

The pituitary gland secretes in bursts: Appraising the nature of glandular secretory impulses by simultaneous multiple-parameter deconvolution of plasma hormone concentrations

(hormone clearance/adenohypophysis)

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ABSTRACT To investigate patterns of endogenous hormone release, we have proposed a biophysical model in which measured hormone concentrations at any given instant reflect the operation of a suitable cumulation function (secretory input) convolved with an appropriate elimination mechanism (metabolic clearance). The cumulation function underlying a macroscopic hormone secretory burst can be represented by a random (Gaussian) distribution of instantaneous molecular secretory rates, which are centered with some finite and determinable standard deviation about a particular moment in time. The hormone elimination mechanism is described by a mono- or biexponential clearance function. The resultant convolution integral is solved by iterative nonlinear least-squares parameter estimation, in which all plasma hormone concentrations and their variances are considered simultaneously. Experiments with human endocrine time series revealed that the spontaneous secretory patterns of any of multiple distinct anterior pituitary hormones (luteinizing hormone, follicle-stimulating hormone, growth hormone, prolactin, thyrotropin, and adrenocorticotrophic hormone) can be described effectively by this parsimonious model. In addition, endogenous hormone disappearance rates determined by deconvolution agreed well with those reported earlier that were determined after exogenous hormone injections. Moreover, this model predicted that durations of underlying secretory impulses are extremely brief; i.e., the standard deviations of the Gaussian distributions of instantaneous secretory rates range from 4.5 min (luteinizing hormone) to 16 min (growth hormone) compared to plasma hormone concentration peaks of 90–140 min in duration. Accordingly, we conclude that observed physiological patterns of fluctuating plasma hormone concentrations can be accounted for by distinct, highly delimited, random bursts of hormone release separated by intervals of secretory quiescence.

Endocrine glands are believed to signal their remote target organs by an intermittent rather than a continuous mode of hormone secretion (1, 2). This presumptively episodic pattern of agonist release might avoid down-regulation of target tissue responses, which could otherwise occur if cells were exposed to unvarying concentrations of a trophic agent. Despite this general inference, few if any analyses have addressed the following physiological question: What is the nature of underlying secretory events that are translated into episodic changes in circulating hormone concentrations?

In the present work, we suggest that the secretion of biological macromolecules by an endocrine gland comprises a population of random instantaneous molecular release events, with finite probable amplitude, standard deviation,

and temporal position. Since metabolic removal mechanisms operate simultaneously with hormone secretion, these two processes are related by a convolution integral, which can be solved by iterative nonlinear least-squares parameter estimation in which all plasma hormone concentrations and their variances are considered simultaneously. The resultant mean parameter estimates and their corresponding statistical confidence limits represent predicted characteristics of the underlying secretion impulses and elimination functions that gave rise to observed fluctuations in plasma hormone concentrations. Here we have defined this experimental model of hormone secretion and tested its applicability to diverse endocrine time series.

METHODS

Collection of Endocrine Data. To obtain detailed endocrine time series, blood samples were withdrawn from healthy young male volunteers at frequent equally spaced intervals (e.g., every 5 min) for extended times (2). The sera were subjected to radioimmunoassay to estimate concentrations of luteinizing hormone (LH), growth hormone (GH), prolactin (PRL), thyrotropin (TSH), or adrenocorticotrophic hormone (ACTH). All samples from an individual were evaluated in the same assay in order to avoid interassay variations. Samples were assayed in duplicate or triplicate, and within-sample (between replicate) variances were related to the sample mean by a power function (2).

Specifying the Convolution Integral. A model of hormone secretion and elimination was postulated as described by the following convolution integral:

$$C(t) = \int_0^t S(z)E(t-z)dz, \quad [1]$$

where $C(t)$ is the concentration of hormone in the serum at any instant in time, t ; $S(z)$ is the impulse function, or the amount of hormone secreted per unit distribution volume per unit time evaluated at time z ; and $E(t-z)$ is the impulse-response function, or the amount of hormone elimination (clearance) that occurs in the time interval $(t-z)$ (3).

The impulse function, $S(z)$, which denotes the secretory rate as a function of time, was assumed to consist of a finite series of pulses, with nonzero positive amplitudes, that occurred at various times throughout the sampling interval. (An underlying "tonic" or invariant mode of secretion would add a baseline component to the data.) In its simplest form,

Abbreviations: LH, luteinizing hormone; GH, growth hormone; PRL, prolactin; TSH, thyrotropin; ACTH, adrenocorticotrophic hormone; FSH, follicle-stimulating hormone.

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a pulse was defined as a random Gaussian distribution of instantaneous molecular secretory rates. Accordingly, the mathematical form of $S(z)$ can be given as

$$S(z) = \sum_{i=1}^n A_i e^{-1/2 \left(\frac{z - PP_i}{SD} \right)^2}, \quad [2]$$

where A_i is the amplitude of the i th secretory impulse (mass of hormone released per unit distribution volume per unit time), PP_i is the i th peak (impulse) position in time, z is time, i is the peak number in the series, and SD is the standard deviation of the Gaussian distribution of instantaneous molecular secretion rates comprising a burst. The burst half-duration (or half-width) equals 2.354 times this SD.

The $E(t - z)$ term can be described reasonably by either a one- or two-component model with two half-lives, HL_1 and HL_2 , and a fraction, f , which describes their relative contributions:

$$E(t - z) = f e^{-[0.693(t-z)/HL_1]} + (1 - f) e^{-[0.693(t-z)/HL_2]}. \quad [3]$$

Reconvolution Fitting of Endocrine Data. Numerical integration of the convolution integral (Eq. 1) was undertaken by an adaptive, nine-point, Newton-Cotes numerical quadrature integration subroutine (4). Nonlinear least-squares parameter estimation was applied to minimize the variance of fit between the observed data and the calculated reconvolution curve (5). The dose-dependent variances associated with each sample mean were used in a weighting function when calculating the best-fit reconvolution curve.

Mean parameter estimates were obtained with their corresponding statistical confidence limits assuming asymmetric highly correlated variance spaces (5). The following independent parameter values were estimated in each time series: base (hormone concentration at time 0); standard deviation of the secretory Gaussian [if the reconvolution fit and/or calculated endogenous half-times of hormone disappearance are biased, one should consider non-Gaussian distributions of instantaneous secretory rates (e.g., skewed or other non-normal distributions)]; amplitudes of all individual secretory impulses; temporal positions of all secretory impulses; and single or dual component disappearance rate constants with their relative amplitudes. Assessment of parameter cross-correlation coefficients indicated that parameters of interest were virtually orthogonal, which permitted the effective use of nonlinear least-squares parameter estimation (5).

Based upon prior studies with pure human LH infusions, we assumed that hormone clearance mechanisms were not saturable over the physiological range (6). In the simplest form of the model, the disappearance rate constant(s), the distribution volume(s), and the standard deviation(s) of the secretory impulses were considered to be uniform for any particular individual time series, whereas secretory burst amplitudes and positions were allowed to vary.

Before deconvolution, potentially significant hormone concentration peaks were identified preliminarily by Cluster analysis (7).

RESULTS

Our multiple-parameter deconvolution technique was first tested on a simple earlier experimental paradigm, in which four individuals with LH deficiency had received a 1-min bolus injection of 35 μ g of highly purified human LH intravenously (6). Assuming that the intravenous bolus injection of LH resulted in an approximately Gaussian-distributed injection peak due to dilutional admixture at the leading and trailing edges of the impulse, we used a standard deviation of 0.425 min for the resultant Gaussian. The calculated reconvolution fits were in excellent agreement

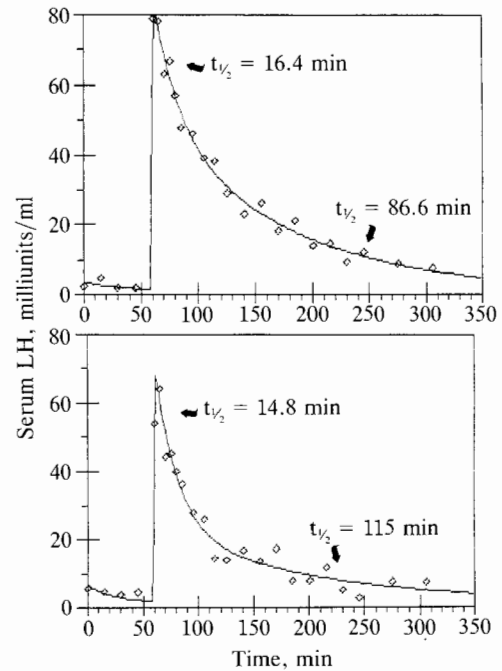


FIG. 1. Reconvolution curves for immunoactive LH time series resulting from the 1-min intravenous bolus injection of 35 μ g of pure human LH in two hypogonadotropic men. The convolution integral comprised a secretion function, $S(z)$, which was convolved with a biexponential clearance function, $E(t - z)$. The curves through the LH concentration data represent reconvolution fits determined by numerical integration of the convolution integral assuming an LH injection-pulse standard deviation of 0.425 min. The indicated half-lives were calculated by nonlinear least-squares simultaneous multiple-parameter estimation.

with the original injection and clearance profiles as shown in Fig. 1. The amplitudes and standard deviations of the secretion impulses were then used to compute the apparent mass of LH injected per unit distribution volume, by using the relationship: impulse peak area = amplitude \times standard deviation of the Gaussian $\times (2\pi)^{1/2}$. Deconvolution estimates of the injection mass of LH were within $\pm 12\%$ of the amount administered.

The deconvolution technique was next tested on endogenous hormone peaks. For intensive time series [serum LH concentrations measured by T. O. F. Wagner (Hannover, F.R.G.) in blood samples withdrawn at 1-min intervals for 400 min], simultaneous multiple-parameter fitting resolved five LH secretory impulses of significant nonzero positive amplitude (Fig. 2A). The mean estimates of these individual secretory and clearance parameters and their statistical (67%) confidence limits were secretory standard deviation of 7.7 (7.6–7.9) min; half-lives of 11 (6.1–2.0) and 119 (111–134) min; fractional amplitude of 0.33 (0.27–0.39); secretory impulse positions of 9.4 (7.6–11), 68 (66–71), 103 (97–110), 176 (175–178), and 318 (316–320) min; and secretory impulse amplitudes of 0.36 (0.30–0.40), 0.24 (0.22–0.27), 0.088 (0.068–0.12), 0.43 (0.38–0.46), and 0.25 (0.20–0.27) milli-units \cdot ml $^{-1}\cdot$ min $^{-1}$. Less frequent blood sampling (every 20 min) also permitted the resolution of discrete secretory episodes, as illustrated in Fig. 2B. In this circumstance, the amplitude (maximal rate) of the secretory bursts (amplitude of the LH secretory impulse) was 0.72 ± 0.084 milli-units \cdot ml $^{-1}\cdot$ min $^{-1}$ (mean \pm SEM) with an average interpulse interval of 79 min. Despite the very few samples used in this analysis, the standard deviation of the LH secretory burst could be estimated as 3.7 (3.0–4.6) min.

To analyze spontaneous GH secretion, blood samples were withdrawn at 5-min intervals for 480 min. The resultant GH

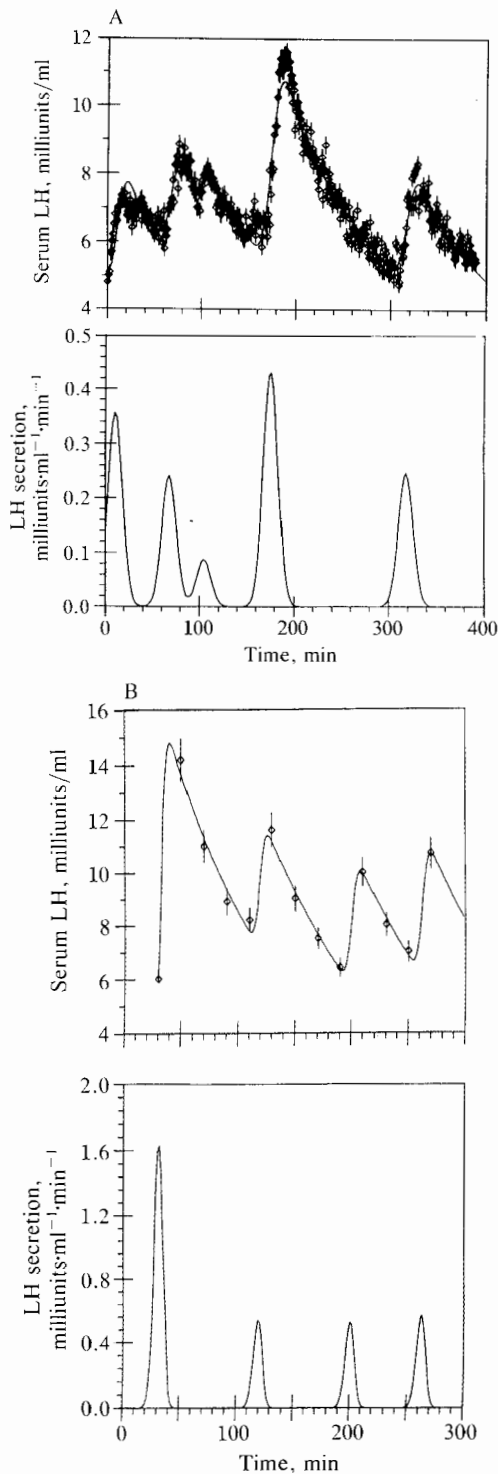


FIG. 2. Instantaneous LH secretory profiles in human volunteers sampled at 1-min (A) or 20-min (B) intervals. The curves in the *Upper* panels of A and B depict the calculated reconvolution fits (continuous curves) traversing the serum immunoreactive LH concentrations with their individual standard deviations (vertical bars). The *Lower* panels of A and B show the resolved instantaneous secretory profiles.

concentration time series (Fig. 3) contained two large peaks and a small intervening plateau that was not identifiable as a "peak," using eight different discrete pulse detection procedures (J.D.V. and M.L.J., unpublished results). However, deconvolution analysis unmasked three distinct and statistically significant (i.e., nonzero positive amplitude) growth hormone secretory bursts. Accordingly, this deconvolution

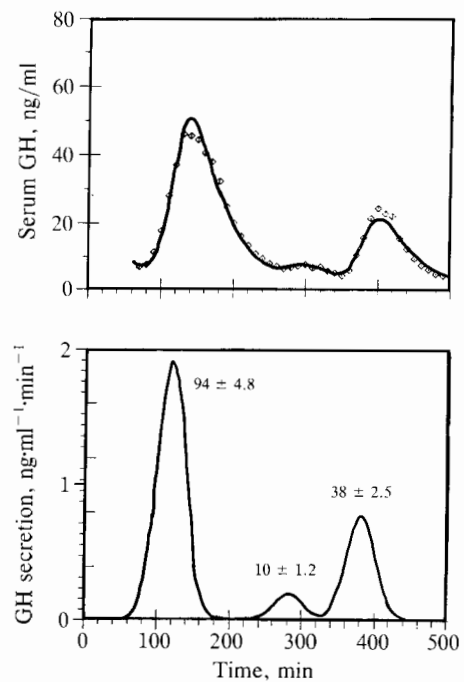


FIG. 3. Deconvolution of immunoreactive (GH) time series in a normal fasted man, who underwent blood sampling at 5-min intervals for 6 hr. (*Upper*) The fitted reconvolution curve (continuous line) in relation to the original GH concentrations (sample means) over time. (*Lower*) The calculated instantaneous GH secretory profile. Values by the peaks denote the estimated mass (means \pm SD) of GH secreted per pulse (ng/ml of distribution volume). The GH profile was provided by M. L. Vance and M. O. Thorner (University of Virginia, Charlottesville).

technique may also help resolve underlying secretory episodes with little if any absolute increase in plasma hormone concentrations, when the temporal profile is inappropriate for the expectation of continued decay (clearance).

The applicability of this convolution integral model to other hormonal [follicle-stimulating hormone (FSH), PRL, TSH, and ACTH] time series was also tested, as illustrated in Fig. 4. In each case, the serum hormone concentration profile could be defined effectively by a model of distinct random secretory bursts acted upon by exponential clearance mechanisms. Moreover, estimates of endogenous half-times of hormone disappearance were in good agreement with available literature estimates derived from exogenous hormone injections (Fig. 5).

DISCUSSION

The nature of endogenous secretory events cannot be readily discerned in the intact animal or human because instantaneous plasma hormone concentrations result from prior and contemporaneous secretion acted upon continuously by metabolic clearance. In an effort to resolve the nature of *in vivo* secretory processes, we propose a conservative model of hormone pulsation in which plasma hormone concentrations are determined by discrete random (Gaussian) secretory bursts of finite number operated upon by exponential clearance mechanisms, as illustrated schematically in Fig. 6. We have determined experimentally that this simple model provides an excellent and general fit of endocrine data. The generality of this paradigm was indicated by its applicability to multiple biological macromolecules discharged by the anterior pituitary gland (LH, FSH, GH, PRL, TSH, and ACTH).

The present model of the episodic nature of endocrine glandular secretion has several important features and im-

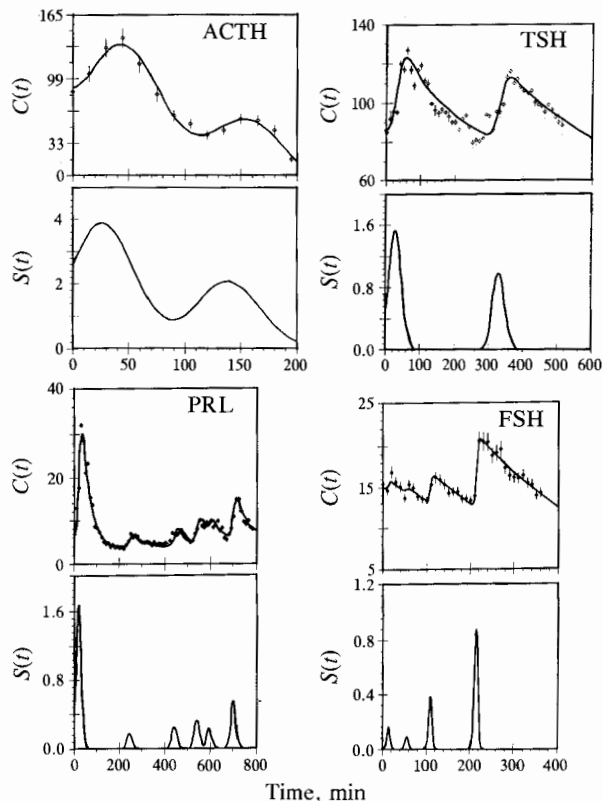


FIG. 4. Individual reconvolution fits of serial plasma hormone concentrations, $C(t)$, and the corresponding deconvolved secretory functions, $S(t)$, for FSH, PRL, TSH in a hypothyroid man, and ACTH. Units for $C(t)$ are milliunits/ml (FSH), ng/ml (prolactin), microunits/ml (TSH), and pg/ml (ACTH). Units for $S(t)$ are in terms of $C(t)$ per min. The ACTH and TSH data were provided, respectively, by B. Sherman (University of Iowa) and A. Iranmanesh (Veterans' Administration Hospital, Salem, VA).

plications. First, the long-held but unproven tenet that hormone secretion occurs in both a tonic and episodic mode

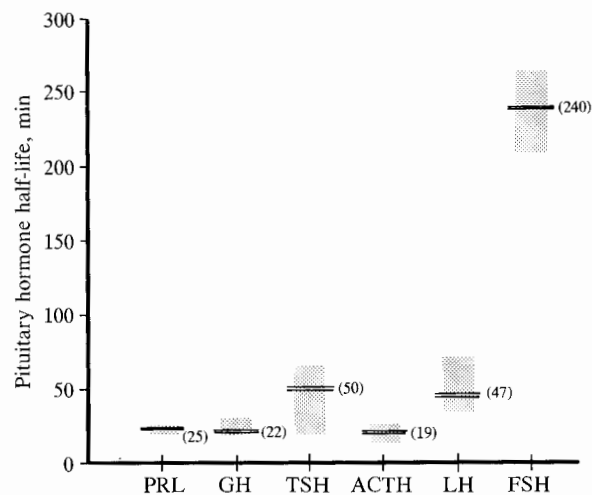


FIG. 5. Calculated endogenous half-lives of disappearance from plasma of six anterior pituitary hormones. Simultaneous multiple-parameter fitting was used to estimate endogenous plasma half-times of clearance from endocrine time series (plots of immunoreactive hormone concentrations over time) without the injection of radiolabeled or unlabeled hormone. Calculated monoexponential hormone half-lives and their 67% confidence limits are given by the stippled bars. Corresponding literature estimates (6, 8) are denoted by the two horizontal bars (numerical values of mean literature estimates are given in parentheses).

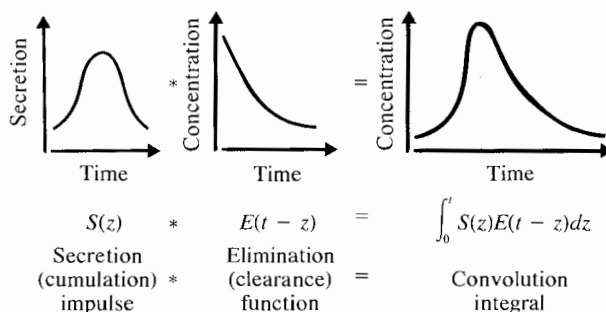


FIG. 6. Schematic illustration of the multiple-parameter deconvolution model proposed here for analyzing endocrine pulse signals. Plasma hormone concentrations at any given instant are assumed to reflect the simultaneous operation of four sets of determinable parameters: (i) the temporal position(s), (ii) standard deviation(s), and (iii) amplitude(s) of all underlying secretory impulses, acted upon continuously by (iv) endogenous metabolic clearance. Simultaneous values of multiple secretion and clearance parameters with statistical confidence limits are determined by nonlinear least-squares analysis, in which the combined influences of all plasma hormone concentrations and their variances are considered.

is not required to account adequately for the behavior of many endocrine time series. Rather, discrete random secretory bursts operated upon by exponential clearance mechanisms are sufficient. Although a random Gaussian distribution of instantaneous molecular release rates fits the present experimental data well, our model may be adapted to accommodate non-Gaussian (e.g., skewed, Poisson, and Lorentzian) distributions as well. In addition, the model can be expanded to allow for multiple distinct secretory impulse half-durations and/or multiple distribution and elimination compartments, if indicated for certain hormones or certain pathophysiological states. Second, simultaneous multiple-parameter deconvolution analysis of endocrine data permits one to estimate endogenous production rates and half-lives of hormone clearance without administering radiolabeled or exogenous hormones. Estimating endogenous disappearance rate constants for each individual and for each experimental context obviates the *a priori* assumption inherent in earlier approaches that a single and uniform disappearance rate constant is known for, and applicable to, different individuals (9–12). If the biological rate constants are known *a priori* and absolutely (i.e., values in Eq. 3), then there are several model-independent methods for deconvolution analysis (e.g., based on Laplace or Fourier transforms of the experimental data). However, such methods do not allow one to solve for endogenous rate constants and perform proper statistical analyses that account for error propagation within the time series.

A third feature of the present algorithm of nonlinear least-squares parameter estimation is its provision of mean best-fit values and statistical confidence limits for each secretory and clearance parameter. Thus, for example, the probability that an individual secretory burst has a significant nonzero (positive) amplitude can be estimated. This statistically based approach represents a desirable advance over earlier discrete or continuous deconvolution procedures (9–12). Moreover, the present model will allow testing of various pathophysiological states, which we postulate may exhibit alterations in the frequency, amplitudes and/or half-durations of underlying secretory bursts, and/or changes in endogenous clearance rates.

A fourth important implication of this deconvolution model is that optimal estimates of clearance and secretory parameter values can be accomplished by considering all hormone concentrations and their variances simultaneously (Table 1).

Table 1. Features of the multiple-parameter deconvolution algorithm

1. All experimental data and their variances are considered when estimating optimal parameter values.
2. A mean estimate and corresponding statistical confidence limits are calculated for each secretion and clearance parameter. Only positive nonzero estimates are obtained.
3. Endogenous hormone disappearance rates are computed from a model of combined secretion and clearance. Disappearance rate constants are thus estimated in each individual and/or experimental context.
4. High data densities (e.g., high sampling frequencies) enhance rather than detract from parameter estimation.
5. The temporal locations as well as the amplitudes and half-durations of secretory impulses can be estimated. This allows assessment of secretory concordance for two or more different hormone series.

This effective use of all available data and variance estimates greatly enhances the statistical power of the deconvolution procedure. For example, such an approach eliminates the instability (repetitive positive and negative excursions in secretion estimates) that occurs when other deconvolution methods are tested at high sampling frequencies (2, 10).

In the present paradigm, endogenous hormone disappearance rates are calculated using a model in which underlying secretion and clearance are considered simultaneously. This concept differs from the conventional approach of evaluating clearance only from the descending limbs of plasma hormone concentration peaks (11, 12). As illustrated in Fig. 7, according to our deconvolution model the conventional approach would be expected to result in erroneous overestimates of endogenous half-lives, since the descending limbs of concentration peaks are augmented to various degrees by underlying secretory bursts. Secretory contributions to the decaying limbs of plasma hormone concentration peaks can be resolved correctly from clearance only when temporal locations, amplitudes, and half-durations of all relevant underlying secretory events are determined *simultaneously* with estimates of disappearance rate constants.

In the current multiple-parameter deconvolution model, the temporal locations of all secretory impulses of significantly nonzero amplitude are estimated with statistical confidence limits. Accordingly, investigators can appraise the

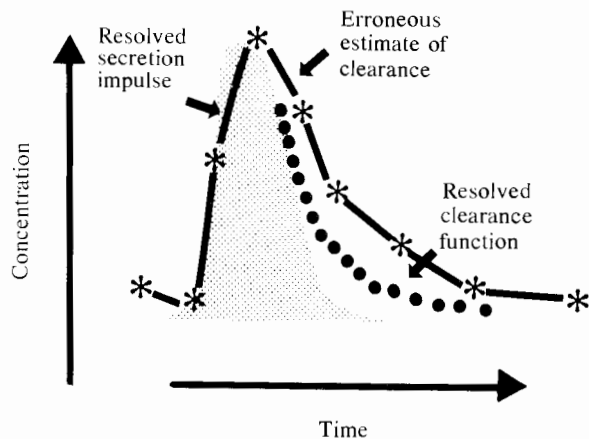


Fig. 7. Methods of estimating endogenous hormone disappearance rates. Note the widely used (but incorrect) procedure of calculating half-life directly from plasma hormone concentrations. In contrast, in our deconvolution analysis the descending limb of the hormone concentration peak reflects the combined influences of nonlinear secretion and clearance functions. The stippled area denotes the resolved secretion impulse.

probability of temporal concordance between two ostensibly cosecreted hormones with different endogenous secretory half-durations, amplitudes, and/or clearance rates. In particular, use of the underlying secretion function over time should eliminate potential artifacts in assessing the concordance between plasma hormone concentration peaks resulting from different hormone concentrations at baseline, dissimilar secretory impulse properties, and/or unequal metabolic clearance rates.

The use of deconvolution modeling should also provide a powerful new tool to model false-positive and false-negative errors in endocrine peak detection. We suggest that reconversion fits through physiological data offer a basis for defining "idealized" hormone peaks mathematically under various experimental conditions. By perturbing idealized endocrine peaks with random measurement variance (noise) or systematic baseline drift (underlying tonic secretion), one can assess false-positive and false-negative error rates for various peak detection procedures. Moreover, investigators can also model particular pathophysiological states of presumptively altered secretory properties and/or clearance rates.

Of particular physiological interest is our observation that the majority of variance inherent in hormone concentration time series can be accounted for by a physiological model of distinct, short-lived, random, burst-like episodes of hormone release, acted upon by endogenous clearance mechanisms. Such observations suggest that episodic signaling of remote target organs by endocrine glands can be achieved at a high efficiency, inasmuch as brief secretory bursts can yield sustained increases in circulating hormone concentrations at the target tissue. The efficiency of this mode of episodic glandular signaling should vary in relation to particular clearance and secretory properties. Accordingly, the present model of episodic burst-like secretion by endocrine glands should provide an additional powerful tool for evaluating these and other physiological aspects of endocrine gland signaling.

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