation in both secretory burst amplitude and interburst nadir (tonic) secretory rates. Importantly, other interburst nadir secretory rates approached zero and were not statistically significant for LH, FSH, ACTH,  $\beta$ -endorphin, and GH. However, aggregate z scores for interpeak nadir secretory rates were significantly nonzero for TSH and PRL (respective median z scores of 2.31 and 1.94), which indicates a measurably tonic mode of release. For TSH, there was no 24-h rhythm in this constitutive level of secretion.

As indicated by the quantitative data summarized in Tables 2 and 3, the largest 24-h rhythms in secretory burst amplitudes (estimated as the percentage ratio of amplitude to mesor) were for GH (81% variation) and ACTH (60% variation). Twenty-four-hour variations in secretory burst frequency were considerably smaller, *viz.* 13% (TSH), 22% ( $\beta$ -endorphin), and 23% (GH). The times of lowest secretory burst frequency (acrophase or maximum of the interburst interval rhythm over 24 h) occurred at approximately midnight for TSH, at 1600 h for GH, and at 2000 h for  $\beta$ -endorphin. The time of maximal secretory burst frequency would be 12 h removed and, therefore, occurred at approximately 1200 h for TSH, 0400 h for GH, and 0800 h for  $\beta$ -endorphin. As shown in Table 2, the acrophase of TSH burst frequency was significantly different (*i.e.* phase shifted) from the acrophase associated with the corresponding 24-h rhythm in secretory burst amplitude. Thus, both the amplitude and frequency of secretory bursts can be modulated, and the phases (timing) of the 24 h rhythms in frequency and amplitude may be different (TSH). In the single case in which a rhythm occurred in both secretory burst amplitude and interburst valley (nadir) secretory rates (prolactin), the timing of the maximal values for these two measures of secretion coincided. Moreover, as shown in Table 2, the acrophases of the 24-h rhythms in

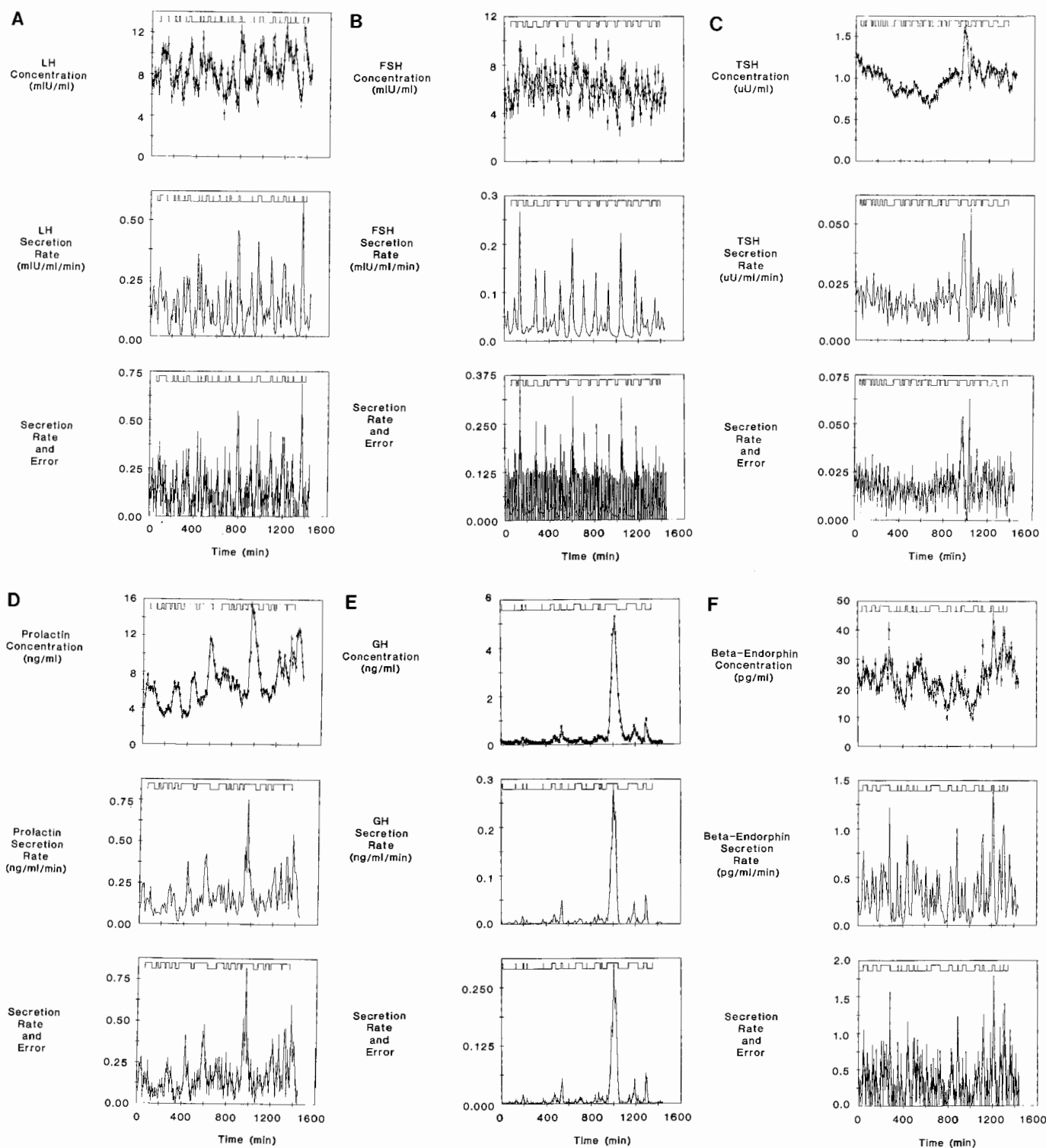
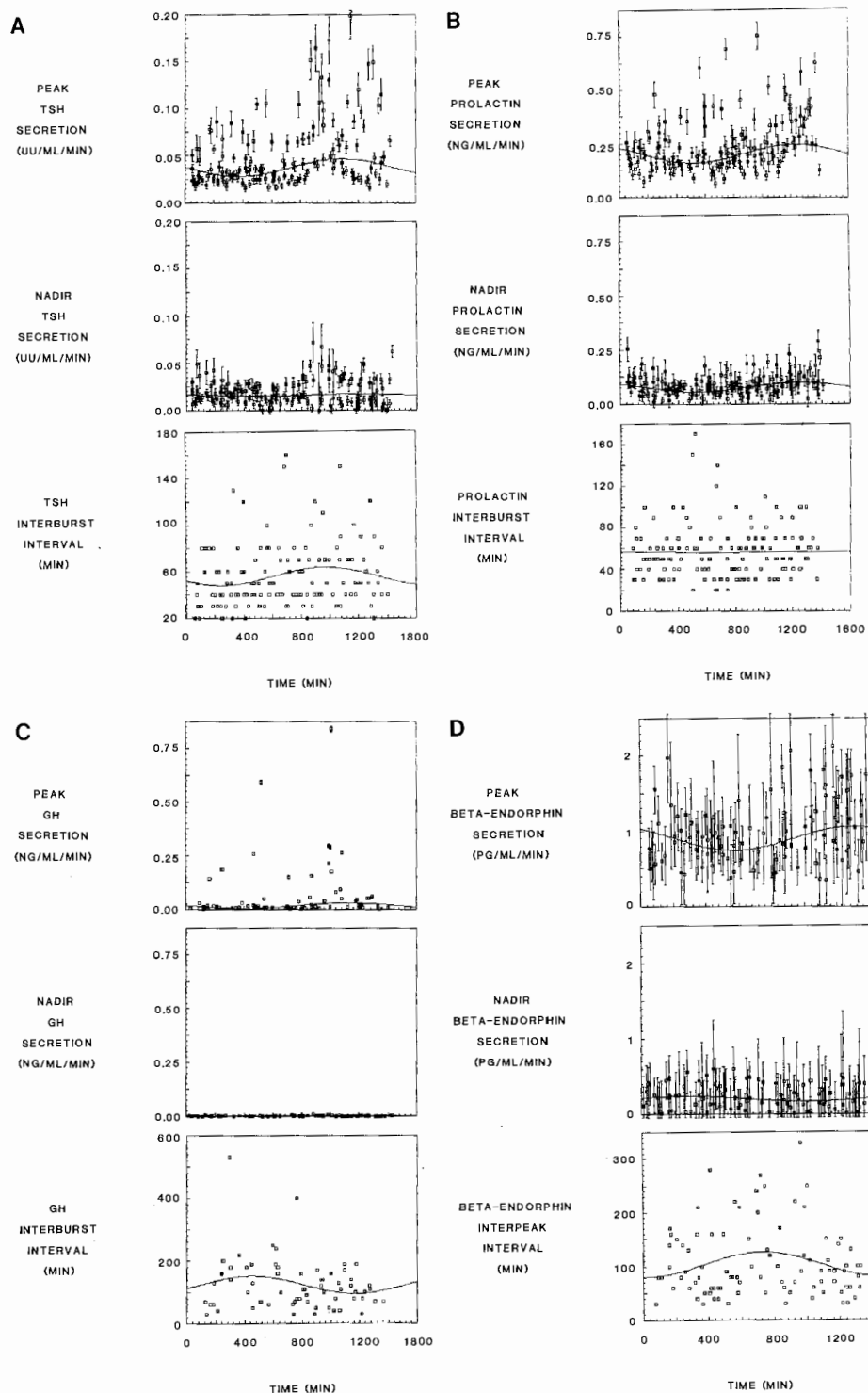


FIG. 1. Individual 24-h time series of six adenohypophyseal hormones and their deconvolution-resolved secretory profiles. Profiles are given for LH, FSH, TSH, PRL, GH, and  $\beta$ -endorphin, each of which is illustrated in one particular subject. For each hormone, the *top panel* gives the observed serial serum hormone concentrations and the intrasample (intrassay) SDs as well as the fitted (predicted) curve specified by the waveform-independent deconvolution technique. The *middle and lower panels* show the calculated sample secretory rates without (*middle*) or with (*lower*) statistical confidence limits ( $\pm$ SD). The *error bars* on the secretion profiles give a measure of how significantly the calculated hormone secretion rate differs from zero, both within and between bursts. Deflections schematized *above* the data denote computer-identified pulses. Table 1 summarizes the mean secretory burst frequencies and interpulse intervals for all seven hormones, each studied in a group of six to eight normal men. For LH and FSH, mIU/mL = IU/L; for PRL and GH, ng/mL =  $\mu$ g/L; for TSH,  $\mu$ U/mL = mU/L; and for  $\beta$ -endorphin, pg/mL = ng/L.

FIG. 2. Twenty-four-hour rhythms in specific deconvolution-resolved secretory features of the adenohypophyseal hormones TSH (A), PRL (B), GH (C), and  $\beta$ -endorphin (D). For each hormone, a waveform-independent deconvolution technique was used to estimate the following: peak (maximal) hormone secretory rates (*upper panels*), interpeak valley or nadir secretory rates (*middle panel*), and interburst intervals in minutes (*lower panel*). Vertical marks are  $\pm$  SD for the individual sample secretion estimates calculated by deconvolution. Data in each panel comprise all observations in the entire group of six to eight normal men who were sampled for that particular hormone. For TSH,  $\mu\text{U/mL} = \text{mU/L}$ ; for PRL and GH,  $\text{ng/mL} = \mu\text{g/L}$ ; and for  $\beta$ -endorphin,  $\text{pg/mL} = \text{ng/L}$ .



plasma hormone concentrations generally agreed well with the acrophases in secretory burst amplitudes.

### Discussion

Although 24-h variations in circulating concentrations of adenohypophyseal hormones have been recognized in

man and experimental animals, the exact neuroendocrine basis for such nyctohemeral rhythmicity<sup>1</sup> has not been elucidated fully. Here, we have used a waveform-independent deconvolution technique to test three spe-

<sup>1</sup> We have used the terms nyctohemeral, diurnal, or 24-h rhythms rather than circadian, since the latter typically denotes free-running, temperature-dependent, and stimulus-entrainable 24-h periodicities.

TABLE 2. Clocktimes of maximal values of significant 24-h rhythms in specific secretory features compared to plasma concentrations of anterior pituitary hormones in normal men

Hormone	Secretory features			Plasma hormone conc. rhythm <sup>a</sup>
	Secretory burst amplitude <sup>b</sup>	Interburst nadir secretory rate <sup>c</sup>	Interburst interval <sup>d</sup>	
LH	1955 (1609–0153) <sup>e</sup>	NS	NS	2228 (1914–0138)
FSH	NS	NS	NS	NS
PRL	0430 (0244–0612)	0442 (0318–0610)	NS	0312 (0223–0402)
TSH	0211 (0133–0359)	NS	0007 (2107–0317)	0255 (0228–0319)
GH	0228 (2304–0541)	NS	1608 (1025–1928)	0041 (2357–0125)
ACTH	0810 (0722–0900)	NS	NS	0853 (0930–0817)
$\beta$ -Endorphin	0532 (0115–0741)	NS	2012 (1726–2251)	0638 (0592–0733)

<sup>a</sup> Cosinor analysis of 24-h plasma/serum hormone concentrations, assuming individual mesors and group best-fit amplitudes and acrophases.

<sup>b</sup> Maximal secretory rate attained per burst.

<sup>c</sup> Secretory rate maintained at lowest point between consecutive secretory bursts.

<sup>d</sup> Time in minutes separating successive secretory burst maxima.

<sup>e</sup> Clocktime when maximal value of the secretory feature (burst amplitude, interburst nadir, or interburst interval) or hormone concentration occurs.

NS, No significant rhythm for the group of six to eight men. Values in parentheses are 67% statistical confidence limits for the parameters considered jointly.

TABLE 3. Mesor (mean) and amplitude of 24-h variations in specific secretory features of anterior pituitary hormones in normal men

Hormone	Secretory burst amplitude <sup>a</sup>			Interburst nadir secretory rate <sup>b</sup>			Interburst interval (min) <sup>c</sup>		
	Mesor <sup>d</sup>	Amplitude <sup>e</sup>	% <sup>f</sup>	Mesor <sup>d</sup>	Amplitude <sup>e</sup>	% <sup>f</sup>	Mesor <sup>d</sup>	Amplitude <sup>e</sup>	% <sup>f</sup>
LH	0.27 <sup>g</sup>	0.033 <sup>g</sup>	12		NS			NS	
FSH	NS	NS	NS		NS			NS	
PRL	0.20	0.043	22	0.077	0.021	27		NS	
TSH	0.037	0.0093	25		NS		60	7.9	13
GH	0.016	0.013	81		NS		123	28	23
ACTH	0.90	0.54	60		NS			NS	
$\beta$ -Endorphin	0.90	0.16	18		NS		104	23	22

<sup>a</sup> Maximal secretory rate attained per burst.

<sup>b</sup> Secretory rate maintained at lowest point between consecutive secretory bursts.

<sup>c</sup> Time in minutes, separating successive secretory burst maxima.

<sup>d</sup> Mean value about which the 24-h cosine rhythm oscillates.

<sup>e</sup> Half the difference between the maximal value and minimal value the 24-h rhythm in the secretory feature.

<sup>f</sup> Ratio of amplitude to mean (mesor), expressed as a percentage.

<sup>g</sup> All secretory rates are given in mass units per unit distribution volume/unit time; viz. IU/L·min for LH and FSH;  $\mu$ g/L·min for PRL and GH; mU/L·min for TSH; and ng/L·min for ACTH and  $\beta$ -endorphin.

sific hypotheses regarding the origin of nyctohemeral rhythms in plasma anterior pituitary hormone concentrations. Our analyses demonstrate that the 24-h rhythms in plasma concentrations of individual pituitary hormones can be generated by distinct mechanisms that typically include diurnal modulation of the amplitudes of underlying pituitary hormone secretory bursts, with or without associated modulation of secretory burst frequency. Specifically, amplitude control of hormone secretory events could be demonstrated for LH, PRL, TSH, GH, ACTH, and  $\beta$ -endorphin in normal men. In the case of LH and ACTH, a 24-h variation in secretory burst amplitude alone gave rise to the nyctohemeral rhythm in serum hormone concentrations. In contrast, both secretory burst amplitude and frequency were significantly regulated over 24 h in the case of TSH, GH, and  $\beta$ -endorphin. FSH secretory features (and plasma concen-

trations) had no significant 24-h rhythmicity in this group of men. A nyctohemeral variation in secretory burst frequency alone was not demonstrated for any of the seven anterior pituitary hormones studied. Of interest, PRL manifested a diurnal rhythm in both tonic (interburst nadir) secretory rates and secretory burst amplitude. Thus, we have found that different hormones exhibit distinctive modes by which their 24-h rhythms in plasma hormone concentrations can be generated.

The most prominent feature of the 24-h variations in specific features of secretory bursts was GH secretory burst amplitude<sup>2</sup>, which exhibited an 81% nyctohemeral variation (ratio of the magnitude of the 24-h rhythm to

<sup>2</sup> A commensurate rhythm in secretory burst mass can be inferred, since the two measures (mass and amplitude) are typically related for any particular waveform.

the mean of 24-h rhythm). These estimates presumably slightly underrepresent the full variation in serum GH concentrations, which appear to remain pulsatile even at levels below our and other conventional assay sensitivity limits (18). ACTH secretory burst amplitude was also highly diurnally specified, since the 24-h percent change in ACTH secretory burst amplitude was 60%. Other hormones typically exhibited a variation of 12–25% in secretory burst amplitude. Secretory burst frequency was also regulated over 24 h, with absolute variations in interburst intervals over 24 h of 56 min for GH, 46 min for  $\beta$ -endorphin, and 16 min for TSH (difference between the maximal value and the minimal interpulse value achieved with the 24-h rhythm). Thus, significant 24-h excursions in secretory burst amplitude and frequency can be specified by individual hypothalamo-pituitary control mechanisms that activate specific pituitary-hormone secretory bursts.

In general, the timing (acrophases) of the maximal values in the 24-h rhythms of secretory burst amplitudes for different anterior pituitary hormones in normal men coincided with or preceded the corresponding times of maximal plasma hormone concentrations (PRL, TSH, GH, ACTH, and  $\beta$ -endorphin). However, of particular interest, TSH secretory burst amplitude reached a maximum at 0211 h, whereas TSH secretory burst frequency reached a maximum at 1207 h ( $P < 0.05$ ). Although GH secretory burst amplitude and frequency peaked at 0228 and 0408 h, respectively, these acrophases were not significantly different. Similarly, the tendency of  $\beta$ -endorphin secretory burst amplitude to reach its maximum earlier (0532 h) than secretory burst frequency (0812 h) was not significant. In the case of TSH, separate neuroendocrine control of secretory burst amplitude and secretory burst frequency can be postulated, since the acrophases of the 24-h rhythms in TSH secretory burst amplitude and secretory burst frequency were statistically distinct. Further studies in additional subjects are required to confirm these findings and provide direct information about the possible mechanisms by which a putative TSH burst-generating system might specify frequency regulation that is temporally distinct from amplitude control. However, our observations are consistent with earlier findings that serum TSH concentrations can increase both before and during sleep (19). In the present studies sleep data were not available as independent measures for possible correlation with secretory parameters.

The exact neuroendocrine mechanisms that give rise to 24-h variations in the frequency and/or amplitude of secretory bursts generated by the anterior pituitary gland *in vivo* in man cannot be stated definitively. In principle, variations in adenohypophyseal secretory burst frequency could arise from neural mechanisms of hypothalamic (or other central nervous system) origin(s) that

activate or inhibit the respective pulse generator systems believed to control the number and timing of pituitary secretory events (20–24). Although some evidence exists that low amplitude episodes of pituitary hormone release can be observed in *in vitro* perifusion systems in the absence of hypothalamic input (25–27), the exact mechanisms generating such release *ex vivo* are not known, and their relevance to *in vivo* pathophysiology has not been defined. In contrast to frequency control, amplitude of secretory bursts is specified presumably by both the net magnitude of the hypothalamic signal and the responsiveness of anterior pituitary cells to that signal. The latter is modulated by the physiological state of the organism (*e.g.* nutrition), the biological activity of cells within the anterior pituitary gland (*e.g.* intracellular granule content, hormone biosynthetic rate, *etc.*), and the systemic endocrine milieu (*e.g.* steroid hormone concentration in plasma) (1–3, 7–11, 25–35). Thus, we suggest that regulation of secretory burst frequency over 24 h is likely to be enacted at the hypothalamic and/or other central nervous system levels, whereas control of 24-h variations in secretory burst amplitude can be exerted via hypothalamic, intrapituitary, and/or systemic mechanisms.

In summary, we have used a new waveform-independent deconvolution technique to evaluate the mechanisms by which 24-h rhythms in plasma concentrations of adenohypophyseal hormones are generated in normal men. We observed that frequency modulation alone did not account for the 24-h rhythms in serum concentrations of LH, FSH, PRL, TSH, GH, ACTH, or  $\beta$ -endorphin. In contrast, amplitude modulation alone was sufficient to give rise to the nyctohemeral variation in plasma LH and ACTH concentrations, and combined amplitude and frequency modulation occurred for TSH, GH, and  $\beta$ -endorphin release. Of interest, both the amplitude of secretory bursts and interburst nadir secretory rates were subject to 24-h control in the case of PRL. The secretory rate of FSH was not modulated in a demonstrably nyctohemeral fashion in this group of men. Thus, we conclude that individually distinct neuroendocrine mechanisms underlie the 24-h rhythms in plasma concentrations of different adenohypophyseal hormones in normal man.

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