Published in final edited form as:

Endocrine. 2014 September ; 47(1): 49–63. doi:10.1007/s12020-014-0212-3.

Episodic hormone secretion: a comparison of the basis of pulsatile secretion of insulin and GnRH

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Abstract

Rhythms govern many endocrine functions. Examples of such rhythmic systems include the insulin-secreting pancreatic beta-cell, which regulates blood glucose, and the gonadotropin-releasing hormone (GnRH) neuron, which governs reproductive function. Although serving very different functions within the body, these cell types share many important features. Both GnRH neurons and beta-cells, for instance, are hypothesized to generate at least two rhythms endogenously: (1) a burst firing electrical rhythm and (2) a slower rhythm involving metabolic or other intracellular processes. This review discusses the importance of hormone rhythms to both physiology and disease and compares and contrasts the rhythms generated by each system.

Keywords

LHRH; Beta-cell; Islet; Oscillations; Rhythms; Metabolism; Endocrine; Hormone

Introduction

Rhythmic activity at various frequencies forms the basis of many neural and endocrine systems. The thalamocortical system relating to sleep [1], vasopressin neurons signaling thirst and osmoregulation, [2, 3], and PreBoetzing neurons governing breathing [4], for example, demonstrate rhythmic firing with periods on the order of seconds. Neurons of the suprachiasmatic nucleus, in contrast, generate much slower circadian (~24 h) rhythms [5, 6], while ultradian rhythms (<24 h, but typically ~ 30–120 min) occur in systems governing, for example, appetite and satiety [7], sleep and dreaming [8, 9], cardiac and respiratory function

[10], as well as the transcription and translation of several genes [11, 12]. Rhythms thus abound in biology as a fundamental means of communication and organization.

This review contrasts two such rhythmic systems: the insulin-secreting pancreatic beta-cell, which regulates plasma glucose, and the gonadotropin-releasing hormone (GnRH) neuron, which governs reproductive function. Each cell type has its own endogenous rhythms, which can be observed in their electrical activity patterns and rhythmical secretory patterns. There are also additional rhythmic complexities to each system [13–18]. The purpose of this introductory review is to demonstrate the importance of rhythmic function to normal physiology and to compare and contrast the distinct mechanisms and functions of rhythmic activity in each of these systems.

Pulsatility in health and disease

For both the GnRH neuron and the insulin-secreting beta-cell, rhythmicity is a *raison d'être*. Properly timed, intermittent bursts of activity are crucial to normal organ function, and the loss of this capacity is associated with dysfunction and disease [19–23]. At a cellular and organismal level, there are several potential advantages to pulsatile signaling: (1) Information can be coded within the frequency and/or amplitude of pulses that permit complex downstream signaling. (2) Pulsatility may help to coordinate the activity of cells within a network that are otherwise isolated from one another, as is the case for GnRH neurons and pancreatic islets. (3) Intermittent cycles of activity and rest may be more energetically efficient and may reduce potential cellular stress. In this section, we introduce these two pulsatile endocrine systems and then highlight pathological conditions that are known to be associated with disrupted pulsatility.

GnRH neurons—A network of some 800–2,000 GnRH neurons is widely distributed throughout the basomedial hypothalamus and preoptic area of the brain [24, 25]. Most GnRH neurons target their axons to the median eminence, where GnRH peptide is secreted into the bloodstream and carried by the portal vasculature to the anterior pituitary (Fig. 1a). Pituitary gonadotropes respond to GnRH signals by secreting both luteinizing hormone (LH) and follicle stimulating hormone (FSH). These pituitary hormones act on the gonads to promote the production of eggs or sperm and the synthesis of different steroids (estrogen, testosterone, etc.). The steroids, in turn, provide both positive and negative feedback at the level of the brain and the pituitary to complete a reproductive regulatory loop called the hypothalamic–pituitary–gonadal (HPG) axis (Fig. 1b). The role of GnRH neurons in the HPG-axis is to integrate the activity of neural afferents, environmental cues, and steroidal cues related to fertility and, in turn, to form the final common pathway for the central control of reproduction.

The output of this final common pathway is the pulsatile release of GnRH peptide [26, 27], a crucial element in maintaining reproductive function. Pulsatile GnRH maintains appropriate levels of LH and FSH, while continuous GnRH suppresses the synthesis and secretion of LH and FSH [28], leading ultimately to infertility [20, 29, 30]. Modulation of the frequency of GnRH pulses is equally critical [31, 32], as GnRH pulse frequency is a primary determinant of differential pituitary responses [33, 34]. Specifically, higher-frequency pulses favor LH

release, whereas lower-frequency pulses favor FSH release (Fig. 2) [31, 32]. To ensure the proper development of the ovarian follicle, GnRH neurons must therefore respond to changes in the steroid milieu, the stage of the reproductive cycle, and other cues by modulating GnRH pulse intervals for a few minutes to hours.

Pulsatility in the GnRH system is required for proper reproductive function, and an inability to modulate GnRH pulse frequency can result in a loss of reproductive function. An imbalance in steroids or stress levels, or a significant change in energy balance toward obesity or anorexia, can result in disruptions or even the outright arrest of the pulsatile GnRH signal. Hyperprolactinemia and hypothalamic amenorrhea, for example, are associated with chronically low-frequency GnRH pulses [35, 36], and polycystic ovary syndrome (PCOS) is associated with persistent high-frequency GnRH pulses [22, 37]. These reproductive disorders thus demonstrate that pulsatility and pulse frequency coding in the GnRH system are crucial to fertility. A summary of reproductive disorders related to dysfunctional GnRH pulsatility can be found in [38].

Beta-cells—Islets of Langerhans are micro-organs that constitute ~ 1–2 % of the total mammalian pancreas by volume and are responsible for regulating plasma glucose and body energy metabolism [39]. Islets vary in size and shape, and possess their own microvasculature to rapidly carry insulin and other secreted factors from the islet cells to the wider circulation, as well as to receive circulating nutrients and regulatory factors involved in glucose homeostasis, providing an additional level of control [40–42]. In addition, islets are innervated by neurons of the autonomic nervous system [43, 44]. The islet is composed of several endocrine cell types, including glucagon-secreting alpha-cells, insulin-secreting beta-cells, somatostatin-secreting delta-cells, and others [45, 46] (see Fig. 3a). The proportion and organization of these cells differ somewhat from species to species [46, 47], but beta-cells compose the majority of the total islet mass (50–80 %).

The primary function of beta-cells is to synthesize and secrete insulin, a critical regulator of blood glucose. In response to a rise in blood glucose concentration following the ingestion of a meal, beta-cells secrete insulin to stimulate the conversion of glucose to glycogen in the liver, suppress hepatic glucose output and facilitate the uptake of glucose into insulin target tissues, particularly skeletal muscle and fat (see Fig. 3b). When blood glucose levels drop as a result of insulin acting at its receptor, insulin secretion is in turn reduced, thus completing the regulatory loop. Blood glucose is also regulated by additional factors including the hormone glucagon, which is secreted by islet alpha-cells and counterbalances the effects of insulin by mobilizing glucose from the liver.

Pulsatility is important to the overall regulation of blood glucose. Delivering intermittent insulin to healthy individuals results in a more effective insulin response than continuous insulin in most [48–51] but not all, studies [52, 53]. In addition, patients with diabetes respond more effectively to pulsatile insulin compared to continuous delivery [54–56]. More recently, a series of elegant studies have shown in both dog and rat that pulsatile insulin is more efficacious in suppressing hepatic glucose production than is continuous insulin as pulsatile presentation potentiated insulin receptor signal transduction in liver [57–59].

Loss of pulsatile insulin secretion also occurs during the early stages of Type 2 diabetes, with a reduction in the amplitude and possibly the frequency of the insulin pulses being linked to subsequent diabetes [60–64]. The close relatives of diabetic patients, even those with normal glucose homeostasis, also demonstrate considerable degradation of pulsatile insulin secretion despite reporting clinically normal responses to glucose challenges and normal levels of insulin resistance [65, 66]. Even among islets observed under control conditions in vitro, oscillatory islets are associated with greater glucose sensitivity than non-oscillatory islets [67]. Aberrant insulin pulsatility has also been reported in specific forms of diabetes and in other metabolic disorders, including maturity onset diabetes of the young type 2 (MODY2) [68], Tarui's disease or glycogen-storage disease type-VII [62], and maternally inherited diabetes and deafness (MIDD) [69], as well as hypertension [70] and obesity [71, 72]. Thus, considerable evidence suggests that pulsatile insulin secretion is important to the proper regulation of blood glucose and is a potential early warning sign of emerging disease [73]. A review of metabolic disorders related to dysfunctional pulsatile insulin secretion can be found in [74, 75].

Anatomical organization and communication

One similarity between GnRH neurons and pancreatic islets is their overall anatomical organization. GnRH neurons, as mentioned previously, form a loose network of cells spread throughout the medial basal hypothalamus that act in unison to release GnRH in pulses at the median eminence (Fig. 4a). Islets are similarly distributed throughout the pancreas, and although islets are composed of hundreds of endocrine cells, each islet acts as a discrete unit that secretes insulin pulses into the vasculature (Fig. 4b).

The diffuse distribution of intrinsic oscillators in the body or within specific tissues represents a communications dilemma. How these systems communicate to produce synchronized hormone pulses is not well understood for either GnRH neurons or for islets. It has been proposed that islets can be entrained to a single-pancreatic secretory pattern by a number of putative synchronizing mechanisms, such as an intra-pancreatic ganglion pacemaker [76–78], circulating inter-islet factors [79], or feedback interactions with the liver [80, 81]. No matter what mechanism is involved in synchronizing islets, the ability to maintain similar pulse frequencies may aid in the process. Of interest, isolated islets lacking any in vivo inputs still maintain calcium oscillations with remarkably similar frequencies to one another; this “imprinted” frequency is tightly maintained among islets isolated from an individual mouse and reflects insulin pulsatility in vivo, but may differ markedly from one mouse to the next [82, 83].

GnRH neurons, likewise, are separated from one another within the hypothalamus and appear to have some contact, although it is limited [84–86]. The neurons may not need to be in physical contact in order to secrete GnRH in a pulsatile fashion, however, as GnRH pulses can still be observed from acutely dissociated hypothalamic neurons [87]. Because most GnRH neurons project their axons to the median eminence, this is one potential point of contact or near contact. Of interest in this regard, isolated median eminence tissue containing only GnRH axons and synaptic terminals has also been shown to maintain pulsatile GnRH release [88]. Further, GnRH neuron projections to the median eminence

appear to have a spike initiation site for action potentials as well as spines and synaptic appositions along its entire length [89], which could aid both in generating and synchronizing pulses. This suggests that the synchronization of GnRH secretion could occur by any number of methods, including the release of diffusible factors such as nitric oxide [90, 91] and GnRH [92, 93].

Although the advantage of having a dispersed distribution of intrinsic oscillators is not known, one possibility is that the distributed network could be a nexus for gathering and integrating different inputs from different brain regions. Neuronal afferents to GnRH neurons transmit numerous cues related to metabolism, stress, steroids, and circadian cues to the reproductive axis, among other effects [94]. It is reasonable to suspect that an individual GnRH neuron, for example, might receive certain inputs related to reproduction such as photoperiod [95] or nutrition [96], but not others, based on the anatomical location of the specific GnRH neuron in the hypothalamus. Under various physiological and pathological conditions, hormonal and metabolic signals either regulate GnRH neurons directly or act on upstream neuronal circuits to influence the pattern of pulsatile GnRH secretion into the hypophyseal portal circulation. Particularly, powerful inputs to GnRH neurons include arcuate projections from cells co-expressing kisspeptin, neurokinin, and dynorphin, collectively known as KNDy neurons [97, 98].

A network of many widely distributed GnRH neurons might thus collect a wider array of inputs in order to integrate their activity into the overall reproductive output signal. Islets, likewise, might receive different signals based on their anatomical location within the pancreas, although this remains to be established. It is known though that individual islets from different parts of the pancreas exhibit certain defining characteristics with regard to their size and intra-islet architecture [99]. Also, certain circulating factors are closely linked to both reproduction and energy metabolism. Adipose-derived adiponectin and leptin, in particular, can modulate the activity of both GnRH neurons [100–102] and beta-cells [103], suggesting a potential common thread between these two endocrine systems. Dysfunction in such inputs could help in explaining the reproductive features of metabolic disorders and the metabolic features of reproductive disorders [101, 104].

Unlike GnRH neurons, beta-cells are contained within micro-organs, the islets of Langerhans, containing their own microvasculature and neuronal inputs together with multiple types of endocrine cells. This scenario provides an additional layer of anatomical organization and possibilities for integration. Within the islet, beta-cells form an electrical syncytium as they are coupled to one another via gap junctions that are mediated by connexin 36 [105–107]. This coupling allows the network of beta-cells to function as a unit, with near synchronous activity of beta-cells preserved across the islet [108]. This strong coordination is important, as its loss in CX36 knockout mice leads to asynchronous beta-cell activity within the islet, a loss of the initial, transient phase of insulin secretion, and a reduced amplitude of pulsatile plasma insulin [109].

The generation of pulsatile secretion (period in minutes)

Secretory rhythms on the order of minutes to hours have long been associated with reproductive function. Endogenous secretory rhythms related to reproductive function were

first observed in LH at circadian (~ hourly) intervals [110]. These hormone pulses were subsequently shown to correlate with episodic volleys of hypothalamic electrical activity (Fig. 5a) [18, 111]. The collection of hypothalamic neurons producing these rhythms has been termed the “GnRH pulse generator.”

The molecular basis of the low-frequency rhythm comprising the GnRH pulse generator is largely unknown, in part, because GnRH neurons are few in number and widely distributed in a configuration that is neither nuclear nor laminar in organization, making the identification of GnRH neurons difficult. Thus, the only electrophysiological report to appear in the first 25 years following the identification of GnRH described results obtained in four immunocytochemically identified GnRH neurons out of 102 attempts total using guinea pig hypothalamic slices [112]. Subsequent techniques developed to identify or isolate GnRH neurons have demonstrated pulsatile GnRH release from cultures of immortalized GnRH (GT1) cells [113, 114] (see Fig. 5b), embryonic GnRH neurons [115], adult hypothalamic cultures [116, 117], and hypothalamic explants [87]. Episodic electrical activity, a correlate to secretion in this system, has also been reported from recordings of individual GnRH neurons in brain slices (see Fig. 5c) and in dissociated GnRH neurons [27, 118, 119]. These findings collectively suggest that the low-frequency rhythm observed for GnRH secretion is indeed endogenous to individual GnRH neurons.

As for the cellular mechanism of the GnRH pulse generator, there are several possibilities. Autocrine or paracrine feedback of GnRH is one candidate mechanism. GnRH neurons express GnRH-receptors [93, 117, 120], and exogenous GnRH (or GnRH agonists) has both positive and/or negative feedback effects on GnRH secretion [117, 121, 122] and electrical activity [93]. These findings support a model in which GnRH could provide autocrine or paracrine feedback involving GnRH receptors to modulate secretory pulse frequency through a G-protein-coupled-receptor signaling cascade [85, 123]. Alternatively, cycles of gene transcription and translation could occur within cells, as it has been shown that the transcription and translation of GnRH mRNA occur in a pulsatile fashion in GT1 cells [11, 124], and the expression of circadian clock genes modulates the frequency of GnRH pulses [125, 126]. However, GnRH pulses are maintained on a short-term basis of a few hours even when transcription and translation are blocked [124, 127]. Yet another candidate mechanism involves cyclic adenosine monophosphate (cAMP) opening cyclic-nucleotide-gated (CNG)-channels to cause depolarization, increased electrical activity, and GnRH secretion [128]. Rising cAMP levels also subsequently activate a protein kinase-A pathway that in turn reduces cAMP, thus closing the CNG-channels and reducing secretion [128, 129]. There is both support for [130–133] and against this mechanism [134, 135]. Finally, KNDy neurons have emerged recently as a putative external driver of pulsatility in GnRH neurons as reviewed here [136–139]. Although each of these candidate mechanisms shows promise, further evidence is required to determine which mechanism or mechanisms are involved in mediating the underlying oscillatory process responsible for generating GnRH pulses.

In islets, the established period of pulsatile insulin secretion measured *in vivo* ranges from 5 to 10 min and has been reported for many species [76, 140–142]. The two initial studies of rhythms on this timescale showed that there were clusters of more intense electrical bursting in isolated islets that occurred on the order of ~5 min [143, 144]. Subsequent reports using a

variety of techniques have shown that islets produce a wide variety of oscillatory patterns, which may be slow (Fig. 6a), a mixture of fast and slow (b), or fast (c) [145]. Note that even some fast patterns still can display a small, underlying slow component, as shown in (Fig. 6c, lower panel).

As summarized in Table 1, it is now well established that islets generate rhythms in electrical activity, $[Ca^{2+}]_i$, and a variety of metabolic processes that have a period of ~3–5 min, in addition to more rapid bursting activity. Based on observations from isolated beta-cells as well as intact islets, low-frequency oscillations appear to be endogenous to individual beta-cells. Low-frequency islet oscillations have also been shown to closely correlate with oscillations in insulin secretion in vitro [146–148] and in vivo [149], suggesting that islet oscillations are important for physiological insulin secretion. Also, isolated human islets have been reported to display slow oscillations in response to glucose for calcium [150, 151] and insulin release [152].

It has been proposed that the low-frequency rhythms of islets are driven by endogenous oscillations in glycolysis mediated by PFKM, which in turn, drive oscillations in downstream mitochondrial activity, ATP/ADP, K_{ATP} -channel conductance, electrical activity, and ultimately insulin secretion [153–155]. It has long been known that yeast exhibits endogenous oscillations in glycolytic activity on the order of minutes [156, 157], but confirming similar glycolytic oscillations in individual beta-cells has been difficult. While there is ample indirect evidence for the glycolytic model [153, 158, 159], the first direct evidence of oscillatory glycolysis in pancreatic beta-cells has only recently been reported using a FRET-based reporter based on pyruvate kinase M2 activity as the glycolytic product of PFKM, fructose-1,6-bisphosphate as an allosteric activator of this key enzyme [160]. These data confirm a glycolytic mechanism that produces oscillations on the order of ~5 min, the same period of insulin pulses produced in healthy humans.

Electrical bursting (period in seconds)

Rhythms in beta-cells were first identified by electrophysiological recordings of dissected mouse islets. In 1968, Dean and Matthews showed that mouse islets produce rhythmic bursts of repeated action potentials exhibiting periods of ~ 15 s in response to elevated glucose [161]. Subsequent studies determined that these rhythms involved the coordinated activity of calcium and potassium ion currents [162–166] and that their frequency and duration are glucose-dependent [167, 168]. An example of the rhythmic activity of a mouse islet is shown for both electrical activity (Fig. 7a) and by corresponding changes in $[Ca^{+}]_i$ (Fig. 7b). These rhythms in electrical activity have been shown to induce oscillations in $[Ca^{+}]_i$ [169, 170], and both electrical activity and $[Ca^{+}]_i$ have been directly correlated with insulin secretion [146–148, 171, 172].

This phenomenon of rhythmic electrical activity is termed “bursting.” A burst is described as a train of action potentials that occur during a “plateau” or “active phase,” followed by a period of relative quiescence called the “silent phase” (see Fig. 7c). The period of burst firing is measured as the time interval from the start of one burst to the start of the next burst and is also called the “burst interval” or “activity cycle.” In beta-cells, bursts are also characterized by their “plateau fraction,” the relative time spent in the active phase divided

by the total burst interval. Plateau fraction is particularly useful for comparing burst firing patterns in beta-cells at different glucose concentrations. The duration of time spent in the plateau phase progressively increases with glucose concentration, while the time spent in the silent phase is correspondingly reduced. Because secretion in excitable cells is closely linked to electrical activity and calcium influx [173], the plateau fraction yields a reasonable estimate of the relative rates of insulin secretion for islets because it is proportional to mean beta-cell calcium concentration [148, 163, 171, 172].

Although the low-frequency rhythm that produces pulsatile GnRH secretion clearly plays an integral role in maintaining reproductive function, much faster rhythms (periods ~5–60 s) have also been observed. Burst firing from unidentified neurons thought to be GnRH neurons based on their low-frequency rhythms was first reported in electrophysiological studies of sheep [18]. Spontaneous burst firing of action potentials was later described in detail in GT1 cells [174] and in GnRH neurons in brain slices [118, 119, 175], as shown in Fig. 8a. Burst firing has also been observed in acutely dissociated GnRH neurons [118, 176, 177] (Fig. 8b), strongly suggesting that burst firing is an intrinsic property of the GnRH neuron.

By testing the effects of a number of ion channel blockers in cultures of GT1 cells, a critical role for Na⁺-channels in generating bursts of action potentials and for L-type Ca²⁺-channels in generating [Ca²⁺]_i oscillations was established [174, 178]. Potential modulators of burst firing properties were also identified, including potassium-channels, T-type calcium-channels, and [Ca²⁺]_i stores [174, 178–180]. These findings led to a model suggesting that amplitude and/or frequency modulation of burst firing could underlie the secretory patterns in GnRH neurons [174, 180]. The presence of many of the same ion channels expressed in GT1 cells has been subsequently confirmed in native GnRH neurons [175, 176, 181–183] as well as additional channels that could participate in burst firing, as reviewed in [184].

The link between low- and high-frequency rhythms

For burst patterns resulting from oscillations in plasma membrane potential to interact with slow oscillations in intracellular processes as described above, a mechanism is needed which links plasma membrane ion fluxes to biochemical oscillations in the cytoplasm. The K_{ATP}-channel can readily provide this link, as first proposed by Cook and Hales [185]. K_{ATP}-channels were first shown to be metabolically sensitive in beta-cells by demonstrating the direct effects of exogenous ATP on single-channel activity in inside-out patches [185, 186] or by exposing beta-cells to bath glucose while recording K_{ATP}-channel activity in cell-attached patches [187]. Spontaneous changes in single-channel K_{ATP}-conductance in situ were later observed to occur at similar intervals as low-frequency metabolic oscillations [188, 189] (more recently for whole cells [155]), implying that the activity of K_{ATP}-channels may be responsive to endogenous changes in beta-cell fuel metabolism.

Although the K_{ATP}-channel appears to be the dominant link between metabolic processes and the plasma membrane of the beta-cell, low-frequency islet rhythms in electrical activity persist in islets from SUR1^{−/−}-knockout mice, which lack functional K_{ATP}-channels [190]. This suggests metabolic oscillations must also influence other electrical mechanisms. A number of other ion channels are thought to be metabolically sensitive in beta-cells, such as

chloride-channels [191], L-type Ca^{2+} -channels [192], and K_{slow} -channels [193, 194], several of which might in theory be able to function as redundant or secondary mechanisms in the event of the loss of the K_{ATP} -channel. It is also possible that other K-channels that play minor roles when K_{ATP} -expression is significant become dominant when K_{ATP} -channels are lost, although strong evidence for this is currently lacking (but see [195]). The majority of evidence, nevertheless, points to the K_{ATP} -channel as the dominant player in coupling rhythmic processes in the pancreatic beta-cell under physiological conditions. Indeed, mutations have been identified in the genes encoding the K_{ATP} -channel subunits SUR1 and Kir6.2 and these produce gain or loss of function for K_{ATP} -current that have been linked to neonatal diabetes or congenital hypoglycemia, respectively [196].

For GnRH neurons, an analogous mechanism for linking intracellular processes to changes in the membrane potential is far less settled. Without a clear understanding of what constitutes the GnRH pulse generator that drives circulatory secretory episodes, it is difficult to speculate as to what links these rhythms to bursts of electrical activity. Bursts of electrical activity in GnRH neurons have been shown to cluster more frequently or less frequently in patterns on the order of many minutes, leading to conjecture that gradual shifts in membrane potential toward or away from excitability may underlie this phenomenon [119]. Supporting this concept, a small subset of GnRH neurons appears to display a distinct subthreshold membrane potential oscillation with a period on the order of ~20 min [197]. Because these neurons are recorded in slices, not in isolation, it is not possible to discern whether these subthreshold oscillations are intrinsic to the GnRH neurons, driven by inputs, or a combination of the two possibilities.

Frequency versus amplitude coding

Although GnRH neurons and pancreatic beta-cells may generate rhythms in a seemingly analogous fashion, one interesting difference between the two systems is how their rhythmic hormone release is modulated. GnRH neurons govern reproductive function primarily by altering the frequency of GnRH pulses to code for different responses from the pituitary. This is interesting in that proper reproductive function in females requires numerous processing stages of the follicle during each ovulatory cycle; this requires a precise balance of hormones that are specific to each stage [38, 198]. GnRH neurons thus promote continuous changes in the steroidal milieu throughout the ovulatory cycle, which may be most effectively communicated by modulating the frequency of GnRH pulses in response to steroid feedback and cycle stage.

The pancreas, in contrast to GnRH neurons, generates pulses at a fairly steady frequency, and responds to increased glucose or to other fuel stimuli mostly by increasing the amplitude of insulin pulses produced, as demonstrated by in vivo studies [74, 199, 200]. This use of pulse amplitude modulation instead of frequency modulation could be indicative of a different mechanism of action and purpose [201]. Rather than promoting complex shifts in the hormone milieu, the role of islets is to secrete appropriate amounts of insulin on a minute-to-minute basis in order to maintain blood glucose within a tight range. The advantage of amplitude rather than frequency coding in this system is not known, although

perhaps the targets of insulin action, liver, muscle, and fat tissue, may be better able to respond appropriately to changes in pulse amplitude.

Conclusions

Several common themes have emerged from this comparison of the GnRH and insulin endocrine systems. First, rhythmic activity takes on several forms, including a low-frequency rhythm synonymous with secretory pulses and also a higher-frequency burst pattern, which may facilitate secretory efficiency and/or communication with downstream targets. Second, these rhythms appear to be functionally connected. Third, secretory rhythms from GnRH neurons and pancreatic beta-cells are functionally important to their respective systems, as evidenced by the disorders and disease states that can result from the loss of pulsatile activity. The GnRH neuronal network and the pancreatic islet may thus serve as models for study of other endocrine systems.

Acknowledgments

Supported by NIH Grants RO1DK46409 to LSS and RO1DK089182 to CSN.

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Fig. 1. GnRH neurons and the HPG-axis. **a** GnRH neurons at the base of the hypothalamus target axons to the median eminence where GnRH is released and travels to the pituitary gland. **b** The HPG-axis.

Fig. 2.

The frequency of GnRH pulses codes for different rates of LH and FSH secretion from pituitary gonadotropes. Adapted from Wildt et al. [216] with permission of the Endocrine Society, © 1981

Fig. 3.
a Illustration of the cellular composition of a pancreatic islet. **b** The actions of insulin and glucagon to regulate blood sugar

Fig. 4.

Anatomy and organization of two rhythmic endocrine systems, **a** GnRH neurons are located in the base of the hypothalamus and target axons to the median eminence, where GnRH is released in pulses. It is not known whether GnRH neurons communicate either at the level of the cell body or at median eminence. *Blue arrows* illustrate the unknown mechanism(s) for communication and synchronization in each system. **b** Islets are spread throughout the pancreas and typically are not in direct contact with one another. Islets are highly vascularized, so that pulses of insulin and other secretory products can be carried to the liver

(A) Hypothalamic electrical activity and LH secretion **(B) GT1 cell** **(C) GnRH neuron** **Fig. 5.**

a Pulses of LH, the downstream target of GnRH signaling, directly follow episodes of neural activity from the hypothalamus, detected as multiunit activity (MUA) from an implanted electrode. Adapted from Wilson et al. [111] with permission of S. Karger AG, Basel © 1984.

b Pulsatile GnRH secretion from cultures of GT1-1 cells. Adapted from Pitts et al. [127]

with permission of the Endocrine Society, ©2001. **c** Episodes of electrical activity from a single-GnRH neuron recorded within a coronal brain slice of an estradiol-treated ovariectomized mouse. Modified from data originally presented in Nunemaker et al. [217]

Fig. 6. Examples of the multiple modes of oscillations in $[Ca^{2+}]_i$, including purely slow oscillations (a), a mixture of fast and slow (b), and fast (c, *top*) with an underlying slow oscillations revealed (c, *bottom*) by signal averaging (Nunemaker and Satin, unpublished example)

Fig. 7.

Bursting in islets, **(a, b)** A simultaneous recording of electrical activity **(a)** and $[Ca^{2+}]_i$ **(b)** illustrates islet bursting (Zhang and Satin, unpublished example). The periodic firing of action potentials results in corresponding changes in $[Ca^{2+}]_i$, which is also reflective of insulin secretion, **c** A description of the components of a burst.

Fig. 8.
a Burst firing from GnRH neurons within a 200- μ m coronal brain slice. Adapted from Nunemaker et al. [218] with permission of the Endocrine Society, ©2002. **b** Burst firing from an isolated GnRH neuron (Nunemaker and Moenter, unpublished example)

Table 1

A summary of slow oscillatory cellular processes

Rhythmic process	Period (min)	Glucose (mM)	Beta-cells or islets tested	Reference (1st author, year, citation)
Electrical activity	3–4	10, 15	Beta-cell	Henquin, 1982 [143]
	4.6	11	Islet	Cook, 1983 [144]
	1–6	11	Islet	Manning-Fox, 2006 [202]
Intracellular calcium	2–6	11	Beta-cell	Grapengiesser, 1988 [203]
	1–5	11 or 16.7	Islet	Valdeomillos, 1989 [204]
Mitochondrial membrane potential	N/A	11	Beta-cell	Krippeit-Drews, 2000 [205]
	N/A	10	Islet	Kindmark, 2001 [206]
	4.5, 3.0	11	Islet, beta-cell	Nunemaker, 2004 [207]
	~3–6	6, 12	Beta-cells in islets	Katzman, 2004 [208]
Oxygen consumption	5.3	20	Beta-cell	Longo, 1991 [159]
	3.3	10	Islet	Jung, 1999 [209]
	~4	3, 11	Islet	Ortsater, 2000 [210]
	3.1	15	Beta-cell (HIT)	Porterfield, 2000 [211]
NAD(P)H	~5	10	Islet	Luciani, 2006 [212]
	~5	11	Islet	Nunemaker, 2006 [213]
Glycolytic enzyme activity				Merrins, 2013 [160]
Glucose consumption	3.1	10	Islet	Jung, 2000 [214]
ATP/ADP	3.6	10	Beta-cell (HIT)	Deeney, 2001 [215]
K _{ATP} -current	2.5–4	0, 3	Beta-cell	Dryselius, 1994 [188]
	(1–4)	10	Beta-cell	Larsson, 1996 [189]
	3.2	11	Beta-cell	Ren, 2013 [155]

First reports and/or detailed studies of oscillations are listed; apologies for any omissions