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Biomathematical Modeling of Pulsatile Hormone Secretion: A Historical Perspective

William S. Evans^{*}, Leon S. Farhy[†], and Michael L. Johnson[‡]

^{*} *Endocrinology and Metabolism Department of Medicine, and Department of Obstetrics and Gynecology, University of Virginia Health System, Charlottesville, Virginia*

[†] *Endocrinology and Metabolism Department of Medicine, University of Virginia Health System, Charlottesville, Virginia*

[‡] *Departments of Pharmacology and Medicine, University of Virginia Health System, Charlottesville, VA*

Abstract

Shortly after the recognition of the profound physiological significance of the pulsatile nature of hormone secretion, computer-based modeling techniques were introduced for the identification and characterization of such pulses. Whereas these earlier approaches defined perturbations in hormone concentration-time series, deconvolution procedures were subsequently employed to separate such pulses into their secretion event and clearance components. Stochastic differential equation modeling was also used to define basal and pulsatile hormone secretion. To assess the regulation of individual components within a hormone network, a method that quantitated approximate entropy within hormone concentration-times series was described. To define relationships within coupled hormone systems, methods including cross-correlation and cross-approximate entropy were utilized. To address some of the inherent limitations of these methods, modeling techniques with which to appraise the strength of feedback signaling between and among hormone-secreting components of a network have been developed. Techniques such as dynamic modeling have been utilized to reconstruct dose–response interactions between hormones within coupled systems. A logical extension of these advances will require the development of mathematical methods with which to approximate endocrine networks exhibiting multiple feedback interactions and subsequently reconstruct their parameters based on experimental data for the purpose of testing regulatory hypotheses and estimating alterations in hormone release control mechanisms.

1. Introduction

The second half of the 20th century witnessed an extraordinary number of scientific advances that coalesced to provide a much improved understanding of how endocrine systems in general—and the reproductive axis in particular—function as “networks” in which individual components communicate with one another in a highly coordinated fashion. Central to the reproductive system are cells within the anterior pituitary gland, which secrete two so-called “gonadotropins”—luteinizing hormone (LH) and follicle-stimulating hormone (FSH)—which control both gametogenesis and hormone production in the testes and ovary. Although our recognition of the role played by LH and FSH in relation to gonadal function dates back to the 1920s (Fluhmann, 1929), it was midcentury before the possibility that the hypothalamus might serve to regulate pituitary function, including that of the gonadotropin-secreting cells, was put forward by Green and Harris (1947). As depicted in Fig. 14.1, these investigators suggested that neuronal systems within the hypothalamus could both synthesize and secrete chemical (hormonal) messengers, which could then travel to the pituitary gland via the hypothalamic–hypophyseal portal circulation where they would influence the secretion of pituitary hormones. However, another two decades would elapse before the first of these hypothalamic hormones,

including gonadotropin-releasing hormone (GnRH), which stimulates the synthesis and release of LH and FSH, and thyrotropin-releasing hormone (TRH), which is responsible for thyrotropin stimulating hormone (TSH) production, were isolated, characterized, and synthesized by Guilleman (Burgus *et al.*, 1972) and Schally (Schally *et al.*, 1971; Matsuo *et al.*, 1971). The significance of this work was recognized by the Nobel Prize for Physiology or Medicine being awarded to these investigators in 1977.

Shortly after the discovery of GnRH, and capitalizing on the availability of a relatively new measurement technique known as radioimmunoassay, studies in the rat (Gay and Midgley, 1969), Rhesus monkey (Atkinson *et al.*, 1970), and the human (Midgley and Jaffee, 1971; Santen and Bardin, 1973; Yen *et al.*, 1972) suggested that LH and FSH were secreted in a pulsatile rather than a tonic fashion. The profound physiologic significance of this observation was demonstrated by Knobil and colleagues using the Rhesus monkey as a model (Knobil, 1980). Knobil's seminal studies demonstrated that whereas the administration of GnRH in a *pulsatile* fashion to monkeys in which the GnRH-secreting cells had been ablated did indeed effect the secretion of LH and FSH, the administration of the peptide as a *nonvarying infusion* failed to stimulate secretion of the gonadotropins (Fig. 14.2). Moreover, GnRH infused into monkeys with an intact hypothalamic–pituitary system actually *diminished* the secretion of the gonadotropins. These studies strongly suggested that GnRH itself might well be secreted in a pulsatile manner, thus stimulating the pulsatile secretion of LH and FSH. Approximately a decade later, studies in which GnRH and LH were measured simultaneously in the rat (Levine and Ramirez, 1982) and sheep (Clarke and Cummins, 1982; Levine *et al.*, 1982) within the hypothalamic–hypophyseal portal circulation and in the vasculature draining the anterior pituitary gland confirmed this direct relationship.

Subsequent basic science and clinical studies have served to underscore the extraordinary significance of the pulsatile nature of hormone secretion within the reproductive axis. Using the rat as a model, Marshall and colleagues have shown that the *gene expression* of LH and FSH is controlled by the frequency and amplitude characteristics of the pulsatile GnRH signal (Haisenleder *et al.*, 1994). Within the clinical setting, the significance of the characteristics of the GnRH signal has been exploited by clinicians who wish either to *restore* normal gonadotrope function by means of the exogenous pulsatile administration of GnRH (e.g., in cases of primary hypothalamic failure such as Kallmann's syndrome or hypothalamic failure-associated infertility) or to *inhibit* gonadotropin secretion by means of a long-acting GnRH agonist (e.g., in the setting of prostrate cancer, precocious puberty, or endometriosis), which provides a nonpulsatile signal to the pituitary and serves to desensitize the pituitary and essentially abolish LH and FSH secretion.

2. Early Attempts to Identify and Characterize Pulsatile Hormone Release

A logical extension of this improved understanding of both the fundamental physiological underpinnings of pulsatile hormone release noted earlier and the potential importance of pulsatile secretion in terms of clinical applications was the recognition of the need to define and subsequently characterize in an objective manner hormone pulses within concentration-time series. Examples of some initial efforts to achieve this goal included defining pulses as an increase followed by a decrease of an arbitrary number of units (e.g., 5 mIU in the case of LH; Yen *et al.*, 1972) or an increase in the serum concentration of a hormone of at least 20% over the proceeding nadir (the so-called “Santen and Bardin” algorithm; Santen and Bardin, 1973). For whatever the reason, however, nearly a decade passed before the power of computer-assisted algorithms for the identification and characterization of pulsatile hormone secretion—and importantly the impact of how blood sampling protocols may affect the ability to define such pulses—began to receive investigative attention.

3. Impact of Sampling Protocol and Pulse Detection Algorithm on Hormone Pulse Detection

The early to mid-1980s saw a flurry of activity focused on the development of a range of sampling protocols (e.g., protocols in which blood samples were obtained anywhere from every 1 to every 60 min over intervals of 2–24 h) and of computerized pulse analysis techniques, each subserved by quite different mathematical constructs. The latter included *Ultra* developed by Van Cauter and colleagues (1981), *Pulsar* by Merriam and Wachter (1982), and *Cycle Detector* by Clifton and Steiner (1983). The use of sampling protocols of differing intensity and interval—and analysis of the resultant hormonal concentration-time series with computer-assisted methods based on nonuniform mathematical and statistical approaches—perhaps not surprisingly resulted in quite diverse estimates of pulsatile hormone secretion, in many cases under theoretically similar physiological conditions. Thus, application of these earlier pulse detection programs to LH concentration-time series obtained in normal women at several phases of the menstrual cycle resulted in the identification of anywhere from 14 to 24 LH pulses/24 h in early follicular phase women, from 17 to 29 in late follicular phase women, and from 4 to 16 in midluteal phase women (Backstrom *et al.*, 1982; Burger *et al.*, 1985; Crowley *et al.*, 1985; Filicori *et al.*, 1984, 1986; Murdoch *et al.*, 1989; Reame *et al.*, 1984; Rossmann *et al.*, 1990; Schweiger *et al.*, 1989; Soules *et al.*, 1984).

To define the impact of sampling protocol and of pulse identification method on the ability to detect LH pulses in men and women, we applied several of the available pulse detection algorithms to serum LH concentration-time series obtained from normal men (Urban *et al.*, 1988) and normal women at different phases of the menstrual cycle (Evans *et al.*, 1992). Both the sampling protocol and the pulse detection algorithm were found to have a profound effect on the number of pulses detected. With regard to sampling intensity, up to an eightfold difference in the number of pulses detected within the same data set could be found when the complete data set (sampled at 4-min intervals) was progressively “deintensified” by omitting successive samples to simulate sampling at, for example, 12-, 20-, and 40-min intervals. Similar concerns were apparent when data from normal women during the midluteal phase of the menstrual cycle were analyzed with four of the independently developed computer-based techniques mentioned previously. As is shown in Fig. 14.3, when data obtained with a standard protocol (samples obtained at 10-min intervals over a 24-h interval) were analyzed, estimates for the number of pulses varied from 3 to 17 per 24 h.

Such divergent results prompted the development of yet more pulse detection algorithms. Nearly simultaneously, two novel programs became available to the scientific community: *Detect*, written by Oerter, Guardarraso, and Rodbard (Oerter *et al.*, 1986), and *Cluster*, provided by Veldhuis and Johnson (Veldhuis and Johnson, 1986). Although using distinctly different mathematical approaches, both programs shared the properties of being rigorously statistically based. Whereas *Cluster* identifies statistically significant increases and decreases in the concentration-time series with a sliding *T* test, *Detect* defines pulses as statistically significant increases and decreases in the numerical first derivatives of the concentration-time series. Of interest, when these programs were applied to the LH concentration-time series mentioned earlier, the number of LH pulses identified was statistically indistinguishable.

4. Application of Deconvolution Procedures for the Identification and Characterization of Hormone Secretory Bursts

Although computer-assisted pulse detection algorithms represented a significant advance in our ability to characterize hormone secretion, it was soon recognized that such methods identify *perturbations* in concentration-time series rather than describing actual underlying secretory

events. The appreciation that a hormone “pulse” comprises both a secretory event and factors that affect clearance of the hormone, such as distribution, binding to proteins, metabolism, and excretion, stimulated interest as to whether mathematical methods might exist that would allow for the “separation” of a hormone pulse into its constituent secretory burst and clearance components. Indeed, Turner and colleagues (1972), Rebar and colleagues (1973), and MacIntosh and MacIntosh (1985) were among the first to employ *deconvolution* procedures to achieve this objective. The latter two groups determined that, for the case of LH, secretion did in fact occur in a burst-like fashion without the requirement for a tonic secretory component. The deconvolution procedures used in these earlier studies, however, had their own limitations, including the need for information about the half-life of the hormone under consideration. For example, in the case of one of the methods mentioned previously (Rebar *et al.*, 1973), the deconvolution procedure employed a sinusoidal function for which an estimated two-compartment half-life component was needed. Similarly, in a later report, Genazzani and colleagues (1990) applied “discrete” (sample-by-sample) deconvolution analysis, which again required a literature estimated LH half-life.

Recognizing the need for an approach that would not be constrained by the requirement for a priori information about the half-life of the hormone of interest, Veldhuis and colleagues (1987) developed a deconvolution procedure that allowed for simultaneous assessment of both the characteristics of the hormone secretory burst and provided an estimate of the hormone half-life. Known as *Deconv*, this multiparameter deconvolution method differed from earlier deconvolution procedures in that it describes secretion as a variable number of Gaussian-shaped secretion events convolved with a one- or two-component exponential elimination and then fit directly to the concentration-time series. Several of the earlier approaches as mentioned above evaluated secretion as a discrete amount at each data point but did not locate the distinct secretory events (McIntosh and McIntosh, 1985; Oerter, 1986; Rebar *et al.*, 1973). Algorithms such as *Cluster* or *Detect* were required to identify the position of the peaks in the secretion based on the evaluation at every data point produced by the earlier methods (Oerter, 1986; Veldhuis and Johnson, 1986). In contrast, *Deconv* allows for the identification of significant secretion events directly by the fitting process. The earlier approaches were also very susceptible to measurement errors within the concentration-time series.

Figure 14.4 shows a LH concentration-time series obtained from a normal woman during the early follicular phase of her menstrual cycle (top) and the LH secretory events as resolved by application of *Deconv* (bottom). In addition to identifying the secretory bursts, *Deconv* also provides information about secretory burst mass, half-duration, and an estimate of hormone half-life. Indeed, shortly after *Deconv* became available, this method was used to analyze data obtained from normal women at several phases of the menstrual cycle, given that our understanding of physiology predicted differences in (1) the *frequency* of secretory events, (2) the *characteristics* of these events underlying the secretory process itself, and (3) the *half-life* of the hormone (reflecting post-translation processing of the protein)(Sollenberger *et al.*, 1990). As can be seen in Fig. 14.5, although the total daily secretion of LH did not vary across the menstrual cycle, the deconvolution-resolved number of secretory bursts, their characteristics, and their half-life varied considerably as a function of the phase of the cycle in the younger women and in the postmenopausal group.

5. Limitations and Subsequent Improvements in Deconvolution Procedures

While deconvolution procedures represented a major advance in our ability to model hormonal data, it became readily apparent that the earlier versions had significant limitations, including the subjective nature of the choice of candidate secretory bursts, the lack of robust statistical verification of resolved secretory bursts, and the user-unfriendly interface of the programs. To address these concerns, a novel deconvolution procedure known as *AutoDecon* has been

developed and validated for LH (Johnson *et al.*, 2008) and growth hormone. This program finds the optimal number of secretory bursts and initial parameter estimates while simultaneously performing the deconvolution. Moreover, the fact that *AutoDecon* is fully automated and statistically based addresses the issues noted earlier, including the user-unfriendly nature of prior deconvolution methods and the previous lack of statistical verification of resolved secretory events. Analysis with *Auto-Decon* results in a substantially higher true-positive rate of identification of hormone secretory events than analysis with *Cluster*. Moreover, both false-positive and false-negative rates are lower with *AutoDecon* compared to *Cluster* and sensitivity is substantially higher with *AutoDecon*.

6. Evaluation of Pulsatile and Basal Hormone Secretion Using a Stochastic Differential Equations Model

An alternative method proposed by Keenan and Veldhuis (1997) to appraise hormone secretion uses a combination of a model of random, but structured, variations in pulse amplitudes superimposed on basal hormone release with a nonstationary Poisson process generating the timing of the hormone pulses. This approach utilizes stochastic differential equations to evaluate certain properties of irregular hormone time series, including pulse onset times, basal secretion, and pulse shape, and has the advantage of using an automated procedure for initial estimate of the pulse positions. However, in this method the amplitude of each pulse is assumed to depend on the previous interpulse length, which is a restrictive condition that may not be valid in all hormone release control systems.

7. Characterization of Regulation of Signal and Response Elements: Estimation of Approximate Entropy

Following the development of methods for hormone pulse and secretory burst identification as described previously, attention focused on whether quantitative techniques could be utilized to appraise the regulation of individual “nodes” (i.e., hormone-secreting components) within overall hormone networks. Such components often respond to a primary input signal but are, at the same time, subject to modulation by a separate signal in the form of “feedback” from the target gland. Again using the reproductive network as an example, LH- and FSH-secreting gonadotropes within the anterior pituitary gland respond to GnRH from the hypothalamus (acting as the primary signal), but the pituitary response to the GnRH signal may be influenced significantly by ambient concentrations of hormones such as estrogen and progesterone secreted by the ovary (i.e., the target gland for LH and FSH). An early attempt to characterize regulation of a single hormone secretory component sought to determine, using quantitative methods, the degree of “orderliness” within a hormone concentration-time series, which was considered to reflect the degree to which the gland is regulated by both primary and feedback signals. To assess such orderliness, Pincus (1991) developed a mathematical approach with which to estimate “approximate entropy” within the data series. This approach (known as *ApEn*) is complementary to hormone pulse and secretory burst activity in that it evaluates both dominant and subordinate patterns in concentration-time series. Given its ability to detect changes in underlying episodic behavior, which will not be reflected in pulse occurrences or their characteristics, *ApEn* acts as an explicit “barometer” of feedback system change in many coupled systems. In addition to its application to components of the reproductive system (Pincus *et al.*, 1997), *ApEn* has been utilized to probe the regulation of other hormones, including growth hormone (Friend *et al.*, 1996; Hartman *et al.*, 1994), aldosterone (Siragy *et al.*, 1995), cortisol (van den Berg *et al.*, 1997), and insulin (Meneilly *et al.*, 1997).

8. Evaluation of Coupled Systems

In the same general time frame that attention was increasingly being focused on how the regulation of individual hormone-secreting components might be appraised in quantitative terms, methods with which to define the activity of *coupled hormonal systems* were being proposed. One of the earliest such approaches utilized cross-correlation, a standard and widely applied method for estimating the degree to which two data series are related. It was argued that cross-correlation could be used to support the existence of a relationship between two nodes in the hormone network and indeed this method has been applied successfully in various circumstances (Booth *et al.*, 1996; Veldhuis *et al.*, 1988). However, the straightforward use of cross-correlation suffers from several disadvantages, including spurious values due to autocorrelation and the linear nature of the assumed relationship. These limitations prompted the development of other methods, specifically adapted to endocrine applications.

Within this context, and shortly after the development of *ApEn* to appraise regulation of a single hormone-secreting node, Pincus and Singer (1996) suggested that the signal-response relationship between coupled nodes within a hormonal network could be described by estimating their cross-approximate entropy. Indeed, the program *Cross-ApEn* assesses the degree of asynchrony between two hormonal concentration-time series known to be coupled and determines whether the coupling is more “ordered” or more “random.” Taking the LH–testosterone system as an example, application of *Cross-ApEn* demonstrated more irregularity or “asynchrony” in data obtained from older vs younger men, even though the mean serum concentrations of LH and testosterone were not found to differ as a function of age (Pincus *et al.*, 1996). Thus, *Cross-ApEn* reinforced the notion that information of potentially significant physiologic interest could be obtained by quantitating the interactions of coupled systems within the overall hormonal network.

Quite recently, approaches using distinctly different mathematical methodologies from those subserving cross-correlation or *Cross-ApEn* have been developed. Farhy and colleagues have proposed a method that seeks to define and quantitate relationships whereby one hormone modulates the release of a second hormone (Nass *et al.*, 2008). This method (referred to as *FeedStrength*) overcomes some of the problems associated with the application of the more traditional statistical methods (e.g., cross-correlation) for estimation of the relationships between two simultaneously sampled hormones. To test the hypothesis that hormone A modulates the secretion of hormone B, *FeedStrength* first determines the individual secretion bursts of hormone B using deconvolution procedures. The amplitude of each secretory burst of hormone B is compared to the average concentration of hormone A over a fixed time interval during and before each hormone B burst. Rather than comparing the entire hormone A and B concentration profiles (as would be the case if cross-correlation is used), the method relates the amplitude of an individual hormone B burst to the concentration of hormone A accompanying the development of this burst. This approach has the advantage that it disregards those parts of the release profile of hormone A during which there is no pulsatile secretory activity of hormone B. Consequently, the method is suitable for analyzing endocrine relationships in which one of the hormones is presumed to be an amplifier/modulator and not the sole regulator of the secretory activity of the second hormone.

The application of *FeedStrength* to estimate the amplifying effect of ghrelin on growth hormone secretion resulted in the first clear demonstration of a relationship between these two hormones in normal subjects (Nass *et al.*, 2008). That this approach is also suitable for estimating the variability in feedback relationships was proposed when the method was applied to synthetic data (Farhy *et al.*, 2006). As shown in Fig. 14.6, network feedback modeling was used to create a system mimicking the feedback effect of progesterone on LH secretion. Synthetic data were modeled to mimic serum LH (hormone B) stimulating progesterone

(hormone A) secretion in normal midluteal phase women. The network also assumes a self-entrained GnRH pulse generator (G) stimulating the secretion of B, an ovarian-hypothalamic delayed feedback component, and a system-independent external regulation (F). Estimates of the level of feedback control (LFC) were computed using *FeedStrength* as described (Nass *et al.*, 2008). Also shown in Fig. 14.6 are results of the application of this method to synthetic data sets in which the strength of the feedback signal (A-to-G) is progressively degraded and the external regulation (F-to-G) becomes dominant. As can be seen, the LFC for the A-to-B coupling identifies correctly gradual changes of the impact of A on the system relative to the effect of F across 11 different levels (L0–L10): (i) minimal LFC when only F, but not A, affects G (L0); (ii) a gradual LFC increase when the feedback becomes progressively dominant over the impact of F (L1–L9); and (iii) maximal LFC when only A, but not F, controls G (L10). Thereby, the LFC as calculated by *FeedStrength* provides a robust estimate of the feedback control exerted by one hormone on another when the main pulse-generating hormone is unknown and can estimate feedback alterations associated with such factors as age, gender, and pathophysiologic conditions.

An additional approach with which to assess the possibility that one hormone regulates the secretion of another hormone in a quantitative manner is to use dynamic modeling. To this end, Johnson and colleagues (2008) developed a computer-based approach for Signal-Response Quantification (*SRQuant*), which reconstructs the dose–response interactions between hormones by fitting parameters of a dynamic model to experimental data. Within this framework, several input signals can be individually delayed, spread in time, transformed by dose–response relationships, combined, and then convolved with a pharmacokinetic elimination function to predict the time course of the concentration of an output hormone. As an example, *SRQuant* has been used to estimate the effect of the endogenous opioid peptide antagonist naloxone on GnRH (and consequently LH) secretion using an animal model (the ovariectomized ewe) in which GnRH and LH were measured simultaneously in blood samples obtained from the hypothalamic–hypophyseal portal circulation (GnRH) and the jugular vein (LH) (Goodman *et al.*, 1995). A minimal model was constructed based on the assumption that GnRH stimulates the secretion of LH, which is, in turn, eliminated after its release into the circulation. The model has several physiological parameters (rate of elimination and secretion of LH, ED_{50} , and slope of the GnRH–LH dose–response interaction) (Farhy, 2004), which were determined simultaneously by *SRQuant* via an iterative procedure to achieve the best possible fit of the model output to the LH data if GnRH was used as input. The result of this process is illustrated in Fig. 14.7 and depicts a very good coincidence between the model predicted and observed LH concentration: 80% of the variance was explained, confirming the existence of a strong feed-forward dose–response relationship between GnRH concentration and LH secretion in this animal model. Further analysis showed that before naloxone administration, the model provides a much better fit of data by explaining approximately 88% of the variation in LH, whereas during the treatment only 68% of the variation was explained. In addition, a clear change in the “shape” of the dose–response interaction was detected after the treatment. This outcome suggests that at least part of the effect of naloxone treatment on the GnRH–LH axis reflects a direct effect on GnRH–LH relationships. It is interesting to note that the analysis of such GnRH–LH relationships with other methods, including cross-correlation and *Cross-AppEn*, does not provide satisfactory results: In this particular case the cross-correlation was only 0.64, which would account for about 36% of the variance in LH by the linear correlation model. The (ratio) *Cross-AppEn* values were 0.94 ± 0.08 ($m = 1$) and 1.07 ± 0.2 ($m = 2$), which are typical for a random rather than a strong dose-dependent relationship between the two hormones. Thus, *SRQuant* provides a viable alternative and a powerful addition to the more traditional methods for the appraisal of coupled endocrine systems.

9. Evaluation of Hormonal Networks with Feedback Interactions

Whereas the methods described earlier have allowed for a detailed appraisal of systems in which hormone-secreting component A stimulates or inhibits hormone-secreting component B or vice versa, it is obvious that in many cases endocrine systems function in a *dynamic* fashion in which there are multiple feedback interactions among the system components. Given that such systems fulfill commonly accepted criteria to be considered as *networks*, an increasing amount of investigative interest has focused on defining such networks in quantitative terms.

A separate approach with which to address this issue and as adopted by Farhy and colleagues (Farhy, 2004; Farhy and Veldhuis, 2005; Farhy *et al.*, 2007, 2008; Kovatchev *et al.*, 1999) and others (Bergman *et al.*, 1979; Dalla Man *et al.*, 2005; Keenan and Veldhuis, 1997, 1998, 2004; Wagner *et al.*, 1998) suggests that endocrine networks can be appraised by deterministic differential equations that describe the rate of change of individual hormones and their interaction. Utilization of this method has the potential to test whether a physiological regulatory hypothesis is consistent with the experimental observations and typically results in formulation of physiological assumptions (additional to the main hypothesis) that would explain the observed data specifics (Farhy, 2004). In effect, this is *in silico* testing of physiological hypotheses, which addresses specific features of the *in vivo* behavior of endocrine systems. This approach permits estimation of the integrative action of individual inferred or postulated feedback and feed-forward interactions within an endocrine axis and combines multiple steps (Farhy, 2004), including (i) preliminary analysis of available experimental data, (ii) design of a formal network describing the axis linkages and its approximation with coupled differential equations, and (iii) determination of the model parameters either functionally or by fitting the model output to available data. As an example, application of this approach may allow insights into the physiological parameters of a specific endocrine system, which may subserve its oscillatory behavior.

Evaluation of simple two- or three-hormone feedback networks, such as the one shown in Fig. 14.8, illustrates the capacity of delayed nonlinear feedbacks to generate hormone pulsatility (Farhy, 2004). In these examples (that may appear in different real endocrine axes), hormones (A, B, and C) are secreted continuously and are subject to elimination. However, in contrast to the previous examples, two or more (delayed; D_1 , D_2) feedback control loops exist between the system components. It is these loops that generate the oscillatory secretory patterns. For example, the two delayed control loops in the network shown in the middle left panel of Fig. 14.8 can drive a particular hormone oscillatory pattern if certain physiological conditions are met. As is depicted in Fig. 14.9, this complex multiple-volley secretory pattern is typical for the growth hormone dynamics in the adult male rat, and results of the simulations show that the underlying construct (Fig. 14.8, middle left panel) wherein B may be thought to be growth hormone (GH), A GH-releasing hormone, and C somatostatin, represents one minimal set of interactions capable of reproducing the typical *in vivo* GH pattern in the male rat. However, as evident from additional model-based analysis, this network is too simplistic and needs to be extended and modified to address other key experimental observations (Farhy and Veldhuis, 2005; Farhy *et al.*, 2007).

Another model-associated outcome could be a detailed reconstruction of the endocrine network dose–response interactions (similarly to the use of the aforementioned procedure *SRQuant*). This would be the case when suitable data exist to permit the precise iterative determination of the model parameters (Bergman *et al.*, 1979; Dalla Man *et al.*, 2005). In this case the physiological hypothesis that *the postulated network unifies the primary endocrine axis control mechanisms* would be strongly supported if the model can provide a good fit of data based on objective statistical criteria.

10. Future Directions

Given the complexity of endocrine axes, a logical extension of previous attempts to assess isolated signal–response endocrine interactions would include the development of automated methods with which to reconstruct the dose–response interactions within an entire hormone network. To this end, a dynamic modeling method, such as that described earlier, could be utilized within a computer-based model similar to *SRQuant*. This approach could make possible the approximation of endocrine networks exhibiting multiple feedback interactions and the subsequent reconstruction of their parameters based on existing experimental data. The result would be a powerful tool capable of testing regulatory hypotheses and estimating the alterations in hormone release control mechanisms related to various factors including, but not limited to, inter- or intraspecies differences, natural processes such as aging, pathophysiologic situations, and interventional procedures.

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References

- Atkinson LE, Bhattacharya AN, Monroe SE, Dierschke DJ, Knobil E. Effects of gonadectomy on plasma LH concentration in the rhesus monkey. *Endocrinology* 1970;87:847–849. [PubMed: 4991671]
- Backstrom CT, McNeilly AS, Leask RM, Baird DT. Pulsatile secretion of LH, FSH, prolactin, oestradiol and progesterone during the human menstrual cycle. *Clin Endocrinol* 1982;17:29–42.
- Bergman RN, Ider YZ, Bowden CR, Cobelli C. Quantitative estimation of insulin sensitivity. *Am J Physiol* 1979;236:E667–E677. [PubMed: 443421]
- Booth RA Jr, Weltman JY, Yankov VI, Murray J, Davison TS, Rogol AD, Asplin CM, Johnson ML, Veldhuis JD, Evans WS. Mode of pulsatile follicle-stimulating hormone secretion in gonadal hormone-sufficient and -deficient women: A clinical research center study. *J Clin Endocrinol Metab* 1996;81:3208–3214. [PubMed: 8784071]
- Burger CW, Korsen T, van Kessel H, van Dop PA, Caron FJ, Schoemaker J. Pulsatile luteinizing hormone patterns in the follicular phase of the menstrual cycle, polycystic ovarian disease (PCOD) and non-PCOD secondary amenorrhea. *J Clin Endocrinol Metab* 1985;61:1126–1132. [PubMed: 3932449]
- Burgus R, Butcher M, Amoss M, Ling N, Monahan M, Rivier J, Fellows R, Blackwell R, Vale W, Guillemin R. Primary structure of the ovine hypothalamic luteinizing hormone-releasing factor (LRF). *Proc Natl Acad Sci USA* 1972;69:278–282. [PubMed: 4550508]
- Clarke IJ, Cummins JT. The temporal relationship between gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. *Endocrinology* 1982;111:1737–1739. [PubMed: 6751801]
- Clifton DK, Steiner RA. Cycle detection: A technique for estimating the frequency and amplitude of episodic fluctuations in blood hormone and substrate concentrations. *Endocrinology* 1983;112:1057–1064. [PubMed: 6822203]
- Crowley WF Jr, Filicori M, Spratt DI, Santoro NF. The physiology of gonadotropin-releasing hormone (GnRH) secretion in men and women. *Rec Prog Horm Res* 1985;41:473–531. [PubMed: 3931190]
- Dalla Man C, Caumo A, Basu R, Rizza R, Toffolo G, Cobelli C. Measurement of selective effect of insulin on glucose disposal from labeled glucose oral test minimal model. *Am J Physiol* 2005;289:E909–E914.
- Evans WS, Sollenberger MJ, Booth RA Jr, Rogol AD, Urban RJ, Carlsen EC, Johnson ML, Veldhuis JD. Contemporary aspects of discrete peak-detection algorithms. II. The paradigm of the luteinizing hormone pulse signal in women. *Endocr Rev* 1992;13:81–104. [PubMed: 1348225]

- Farhy LS. Modeling of oscillations in endocrine networks with feedback. *Methods Enzymol* 2004;384:54–81. [PubMed: 15081681]
- Farhy LS, Bowers CY, Veldhuis JD. Model-projected mechanistic bases for sex differences in growth hormone regulation in humans. *Am J Physiol* 2007;292:R1577–R1593.
- Farhy LS, Du Z, Zeng Q, Veldhuis PP, Johnson ML, Brayman KL, McCall AL. Amplification of pulsatile glucagon counterregulation by switch-off of α -cell-suppressing signals in streptozotocin (STZ)-treated rats. *Am J Physiol Endocrinol Metab* 2008;295:E575–E585. [PubMed: 18577688]
- Farhy, LS.; Johnson, ML.; Veldhuis, PP.; Boyd, DG.; Evans, WS. Control Strength (CS) of Hormone Interactions in Endocrine Feedback Networks. 88th Annual Meeting of the Endocrine Society; 2006. p. 2-21.[Abstract]
- Farhy LS, Veldhuis JD. Deterministic construct of amplifying actions of ghrelin on pulsatile growth hormone secretion. *Am J Physiol* 2005;288:R1649–R1663.
- Filicori M, Butler JP, Crowley WF Jr. Neuroendocrine regulation of the corpus luteum in the human: Evidence for pulsatile progesterone secretion. *J Clin Invest* 1984;73:1638–1647. [PubMed: 6427277]
- Filicori M, Santoro N, Merriam GR, Crowley WF Jr. Characterization of the physiological pattern of episodic gonadotropin secretion throughout the human menstrual cycle. *J Clin Endocrinol Metab* 1986;62:1136–1144. [PubMed: 3084534]
- Fluhmann CF. Anterior pituitary hormone in the blood of women with ovarian deficiency. *JAMA* 1929;93:672–674.
- Friend K, Iranmanesh A, Veldhuis JD. The orderliness of the growth hormone (GH) release process and the mean mass of GH secreted per burst are highly conserved in individual men on successive days. *J Clin Endocrinol Metab* 1996;81:3746–3753. [PubMed: 8855833]
- Gay VL, Midgley AR Jr. Response of the adult rat to orchidectomy and ovariectomy as determined by LH radioimmunoassay. *Endocrinology* 1969;84:1359–1364. [PubMed: 5781126]
- Genazzani AD, Rodbard D, Forti G, Petraglia F, Baraghini GF, Genazzani AR. Estimation of instantaneous secretory rate of luteinizing hormone in women during the menstrual cycle and in men. *Clin Endocrinol* 1990;32:573–581.
- Goodman RL, Parfitt DB, Evans NP, Dahl GE, Karsch FJ. Endogenous opioid peptides control the amplitude and shape of gonadotropin-releasing hormone pulses in the ewe. *Endocrinology* 1995;136:2412–2420. [PubMed: 7750462]
- Green JD, Harris GW. The neurovascular link between the neurohypophysis and adenohypophysis. *J Endocrinol* 1947:136–149.
- Haisenleder, DJ.; Dalkin, AC.; Marshall, JC. Regulation of gonadotropin gene expression. In: Knobil, E.; Neill, J., editors. *The Physiology of Reproduction*. Vol. 2. Raven Press; New York: 1994. p. 1793-1832.
- Hartman ML, Pincus SM, Johnson ML, Matthews DH, Faunt LM, Vance ML, Thorner MO, Veldhuis JD. Enhanced basal and disorderly growth hormone secretion distinguish acromegalic from normal pulsatile growth hormone release. *J Clin Invest* 1994;94:1277–1288. [PubMed: 8083369]
- Johnson ML, Pipes L, Veldhuis PP, Farhy LS, Boyd DG, Evans WS. AutoDecon, a deconvolution algorithm for identification and characterization of luteinizing hormone secretory bursts: Description and validation using synthetic data. *Anal Biochem* 2008;381:8–17. [PubMed: 18639514]
- Keenan DM, Veldhuis JD. Stochastic model of admixed basal and pulsatile hormone secretion as modulated by a deterministic oscillator. *Am J Physiol* 1997;273:R1182–R1192. [PubMed: 9321902]
- Keenan DM, Veldhuis JD. A biomathematical model of time-delayed feedback in the human male hypothalamic-pituitary-Leydig cell axis. *Am J Physiol* 1998;275:E157–E176. [PubMed: 9688887]
- Keenan DM, Veldhuis JD. Divergent gonadotropin-gonadal dose-responsive coupling in healthy young and aging men. *Am J Physiol* 2004;286:R381–R389.
- Knobil E. The neuroendocrine control of the menstrual cycle. *Rec Prog Horm Res* 1980;36:53–88. [PubMed: 6774388]
- Kovatchev BP, Farhy LS, Cox DJ, Straume M, Yankov VI, Gonder-Frederick LA, Clarke WL. Modeling insulin-glucose dynamics during insulin induced hypoglycemia: Evaluation of glucose counterregulation. *Comput Math Meth Med* 1999;1:313–323.

- Levine JE, Pau KY, Ramirez VD, Jackson GL. Simultaneous measurement of luteinizing hormone-releasing hormone and luteinizing hormone release in unanesthetized, ovariectomized sheep. *Endocrinology* 1982;111:1449–1455. [PubMed: 6751794]
- Levine JE, Ramirez VD. Luteinizing hormone-releasing hormone release during the rat estrous cycle and after ovariectomy, as estimated with push-pull cannulae. *Endocrinology* 1982;111:1439–1448. [PubMed: 6751793]
- Matsuo H, Baba Y, Nair RM, Arimura A, Schally AV. Structure of the porcine LH- and FSH-releasing hormone. I. The proposed amino acid sequence. *Biochem Biophys Res Commun* 1971;43:1334–1339. [PubMed: 4936338]
- McIntosh RP, McIntosh JE. Amplitude of episodic release of LH as a measure of pituitary function analysed from the time-course of hormone levels in the blood: Comparison of four menstrual cycles in an individual. *J Endocrinol* 1985;107:231–239. [PubMed: 3934310]
- Meneilly GS, Ryan AS, Veldhuis JD, Elahi D. Increased disorderliness of basal insulin release, attenuated insulin secretory burst mass, and reduced ultradian rhythmicity of insulin secretion in older individuals. *J Clin Endocrinol Metab* 1997;82:4088–4093. [PubMed: 9398719]
- Merriam GR, Wachter KW. Algorithms for the study of episodic hormone secretion. *Am J Physiol* 1982;243:E310–E318. [PubMed: 6889816]
- Midgley AR Jr, Jaffe RB. Regulation of human gonadotropins: Episodic fluctuation of LH during the menstrual cycle. *J Clin Endocrinol Metab* 1971;33:962–969. [PubMed: 5135635]
- Murdoch AP, Diggle PJ, White MC, Kendall-Taylor P, Dunlop W. LH in polycystic ovary syndrome: Reproducibility and pulsatile secretion. *J Endocrinol* 1989;121:185–191. [PubMed: 2715757]
- Nass R, Farhy LS, Liu J, Prudom CE, Johnson ML, Veldhuis P, Pezzoli SS, Oliveri MC, Gaylinn BD, Geysen HM. Evidence for acyl-ghrelin modulation of growth hormone release in the fed state. *J Clin Endocrinol Metab* 2008;93:1988–1994. [PubMed: 18334589]
- Oerter KE, Guardabasso V, Rodbard D. Detection and characterization of peaks and estimation of instantaneous secretory rate for episodic pulsatile hormone secretion. *Comput Biomed Res* 1986;19:170–191. [PubMed: 3754800]
- Pincus SM. Approximate entropy as a measure of system-complexity. *Proc Natl Acad Sci USA* 1991;88:2297–2301. [PubMed: 11607165]
- Pincus SM, Mulligan T, Iranmanesh A, Gheorghiu S, Godshalk M, Veldhuis JD. Older males secrete luteinizing hormone and testosterone more irregularly, and jointly more asynchronously, than younger males. *Proc Natl Acad Sci USA* 1996;93:14100–14105. [PubMed: 8943067]
- Pincus SM, Singer BH. Randomness and degrees of irregularity. *Proc Natl Acad Sci USA* 1996;93:2083–2088. [PubMed: 11607637]
- Pincus SM, Veldhuis JD, Mulligan T, Iranmanesh A, Evans WS. Effects of age on the irregularity of LH and FSH serum concentrations in women and men. *Am J Physiol* 1997;273:E989–E995. [PubMed: 9374686]
- Reame N, Sauder SE, Kelch RP, Marshall JC. Pulsatile gonadotropin secretion during the human menstrual cycle: Evidence for altered frequency of gonadotropin-releasing hormone secretion. *J Clin Endocrinol Metab* 1984;59:328–337. [PubMed: 6429184]
- Rebar R, Perlman D, Naftolin F, Yen SS. The estimation of pituitary luteinizing hormone secretion. *J Clin Endocrinol Metab* 1973;37:917–927. [PubMed: 4584926]
- Rossmann WG, Liu CH, Laughlin GA, Mortola JF, Suh BY, Yen SSC. Relative changes in LH pulsatility during the menstrual cycle; using data from hypogonadal women as a reference point. *Clin Endocrinol* 1990;32:647–660.
- Santen RJ, Bardin CW. Episodic luteinizing hormone secretion in man: Pulse analysis, clinical interpretation, physiologic mechanisms. *J Clin Invest* 1973;52:2617–2628. [PubMed: 4729055]
- Schally AV, Arimura A, Baba Y, Nair RM, Matsuo H, Redding TW, Debeljuk L. Isolation and properties of the FSH and LH-releasing hormone. *Biochem Biophys Res Commun* 1971;43:393–399. [PubMed: 4930860]
- Schweiger U, Laessle RG, Tuschl RJ, Broocks A, Krusche T, Pirke KM. Decreased follicular phase gonadotropin secretion is associated with impaired estradiol and progesterone secretion during the follicular and luteal phases in normally menstruating women. *J Clin Endocrinol Metab* 1989;68:888–892. [PubMed: 2497129]

- Siragy HM, Vieweg WV, Pincus S, Veldhuis JD. Increased disorderliness and amplified basal and pulsatile aldosterone secretion in patients with primary aldosteronism. *J Clin Endocrinol Metab* 1995;80:28–33. [PubMed: 7829626]
- Sollenberger M, Carlsen EC, Johnson M, Veldhuis J, Evans W. Specific physiological regulation of LH secretory events throughout the human menstrual cycle: New insights into the pulsatile mode of gonadotropin release. *J Neuroendocrinol* 1990;2:845–852. [PubMed: 19215428]
- Soules MR, Steiner RA, Clifton DK, Cohen NL, Aksel S, Bremner WJ. Progesterone modulation of pulsatile luteinizing hormone secretion in normal women. *J Clin Endocrinol Metab* 1984;58:378–383. [PubMed: 6420438]
- Turner RC, Grayburn JA, Newman GB, Nabarro JDN. Measurement of the insulin delivery rate in man. *J Clin Endocrinol Metab* 1972;33:279–286. [PubMed: 4935637]
- Urban RJ, Evans WS, Rogol AD, Johnson ML, Veldhuis JD. Contemporary aspects of discrete peak detection algorithms. 1. The paradigm of the luteinizing hormone pulse signal in man. *Endocr Rev* 1988;9:3–37. [PubMed: 3286234]
- Van Cauter E, L'Hermite M, Copinschi G, Refetoff S, Desir D, Robyn C. Quantitative analysis of spontaneous variations of plasma prolactin in normal man. *Am J Physiol* 1981;241:E355–E363. [PubMed: 7304739]
- van den Berg G, Pincus SM, Veldhuis JD, Frolich M, Roelfsema F. Greater disorderliness of ACTH and cortisol release accompanies pituitary-dependent Cushing's disease. *Eur J Endocrinol* 1997;136:394–400. [PubMed: 9150699]
- Veldhuis JD, Carlson ML, Johnson ML. The pituitary gland secretes in bursts: Appraising the nature of glandular secretory impulses by simultaneous multiple-parameter deconvolution of plasma hormone concentrations. *Proc Natl Acad Sci USA* 1987;84:7686–7690. [PubMed: 2823271]
- Veldhuis JD, Christiansen E, Evans WS, Kolp LA, Rogol AD, Johnson ML. Physiological profiles of episodic progesterone release during the midluteal phase of the human menstrual cycle: Analysis of circadian and ultradian rhythms, discrete pulse properties, and correlations with simultaneous luteinizing hormone release. *J Clin Endocrinol Metab* 1988;66:414–421. [PubMed: 3339114]
- Veldhuis JD, Johnson ML. Cluster analysis: A simple, versatile, and robust algorithm for endocrine pulse detection. *Am J Physiol* 1986;250:E486–E493. [PubMed: 3008572]
- Wagner C, Caplan SR, Tannenbaum GS. Genesis of the ultradian rhythm of GH secretion: A new model unifying experimental observations in rats. *Am J Physiol* 1998;275:E1046–E1054. [PubMed: 9843748]
- Yen SS, Tsai CC, Naftolin F, Vandenberg G, Ajabor L. Pulsatile patterns of gonadotropin release in subjects with and without ovarian function. *J Clin Endocrinol Metab* 1972;34:671–675. [PubMed: 5012772]

Figure 14.1.

Control of pituitary hormone synthesis and secretion by the hypothalamus as hypothesized by Green and Harris (1947). Chemical messengers secreted by nerve cells within the hypothalamus were proposed to travel through the hypothalamic-hypophyseal portal circulation to the anterior pituitary gland where they would stimulate, inhibit, or otherwise modulate the synthesis and release of hormones.

Figure 14.2.

Luteinizing hormone (LH) secretion in Rhesus monkeys with (A) intact hypothalamic function, (B) after ablation of the medial basal hypothalamus, (C) in response to a continuous GnRH infusion after hypothalamic ablation, and (D) in response to pulsatile GnRH infusion after ablation of the hypothalamus. These studies (Knobil, 1980) were the first to demonstrate the profound importance of the pulsatile versus nonvarying nature of GnRH secretion with regard to LH and FSH secretion.

Figure 14.3.

Number of LH pulses identified in concentration-time series obtained over a 24-h period of time during the midluteal phase of the menstrual cycle of normal women. The same data sets were analyzed with independent pulse detection algorithms, including *Santen & Bardin* (*S & B*), *Pulsar*, *Cycle Detector* (*Cycle*), and *Ultra*. Note the considerable difference in number of pulses detected as a function of the pulse analysis program used for the analysis (Evans *et al.*, 1992).

Figure 14.4.

(Top) A serum LH concentration-time series constructed from samples obtained from a normal woman in the early follicular phase of her menstrual cycle. Samples were collected at 10-min intervals over a 24-h period of time. (Bottom) LH secretory bursts resolved from this concentration-time series using the deconvolution procedure *Deconv* (Veldhuis, Carlson and Johnson, 1987).

Figure 14.5.

Deconvolution-resolved number of secretory bursts (/24 h; top), burst mass (IU/liter; middle), and half-life (minutes; bottom) in LH concentration-time series obtained from normal women in the early follicular (EF), late follicular (LF), and midluteal (ML) phases of the menstrual cycle and in postmenopausal (PM) women. For each group, values identified with different superscripts differ significantly ($p < 0.05$) (Booth *et al.*, 1996; Sollenberger *et al.*, 1990).

Figure 14.6.

A synthetic endocrine network (left) and estimate of the level of feedback control (LFC) for the A-to-B negative coupling (table on right) corresponding to the gradual increase in the feedback strength and simultaneous decrease of the independent factor (Farhy *et al.*, 2006).

Figure 14.7.

Modeling of pituitary LH stimulation by hypothalamic GnRH in a representative ewe treated with naloxone (solid bar). (Top) Model fit (solid line) and observed (errorbars) LH (80% of the variance of LH is accounted for). (Bottom) GnRH concentration. (Inset) Reconstructed dose^{response} interaction between GnRH concentration and LH secretion over the range of observed GnRH.

Figure 14.8.
Examples of endocrine networks with multiple delayed feedback loops.

Figure 14.9. Computer-generated output (concentration of B vs time) of the core system shown in Fig. 14.8, middle left.