Published in final edited form as:

Math Biosci. 2010 September ; 227(1): 12–19. doi:10.1016/j.mbs.2010.05.005.

Lessons from Models of Pancreatic β -Cells for Engineering Glucose-sensing Cells

Arthur Sherman

National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Laboratory of Biological Modeling, 12A South Drive, Room 4007, Bethesda, MD 20892-5621

Arthur Sherman: asherman@nih.gov

Abstract

Mathematical models of pancreatic β cells suggest design principles that can be applied to engineering cells to sense glucose and secrete insulin. Engineering cells can potentially both contribute to future diabetes therapies and generate new insights into β cell function. The focus is on ion channels, Ca^{2+} handling, and elements of metabolism that combine to produce the varied oscillatory patterns exhibited by β cells.

Keywords

calcium; oscillations; bursting; insulin; secretion; diabetes; cell engineering

1. Introduction

Insulin is a critical hormone for regulating the body's use of glucose. When consumption of carbohydrates provides an influx of glucose to the circulation, insulin allows muscle cells to take it up for use as a fuel, suppresses production of glucose from stored glycogen in the liver, and suppresses release of fat by adipose cells. In type 1 diabetes mellitus, insulin is absent due to auto-immune destruction of the β cells of the pancreatic islets of Langerhans that secrete it. This results in an inability to maintain energy stores between meals and dramatic fluctuations of plasma glucose. The latter, if not treated, cause terrible morbidity in the form of blindness, kidney failure, neuropathy, and cardio-vascular disease, culminating in premature death.

Type 2 diabetes, the less severe but much more common form, is generally the result of two interacting deficiencies. One is insulin resistance, which is the inefficient use of insulin and usually arises from obesity, perhaps as a way for cells to defend themselves against excess nutrition [1]. This places excess demand on the β cells to secrete insulin. If they are unable to meet the demand, owing to a deficiency in either function (less secretion per cell) or mass (fewer cells) or both, the effects on muscle and liver lead to chronic hyperglycemia, and slow but progressive development of many of the same symptoms as in type 1.

For type 1 diabetes, the classic solution has been injection of exogenous insulin, which is imperfect but fairly effective. However, tight control of glucose is difficult for most patients

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

to achieve. Multiple doses are required per day, carefully calibrated to meal size and exercise level by self-monitoring.

Type 2 diabetics are also sometimes given insulin but more typically first receive one of several oral drugs. The first class of oral drug for type 2 was the sulfonylureas, which enhance β -cell function, at least initially, by blocking the K(ATP) channel (see below), leading to depolarization, calcium (Ca^{2+}) entry, and insulin release. These drugs eventually become ineffective and have to be supplemented by other classes of drugs, often including insulin [2]. Sulfonylureas do not prevent the loss of β -cell mass, and *in vitro* experiments with islets and mice indicate that their effect on function disappears as the β cells stop responding to the rise in cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) [3].

A natural alternative to increasing insulin secretion for type 2 diabetes, is to treat the insulin resistance that places stress on the β cells. Powerful oral agents exist to achieve this, the thiazolidinediones, which increase glucose uptake by muscle and adipose tissue, and metformin, which reduces the excessive glucose output of the liver [2]. However, these drugs are generally given at the onset of clinical diabetes, when it is too late to halt the progressive loss of β -cell mass already well underway. On the other hand, although insulin resistance precedes frank diabetes by years, the drugs have too many side effects to be given to patients who are not yet ill. This has led to interest in preserving or enhancing insulin secretion.

A newer class of drugs to enhance insulin secretion works by increasing the activity of the glucagon-like peptide 1 receptor (GLP-1), which raises cyclic adenosine monophosphate (cAMP) levels in β cells [4]. This increases insulin secretion, in part by enhancing the effect of Ca^{2+} on vesicle exocytosis. The drugs have other beneficial systemic effects, but despite some encouraging evidence from mice, it is not clear that these drugs preserve β -cell mass in humans [5].

Another possibility that has been tried for type 1 diabetes is to transplant β cells from an organ donor. However, the supply of donors is limited, and transplantation requires lifelong immune suppression. Transplantation has not yet proven to be practical or superior to insulin injections in most cases [6].

An alternative proposed means of β -cell replacement is to implant an artificial pancreas, a machine that can automatically monitor blood glucose and deliver the appropriate dose of insulin. Although devices exist to do both arms of the task (continuous glucose monitoring systems and insulin pumps, respectively), marrying them into a reliable unit remains promising but elusive [7,8].

This brings us to the subject of this review, engineering cells that can substitute for endogenous β cells in secreting insulin in a glucose-dependent fashion. The main obstacles to achieving this goal lie in developing cells that can be safely implanted in patients and that can function for the needed prolonged periods of time (see [9,10] for reviews). We will not be able to address these difficult issues here but will limit our discussion to what features replacement cells should have in order to mimic the functionality of native β cells. Our rationale is that, despite their limitations, mathematical models of β cell electrical activity, Ca^{2+} dynamics and metabolism behave in many respects like β cells and islets and can therefore suggest useful design principles.

Moreover, the attempt to engineer cells can potentially aid in understanding the basic science of how native β cells work. It may be easier to learn from engineered cells in which one knows what elements have been added rather than try to reverse engineer native cells. As we will see, one strategy is to start with cells that can already secrete, such as neurons

and other endocrine cells. This can provide important insight into the comparative physiology of secretory cells by showing what is unique to β cells and what is universal and modular.

One limitation is that most of the available electrophysiological data comes from mice, whereas we know that humans, and even dogs and rats, differ from mice in their complement of ion channels. There are important opportunities here for building models for other species, as some of these differences could be important for larger animals.

2. Raising $[Ca^{2+}]_i$

The primary task of the β cell is to secrete insulin in response to glucose, and it is desirable for the output to increase in a graded way with the stimulus, not in an all-or-none fashion. This is achieved mainly by raising Ca^{2+} concentration, similar to neurotransmitter release and secretion of other hormones. Empirically, secretion increases linearly with the bulk $[Ca^{2+}]_i$ [11], but Ca^{2+} local to Ca^{2+} channels also plays a critical role [12,13,14,15]. The rise in $[Ca^{2+}]_i$ is in turn controlled by the extracellular glucose concentration ($[G]$). When $[G]$ is less than the threshold level of about 5 mM, corresponding to fasting conditions *in vivo*, $[Ca^{2+}]_i$ is low (< 100 nM) and there is almost no secretion. After a meal, $[G]$ exceeds the threshold, and, *in vitro*, $[Ca^{2+}]_i$ rises hyperbolically with $[G]$, saturating at about 200 nM when $[G]$ reaches about 10 mM [16,17]. The increase of $[Ca^{2+}]_i$ with $[G]$ is mediated by an ion channel, K(ATP), which senses the ATP/ADP ratio of the cells. The latter rises with $[G]$, completing the feed-forward connection.

Insulin secretion continues to increase beyond the point where $[Ca^{2+}]_i$ is saturated, up to as much as 30 mM $[G]$ [16,17], demonstrating that factors beyond $[Ca^{2+}]_i$ are important, and may account for as much as 50% of total secretion. Those factors depend on Ca^{2+} , however, and work by enhancing the efficacy of Ca^{2+} , so we will discuss Ca^{2+} first.

2.1. Oscillations

In vitro, β cell electrical and Ca^{2+} activity is generally oscillatory when $[G]$ is in the range of 5–15 mM; above that range, a constant high $[Ca^{2+}]_i$ is seen, corresponding to continuous spike activity. These oscillations occur on a variety of time scales, spiking (< 1 s), bursting, sometimes called “fast bursting” (10–60 s), and slow oscillations, (1–6 min). These have all been modeled, and in a variety of ways, but the mechanisms all have in common that they are based on the combination of fast positive feedback with slow negative feedback, with slow being relative to the timescale in question. Fast bursting and slow oscillations in membrane potential have been seen in islets *in vivo* [18], and slow oscillations in insulin are found in the circulation [19].

2.1.1. Spiking—Similar to the neuronal and endocrine cousins of the β cell, secretion is driven by Ca^{2+} entry through voltage-dependent (V -dependent) Ca^{2+} channels. This is unlike non-electrically excitable cells, including the β cell’s developmental sibling, the pancreatic acinar cell, in which secretion is governed by release of Ca^{2+} from internal stores (endoplasmic reticulum or ER). We will see that the ER does, however, play a significant role in shaping the kinetics and amplitude of the $[Ca^{2+}]_i$ signal.

Secretion depends on both vesicles in close association with Ca^{2+} channels and distal vesicles [20,21,15]. A full treatment therefore would require modeling $[Ca^{2+}]_i$ in the nano-domains surrounding Ca^{2+} channels as well as bulk $[Ca^{2+}]_i$. However, time-averaged domain $[Ca^{2+}]_i$ is likely proportional to bulk Ca^{2+} , and since secretion increases linearly with bulk $[Ca^{2+}]_i$ [11], we will use bulk concentration as a surrogate for secretion.

This means that the average $[Ca^{2+}]_i$ has to be increased in a graded fashion. The first step in satisfying this requirement is to have V -dependent Ca^{2+} and K^+ channels to provide fast positive and slow negative feedback, respectively, for spikes that can bring in Ca^{2+} . Next, the cell needs appropriate metabolic machinery to raise the ATP/ADP ratio as $[G]$ increases. Some of the elements are well understood. For example, the replacement cell would need a glucose transporter that can allow glucose into the cell when insulin is low, i.e. the GLUT2 isoform found in β cells and also glucose-sensing neurons in the hypothalamus. Another is a low-affinity hexokinase, i.e. glucokinase, which can increase its activity as glucose rises in the physiological range instead of saturating at low glucose. Other elements such as the mitochondrial shuttles and other mitochondrial features that are specialized in β cells, are less well understood. Assuming these elements are all in place, the final requirement for a minimal glucose-sensitive cell is the K(ATP) channel, which is inhibited by the rise in ATP/ADP and links the spike activity to metabolism. Alternatively, K(ATP) can be blocked pharmacologically, but then the connection to glucose is lost and must be maintained artificially.

Fig. 1 shows a diagram of the mechanisms incorporated in the models or discussed in the text. Mechanisms will be added sequentially; the shaded, encircled letters in the figure indicate in which figure they are introduced.

A minimal set of mechanisms for a spiking cell that can raise $[Ca^{2+}]_i$ in response to glucose is described by simplified equations of the Hodgkin-Huxley class [22], augmented by an equation for $[Ca^{2+}]_i$:

$$\frac{dV}{dt} = -I_{Ca}(V) - I_{K(V)}(V, n) - I_{K(ATP)}(G, V) \quad (1)$$

$$\frac{dn}{dt} = \frac{n_{\infty}(V) - n}{\tau_n} \quad (2)$$

$$\frac{d[Ca^{2+}]_i}{dt} = J_{in} - J_{out} \quad (3)$$

V is the membrane potential, which is raised by the inward (negative) Ca^{2+} current I_{Ca} and lowered by the outward (positive) K^+ . The Ca^{2+} current responds rapidly (here, instantaneously) to V , providing the fast positive feedback to initiate each spike. The V -dependent K^+ current, $I_{K(V)}$, responds to this rise in V more slowly, terminating the spike. Its slow response is controlled by the gating variable, n , which increases the conductance of the channel as V increases with time constant τ_n . $[Ca^{2+}]_i$ is raised by Ca^{2+} influx through the plasma membrane, J_{in} , which is proportional to I_{Ca} , and reduced by J_{out} , which represents the plasma membrane Ca^{2+} pump. Sensing of the glucose concentration is mediated by $I_{K(ATP)}$, the conductance of which decreases as G increases, reducing its inhibition of V .

Figure 2(a) and the dashed curve in Fig. 2(d) display a simulation of the $[Ca^{2+}]_i$ that would be recorded *in vitro*, say, using a fluorescent dye, from a hypothetical spiking cell engineered to include the mechanisms described above. The figure shows that Eqs. 1–3 produce the desired effect. The increase in mean $[Ca^{2+}]_i$ results from increases in both spike frequency and mean membrane potential. Note that in order to have spikes from baseline driven by Ca^{2+} current, the Ca^{2+} channels have to open at relatively low membrane

potential. This then automatically ensures that the spikes bring in significant amounts of Ca^{2+} . In mouse β cells, the spikes are driven by Ca^{2+} entry. Alternatively, one could start with a neuroendocrine cell with classical neuronal Na^+ spikes. These would work by raising the membrane potential to a level at which Ca^{2+} channels open. Secretion of gonadotropin-releasing hormone (GnRH) from GT1 cells has been modeled in this way [23]. Glucagon secretion from the α cells of the islet has been modeled similarly, but with the twist that glucose-induced depolarization via K(ATP) channels inactivates the Na^+ channels and *terminates* Ca^{2+} entry [24]. Further, β cells in humans [25, 26] and other species have Na^+ currents, which presents opportunities for future modeling of how this changes the picture derived from the Ca^{2+} -centric models for mice.

Native β cells accessorize the spike mechanism with additional features, such as Ca^{2+} -dependent and V -dependent inactivation of Ca^{2+} channels and V -dependent inactivation of K^+ channels. Large conductance Ca^{2+} -activated K^+ (BK K(Ca)) channels probably also contribute to shaping the spikes. A recent paper that takes a first step in incorporating more detailed ionic mechanisms is [27], but much more remains to be done in this direction.

Other glucose-sensing cells, such as GLP-1 secreting intestinal L cells and certain hypothalamic neurons, carry out their functions through modulated spiking, though their spikes are probably initiated by Na^+ channels, and the sodium glucose co-transporter is used instead of or in addition to K(ATP) [28,29]. Nonetheless, these examples indicate that modulated spiking is a viable approach to glucose sensing.

2.1.2. Bursting—Bursting is a ubiquitous pattern found in electrically excitable cells and serves to construct slow timescale oscillations out of fast ones. There are many types of bursting in many types of cells, but almost all are based on slow negative feedback. An accessible monograph on the theory of bursting, as well as the various modes of spiking, is [30].

The simplest way to get bursting is to add a K(Ca) current, such as some type of small conductance, SK channel [31,32]. In the model, we $I_{\text{K(Ca)}}$ to Eq. 1:

$$\frac{dV}{dt} = -I_{\text{Ca}}(V) - I_{\text{K(V)}}(V, n) - I_{\text{K(Ca)}}(V) - I_{\text{K(ATP)}}(G, V). \quad (4)$$

This provides additional negative feedback that slowly increases as Ca^{2+} accumulates during a train (burst) of spikes and terminates the activity. With typical Ca^{2+} buffering power, such that about 1% of Ca^{2+} is unbound, this can give bursts with a period of a few seconds (Fig. 2(b)). To distinguish this from fast bursting, we call it “very fast bursting.” The first model to offer a plausible account for how glucose increases β cell insulin secretion was based on the K(Ca) mechanism [33]. In such a model, reducing the K(ATP) conductance, $g_{\text{K(ATP)}}$, increases the duration of the active phase and decreases the duration of the silent phase. The increase in mean $[\text{Ca}^{2+}]_i$, however, is due almost entirely to the upward shift in the silent and active phase $[\text{Ca}^{2+}]_i$ levels (Fig. 2(b)). Note that the $[\text{Ca}^{2+}]_i$ time course is roughly sawtooth shaped, reflecting the essentially exponential kinetics of a single Ca^{2+} compartment. In islets, however, the $[\text{Ca}^{2+}]_i$ time course is more square shaped, with a plateau, and is more complex, with fast and slow components. The burst period is also much larger, typically 10–60 seconds.

Very fast bursts are often seen, nonetheless, in β cells when they are isolated [34] or exposed to muscarinic agonists [35]. Very fast bursting is also seen in pituitary cells, such as somatotrophs, corticotrophs, and lactotrophs. In all those cases, the $[\text{Ca}^{2+}]_i$ timecourse is indeed sawtooth shaped.

In order to get bursts with periods of tens of seconds, an additional element is needed to slow down the kinetics of cytosolic Ca^{2+} . Chay [36] showed that the ER could play this role. In the model, this means adding an equation for ER Ca^{2+} concentration, $[\text{Ca}^{2+}]_{\text{ER}}$, and adding terms for Ca^{2+} uptake and release by the ER to the Eq. 3, along with a correction for the volume ratio of the two compartments (model adapted from [37]):

$$\frac{d[\text{Ca}^{2+}]_i}{dt} = J_{\text{in}} - J_{\text{out}} + J_{\text{release}} - J_{\text{uptake}} \quad (5)$$

$$\frac{d[\text{Ca}^{2+}]_{\text{ER}}}{dt} = -\frac{V_i}{V_{\text{ER}}}(J_{\text{release}} - J_{\text{uptake}}) \quad (6)$$

The slower bursting produced with the augmented model, Eqs. 2, 4–6, is demonstrated in Fig. 2(c). The mean $[\text{Ca}^{2+}]_i$ again increases as $g_{\text{K(ATP)}}$ is reduced (Fig. 2(d)). The response of mean $[\text{Ca}^{2+}]_i$ to $g_{\text{K(ATP)}}$ is similar to the spiking case, but the mechanism is different from both the spiker and the burster without an ER (Fig. 2(b)). Within the burst regime the increase is now mainly due to the increase in plateau fraction, which increases the proportion of time spent at the higher $[\text{Ca}^{2+}]_i$ level.

$[\text{Ca}^{2+}]_i$ rises rapidly at the beginning of the active phase of the burst and then rises more slowly. The rapid rise is due to the attainment within seconds of a new steady state in response to the increased Ca^{2+} influx. The increased $[\text{Ca}^{2+}]_i$ activates the ER Ca^{2+} pump, which increases the term J_{uptake} in Eq. 6 and as a result the ER slowly fills. This slows the further rise of $[\text{Ca}^{2+}]_i$ as some of the incoming Ca^{2+} is diverted to the ER. The process is reversed when Ca^{2+} influx is shut off at the end of the active phase, producing the observed rapid fall in $[\text{Ca}^{2+}]_i$, followed by a slow fall in quasi-steady state with $[\text{Ca}^{2+}]_{\text{ER}}$ as the ER empties, giving back some of the Ca^{2+} it took up during the active phase. This interpretation is consistent with the observation that the slow components are eliminated when the ER Ca^{2+} pump is blocked [38,39].

2.1.3. Slow Oscillations— β cells exhibit oscillations of $[\text{Ca}^{2+}]_i$ and secretion on yet slower timescales of minutes. These are of particular interest because they drive pulses of insulin secretion in the plasma and may be important for optimizing insulin action [19]. A number of proposals have been put forward for these, mostly based on the hypothesis that $g_{\text{K(ATP)}}$ not only modulates electrical activity but also drives it by transmitting slow oscillations in metabolism. This is reflected in the model by making $I_{\text{K(ATP)}}$ a function of cytosolic $[\text{ADP}]_i$ and $[\text{ATP}]_i$, or, assuming that the sum of the nucleotides is constant, just $[\text{ADP}]_i$:

$$\frac{dV}{dt} = -I_{\text{Ca}}(V) - I_{\text{K(V)}}(V, n) - I_{\text{K(Ca)}}(V) - I_{\text{K(ATP)}}([\text{ADP}]_i, V). \quad (7)$$

(The effect of glucose concentration, G , is now assumed to be on the production of ATP rather than the levels of $[\text{ATP}]_i$ and $[\text{ADP}]_i$, see Eq. 8 below.)

Such models can be classified by whether the fluctuations of metabolism are primarily driven by fluctuations in $[\text{Ca}^{2+}]_i$ or *vice versa*. An example of the former is the model of Magnus and Keizer [40]. Their hypothesis was that Ca^{2+} accumulation in the cytosol during spiking increases Ca^{2+} uptake by the mitochondria, which shunts the mitochondrial

membrane potential and inhibits ATP production. This reopens some of the K(ATP) channels that were closed by the introduction of stimulatory glucose and terminates the active phase of the burst. We account for this by adding the following equation to the model:

$$\frac{d[\text{ADP}]_i}{dt} = \frac{A_\infty([\text{ADP}]_i, G, [\text{Ca}^{2+}]_i) - [\text{ADP}]_i}{\tau_A}, \quad (8)$$

where A_∞ is an increasing function of G and a decreasing function of $[\text{ADP}]_i$ and $[\text{Ca}^{2+}]_i$. Slow bursting based on this model (Eqs. 7, 2, 5, 6, 8) is shown in Fig. 3(a and b).

An alternative proposal [41] is that the rise in $[\text{Ca}^{2+}]_i$ stimulates consumption of ATP, which again reopens K(ATP) channels and terminates each burst. Such a model could in principle perform equivalently to the one presented here. An example of a model based on this hypothesis can be found in [42]. In that model, positive feedback by Ca^{2+} via activation of mitochondrial enzymes also plays a role, helping to initiate each active phase.

Another model that can produce slow bursting is that of Fridlyand, Philipson and colleagues [43]. Metabolism here is again driven by $[\text{Ca}^{2+}]_i$, but oscillations in metabolism are not required for oscillations in $[\text{Ca}^{2+}]_i$. Rather, oscillations are primarily due to the slow rise and fall of Na^+ , which enters through the Na^+ - Ca^{2+} exchanger and activates an outward current through the Na^+ - K^+ ATPase. Independent of whether β cells work this way, slow activation of the Na^+ - K^+ ATPase is another means of achieving slow $[\text{Ca}^{2+}]_i$ oscillations that could be engineered into cells.

In contrast to the class of models above is a class in which metabolic oscillations do not require Ca^{2+} oscillations [44]. Here we describe one such model in which the metabolic oscillations arise from oscillations in glycolysis, which drive the rate of mitochondrial respiration.

We focus on two key metabolites in glycolysis, glucose 6-phosphate (G6P) and fructose-1,6-bis-phosphate (FBP), and the enzyme that converts G6P to FBP, phosphofructokinase (PFK). The key element that makes this system oscillate is the fast positive feedback of FBP onto PFK. The slow negative feedback results from the depletion of G6P. (See [42] for a contrary view on PFK.) To represent these reactions, we add two final equations, incorporating a model of Smolen [45]:

$$\frac{d[\text{G6P}]}{dt} = J_{\text{GK}}(G) - J_{\text{PFK}}([\text{G6P}], [\text{FBP}]) \quad (9)$$

$$\frac{d[\text{FBP}]}{dt} = J_{\text{PFK}}([\text{G6P}], [\text{FBP}]) - \frac{1}{2} J_{\text{GPDH}}([\text{FBP}]) \quad (10)$$

The rate of flux through glucokinase, J_{GK} , is assumed to control the availability of G6P, and now represents the point at which G has its effect. We also assume that the reactions downstream of PFK, such as glycerol phosphate dehydrogenase (GPDH), are in equilibrium, so that in the end $[\text{FBP}]$ determines the rate of substrate entering the mitochondria and thence the rate of production of oxidative phosphorylation. This is reflected in a modification of Eq. 8:

$$\frac{d[\text{ADP}]_i}{dt} = \frac{A_{\infty}([\text{ADP}]_i, [\text{FBP}], [\text{Ca}^{2+}]_i) - [\text{ADP}]_i}{\tau_A}, \quad (11)$$

This combined electrical-glycolytic or dual oscillator model (DOM) [46,44] (Eqs. 7, 2, 5, 6, 9, 10, 11) is used for the simulations in Fig. 3(c–f).

Because the metabolic and ionic subsystems can act semi-independently of each other, the model can produce compound oscillations, i.e. episodes of fast bursting separated by periods of inactivity (Fig. 3(c and d)). (Two other models have been shown to produce compound oscillations. In one [47], the slow component results from inhibition of glucose uptake by insulin, and in the other [48], very fast bursting is episodically interrupted.)

The DOM can also produce pure slow oscillations (Fig. 3(e and f)) and other patterns [46, 44]. Because the glycolytic oscillation is not constrained by the balance of ionic currents, the amplitude of the $[\text{Ca}^{2+}]_i$ and $g_{\text{K(ATP)}}$ oscillations can be much larger than in the pure electrical models. The compound and slow oscillations respond to glucose similarly to the fast ones, by increase of the duration of the high $[\text{Ca}^{2+}]_i$ periods and reduction of the low [49].

A particularly revealing pattern produced by the model (not shown) occurs when glucose is high enough to activate the glycolytic oscillator but $g_{\text{K(ATP)}}$ is too large to allow electrical activity. In this case there are very small (“sub-threshold”) $[\text{Ca}^{2+}]_i$ oscillations but full size oscillations in G6P and FBP. Slow sub-threshold oscillations in $[\text{Ca}^{2+}]_i$ have been seen in islets [49], suggesting that metabolic oscillations can occur in the absence of frank $[\text{Ca}^{2+}]_i$ oscillations. At the same time, this also shows that the metabolic oscillator, though it causes large oscillations in $g_{\text{K(ATP)}}$, cannot trigger excursions in $[\text{Ca}^{2+}]_i$ large enough to yield substantial insulin secretion unless the electrical subsystem goes above threshold and amplifies the signal [50]. Thus, although glycolytic oscillations have been proposed as a way for metabolism to drive electrical activity with $[\text{Ca}^{2+}]_i$ participating only as a second messenger to trigger secretion [51], we find that both the electrical and metabolic oscillators are needed.

Conversely, if one wants to equip a cell with slow oscillations, the model suggests that the only additional element required, beyond the ionic components needed for fast bursting, is a glycolytic oscillator. This in turn requires that the cell have the oscillatory isoform of PFK, found in muscle and β cells [51]; this isoform is distinguished by a lower affinity for ATP, which allows it to remain active in the face of increased mitochondrial ATP production.

We cannot say at this time whether the model simulated in Fig. 3 truly encapsulates the way β cells produce slow oscillations. For example, it is possible that the framework of the DOM is correct, but that the metabolic oscillations originate downstream of glycolysis, in the mitochondria. All we can say is that it includes plausible mechanisms known to exist in β cells and can produce almost all the major patterns (fast, slow, compound and others) that are observed. These mechanisms exist individually in other cells, and thus it is reasonable to attempt to assemble such modules in order to engineer cells that can imitate β cells.

3. Beyond $[Ca^{2+}]_i$

3.1. Modulation

In addition to sensing glucose, β cells need to respond to modulatory factors that potentiate the glucose response (acetylcholine (ACh), GLP-1) or restrain insulin secretion when high plasma glucose is needed for emergencies (epinephrine).

ACh binds to M3 muscarinic receptors and enhances secretion in two ways by cleaving phosphatidyl inositol 4,5-bisphosphate (PIP₂). One product, inositol 1,4,5-trisphosphate (IP₃), triggers release of store Ca^{2+} and the other, diacylglycerol (DAG), activates protein kinase C (PKC). PKC increases the rate of secretion obtained at a given $[Ca^{2+}]_i$ level, and the store dumping converts fast bursting to very fast bursting at a more depolarized level. Thus, the combined effect is an increase in both mean $[Ca^{2+}]_i$ and the efficacy of $[Ca^{2+}]_i$. Models, such as the one in Fig. 2(c), have revealed that store dumping *per se* can account for the increased frequency of bursting, provided the ER is sufficiently labile, but not the depolarization [37]. The latter effect can be achieved if an inward current is activated as well. Two candidates have been described, a store-operated channel and a channel directly activated by the G-protein coupled muscarinic receptor [52].

GLP-1 acts through cAMP and has similar effects as ACh, increasing $[Ca^{2+}]_i$, burst frequency, and the efficiency of exocytosis, with the additional feature that the plateau fraction is increased. An interesting model has been developed to explain observations in cell lines that cAMP can be either in-phase or anti-phase with respect to $[Ca^{2+}]_i$ [53]. The model suggests that this depends on the relative Ca^{2+} sensitivity of adenylyl cyclase, the enzyme that produces cAMP, and phosphodiesterase, the enzyme that degrades it.

3.2. Vesicle Dynamics

The models considered here have as their final output cytosolic Ca^{2+} levels, under the assumption that this determines insulin secretion. However, there is more to the story. At a given level of $[Ca^{2+}]_i$, increase in glucose metabolism itself increases secretion [11]. It is postulated that a metabolic amplifying factor enhances the efficiency of Ca^{2+} in driving exocytosis. Put another way, the rate of exocytosis is the product of the probability of release per vesicle and the number of releasable vesicles. Ca^{2+} is the primary determinant of the former, and the amplifying factor is the primary determinant of the latter [14,15]. The triggering effect of Ca^{2+} and the amplifying factor make roughly equal contributions to total insulin secretion [11].

Secretion also has complex kinetics. The first phase consists of a spike of release that lasts about 10 minutes and decays almost back to baseline. It is followed by a second phase that can rise over the course of an hour in response to a maintained step of glucose [54].

This biphasic pattern has been cast in the language of control theory [8]. The first phase is lost if glucose is raised gradually instead of stepped, so this can be considered a form of “derivative control”, i.e. the output depends on the derivative of the input [55,56] (when the derivative is positive). The rising second phase is an example of “integral control”, i.e. the output depends on the duration of the glucose step, or more generally the integral of the input.

In contrast, the simple models for Ca^{2+} in Figs. 2, 3 only exhibit “proportional control” – the output is roughly proportional to the increase of glucose level above basal. Integral control comes about via slow delivery of new vesicles to the plasma membrane [54,57,14,15]. A variety of models that incorporate a pool of readily-releasable vesicles at the plasma membrane can reproduce the loss of first phase when glucose is raised gradually

[54,57,14,15]. The slow rise of glucose slows the response of the readily releasable pool so that it merges with the release of the slowly growing population of incoming vesicles. However, islet behavior is more complex than this – they respond to escalating steps of glucose with repeated first-phase-like spikes of secretion. More elaborate models in which the size of the readily releasable pool or the subset of cells in an islet that respond is a function of glucose can account for repeated steps [54,58].

The cellular mechanisms are not very relevant for designing machines, which have other ways of achieving combined proportional-integral-derivative (PID) control, but could be important for designing cells to have the desired properties. The models suggest that this can be done by exploiting vesicle trafficking mechanisms that are ubiquitous among endocrine and neuronal secretory cells. Although initial attempts to engineer cells have aimed to achieve any kind of insulin secretion at all, the vesicle models show that oscillations may be important for optimizing secretion by allowing the readily releasable pool to recover between episodes of activity [15] as well as enhancing insulin action [19].

4. Robustness

Up to this point we have focused on the elements that models suggest are capable of providing the various functionalities, but have neglected the equally important matter of how the various components combine quantitatively to produce these effects. The poor performance of insulin-secreting cell lines, which nominally have the right components, indicates that this is a real problem.

As an example, Figs. 2(a) shows an example of the calcium timecourse produced by spiking whereas 2(b) shows the calcium response to membrane potential bursting, made possible by the inclusion of a K(Ca) channel. However, increasing the time constant of the V -dependent K^+ channel in 2(b) by 25% would change the solution to spiking little different from than in panel (a). The same effect can be obtained in a more plausible manner if one assumes that the K^+ channel in the model is an amalgam of a relatively slow voltage-dependent K^+ (K_v) channel and a faster large conductance (BK) K(Ca) channel [59]. In that case, bursting could be converted to spiking by blocking the BK component. Thus, the apparent discrepancy between reports that rat islets respond to glucose by modulating spiking [60] vs. bursting [61] could reflect small differences in BK channel expression, as has been reported for pituitary somatotrophs [62]. Caution must be applied in interpreting these simulation results because the models are much more sensitive than living cells. For example, in Fig. 2, a 10% change in $g_{K(ATP)}$ is sufficient to take a silent cell to a fully active cell. In contrast, measurements of $g_{K(ATP)}$ in isolated β cells suggest that changes on the order of 100% would be required [63]. This may be partly due to the fact that the models have been simplified to the minimal mechanisms required to produce given behaviors. Whereas this has been successful in many ways, further research and possibly novel approaches are needed to improve robustness.

Studies of invertebrate neuronal networks have inspired the hypothesis that such sensitivity to modest variation in cell parameters is overcome via regulation of channel conductances by cell activity [64]. For example, if $[Ca^{2+}]_i$ is too low, g_{Ca} could be increased and/or g_K decreased. The mechanisms for achieving this remain unclear, however. Similar considerations could apply to any cellular process, including metabolism.

Although the mechanisms are not well understood, there is experimental evidence that the self-regulating and modular character of cells can help. The modularity of the action-potential generating system has been demonstrated by transfecting the Na^+ and K^+ channels found in neurons into non-excitable Chinese hamster ovary cells [65]. Similarly, insulin secretion sufficient to protect mice from diabetes when their native β cells were destroyed

was achieved by adding the insulin gene to intestinal K cells; these cells normally secrete glucose-dependent insulintropic polypeptide (GIP) in response to glucose in the lumen of the gut [66]. Another class of professional secretory cells, in the pituitary, was engineered to secrete insulin in response to GLP-1 by supplying them with the genes for insulin and the GLP-1 receptor [67]. *In vitro*, the cells secreted insulin in response to GLP-1 but not glucose. *In vivo*, however, the cells were able to secrete insulin in response to an oral glucose load, presumably due to GLP-1 secretion from intestinal L cells that sensed the glucose. Unfortunately, the cells also secreted pituitary hormones in response to GLP-1, but the experiment showed that the added components were sufficient to achieve regulated insulin secretion because of the pre-existing secretory system.

5. Coupling and Synchronization

Even allowing for some mechanism for regulating cell properties so as to produce a functional output, small cells such as β cells seem to require additional mechanisms to keep them near the target mean. One way to achieve this is to couple the cells by gap junctions. This synchronizes the population so that it behaves roughly like a cell with the mean parameters, and each cell then need not be engineered to exacting tolerances [68]. As a byproduct of synchronization, the glucose dose response curves of the individual cells, which have heterogeneous thresholds, become unified as well. At low glucose, the less active cells suppress the more active ones, reducing basal activity, whereas at high glucose, enough cells have become active to pull the more reluctant ones along, enhancing the stimulated response. Thus, the collective response curve becomes sharper, as predicted by modeling [68] and confirmed by measurements of $[Ca^{2+}]_i$ in single cells vs. clusters [69, Fig. 3]. It is not as clear how synchronized secretion is, as cells may secrete differently in response to the same $[Ca^{2+}]_i$ [58]. Nonetheless, there is some evidence that the secretion dose response is also sharper in islets with gap junctions than without (Cx36 +/+ vs. Cx36-/-) [70, Fig. 9]. In other words, the response is “democratized” – the cells vote according to their individual sensitivities to glucose but either all respond or all remain silent. Synchronizing secretion would have the beneficial effect of distributing the load evenly, whereas in the absence of coupling, the burden would fall mainly on the most active cells. In such a case, the most active cells could be subject to failure due to stress resulting from, say, excessive traffic through the endoplasmic reticulum or production of reactive oxygen species. A similar concept of load sharing has been proposed for vasopressin-secreting neurons of the hypothalamus in the context of diabetes insipidus [71].

Coupling also buffers the population against the effects of stochastic fluctuations in channel conductances or metabolite levels [72]. The models suggest that fast bursting is more sensitive than slow oscillations because it depends on small variations in the driving conductances, such as K(Ca) and K(ATP) [73]. On the other hand, very fast bursting in the models is not so delicate. This may account for why slow and very fast bursting are readily seen in isolated β cells, but not fast bursting.

The above features in principle require only gap junctions. A final level of coordination is the synchronization of large numbers of islets or cell clusters separated in space to obtain pulsatile whole-body insulin secretion. This seems difficult to engineer because it involves organ level interactions, possibly through plasma insulin and glucose [74] and/or pancreatic ganglia [75].

6. Summary

β cells are dauntingly complex, as they must be to produce the great diversity of patterns they do and have proper regulation. On the other hand, a relatively simple set of mechanisms

is sufficient to produce a $[Ca^{2+}]_i$ that rises linearly with $[G]$ (Fig. 2(a and d)). It is not clear how much more is required beyond this to achieve adequate glycemic control *in vivo*. Modeling, nonetheless, offers some encouragement that the full complexity exhibited by β cells result from the combination of relatively simple modules.

This complements the biological understanding that the different functionalities likely evolved in a step-wise manner rather than all at once. Some may have arisen sequentially (eg. spiking followed by bursting) whereas others may have arisen by combining modules that previously evolved in parallel (eg. ionic and glycolytic oscillators). Put another way, the β cell is not *irreducibly* complex – half a β cell may be better than none – and capturing the minimal functionality of glucose sensing may not require much.

On the other hand, the models also show that it is not sufficient for cells to have the proper ingredients – they also need to have them in the right proportions. Modeling has suggested some possible ways for cells to regulate the proportions, but only the surface of this area has been scratched. It may be that this question will in fact be best illuminated by the attempt to design cells.

Acknowledgments

The advances in modeling described here would not have been possible without the collaboration on both theory and experiment over more than 15 years with Les Satin and Richard Bertram. I thank Anmar Khadra and Kevin Hall and two anonymous reviewers for helpful comments on the manuscript. This work was supported by the Intramural Research Program of the National Institutes of Health, USA, NIDDK.

References

1. Hoehn KL, Salmon AB, Hohnen-Behrens C, Turner N, Hoy AJ, Maghzal GJ, Stocker R, Remmen HV, Kraegen EW, Cooney GJ, Richardson AR, James DE. Insulin resistance is a cellular antioxidant defense mechanism. *Proc Natl Acad Sci U S A* 2009;106(42):17787–17792. [PubMed: 19805130]
2. Brown JB, Nichols GA, Perry A. The burden of treatment failure in type 2 diabetes. *Diabetes Care* 2004;27(7):1535–1540. [PubMed: 15220224]
3. Remedi MS, Nichols CG. Chronic antidiabetic sulfonylureas in vivo: reversible effects on mouse pancreatic beta-cells. *PLoS Med* 2008;5(10):e206. [PubMed: 18959471]
4. Lovshin JA, Drucker DJ. Incretin-based therapies for type 2 diabetes mellitus. *Nat Rev Endocrinol* 2009;5(5):262–269. [PubMed: 19444259]
5. Wajchenberg BL. beta-cell failure in diabetes and preservation by clinical treatment. *Endocr Rev* 2007;28(2):187–218. [PubMed: 17353295]
6. Harlan DM, Kenyon NS, Korsgren O, Roep BO. Current advances and travails in islet transplantation. *Diabetes* 2009;58(10):2175–2184. [PubMed: 19794074]
7. Saudek CD. Can diabetes be cured?: potential biological and mechanical approaches. *JAMA* 2009;301(15):1588–1590. [PubMed: 19366779]
8. Steil GM, Panteleon AE, Rebrin K. Closed-loop insulin delivery-the path to physiological glucose control. *Adv Drug Deliv Rev* 2004;56(2):125–144. [PubMed: 14741112]
9. McClenaghan NH, Flatt PR. Engineering cultured insulin-secreting pancreatic b-cell lines. *J Mol Med* 1999;77(1):235–243. [PubMed: 9930971]
10. Soria B, Andreu E, Berna G, Fuentes E, Gil A, Leon-Quinto T, Martin F, Montanya E, Nadal A, Reig JA, Ripoll C, Roche E, Sanchez-Andres JV, Segura J. Engineering pancreatic islets. *Pflugers Arch* 2000;440(1):1–18. [PubMed: 10863992]
11. Henquin JC. Triggering and amplifying pathways of regulation of insulin secretion by glucose. *Diabetes* 2000;49:1751–1760. [PubMed: 11078440]
12. Rorsman P, Renström E. Insulin granule dynamics in pancreatic beta cells. *Diabetologia* 2003;46(8):1029–1045. [PubMed: 12879249]

13. Eliasson L, Abdulkader F, Braun M, Galvanovskis J, Hoppa MB, Rorsman P. Novel aspects of the molecular mechanisms controlling insulin secretion. *J Physiol* 2008;586(14):3313–3324. [PubMed: 18511483]
14. Chen YD, Wang S, Sherman A. Identifying the targets of the amplifying pathway for insulin secretion in pancreatic beta-cells by kinetic modeling of granule exocytosis. *Biophys J* 2008;95(5):2226–2241. [PubMed: 18515381]
15. Pedersen MG, Sherman A. Newcomer insulin secretory granules as a highly calcium sensitive pool. *Proc Natl Acad Sci (USA)* 2009;106(18):7432–7436. [PubMed: 19372374]
16. Heart E, Corkey RF, Wikstrom JD, Shirihai OS, Corkey BE. Glucose-dependent increase in mitochondrial membrane potential, but not cytoplasmic calcium, correlates with insulin secretion in single islet cells. *Am J Physiol Endocrinol Metab* 2006;290(1):E143–E148. [PubMed: 16144817]
17. Henquin JC, Nenquin M, Stiernet P, Ahren B. In vivo and in vitro glucose-induced biphasic insulin secretion in the mouse: pattern and role of cytoplasmic Ca^{2+} and amplification signals in β -cells. *Diabetes* 2006;55:441–451. [PubMed: 16443779]
18. Sanchez-Andres JV, Gomis A, Valdeolmillos M. The electrical activity of mouse pancreatic beta-cells recorded in vivo shows glucose-dependent oscillations. *J Physiol* 1995;486(Pt 1):223–228. [PubMed: 7562637]
19. Schmitz O, Brock B, Hollingdal M, Juhl CB, Porksen N. High-frequency insulin pulsatility and type 2 diabetes: from physiology and pathophysiology to clinical pharmacology. *Diabetes Metab* 2002;28(6 Suppl):4S14–4S20. [PubMed: 12703061]
20. Yang Y, Gillis KD. A highly Ca^{2+} -sensitive pool of granules is regulated by glucose and protein kinases in insulin-secreting ins-1 cells. *J Gen Physiol* 2004;124(6):641–651. [PubMed: 15572344]
21. Wan QF, Dong Y, Yang H, Lou X, Ding J, Xu T. Protein kinase activation increases insulin secretion by sensitizing the secretory machinery to Ca^{2+} . *J Gen Physiol* 2004;124(6):653–662. [PubMed: 15572345]
22. Hodgkin AL, Huxley AF. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J Physiol (London)* 1952;117:205–249.
23. LeBeau AP, Goor FV, Stojilkovic SS, Sherman A. Modeling of membrane excitability in gonadotropin-releasing hormone-secreting hypothalamic neurons regulated by Ca^{2+} -mobilizing and adenylyl cyclase-coupled receptors. *J Neurosci* 2000;20:9290–9297. [PubMed: 11125008]
24. Diderichsen PM, Göpel SO. Modelling the electrical activity of pancreatic α -cells based on experimental data from mouse islets. *J Biol Physics* 2006;32:209–229.
25. Barnett DW, Pressel DM, Misler S. Voltage-dependent Na^{+} and Ca^{2+} currents in human pancreatic islet beta-cells: evidence for roles in the generation of action potentials and insulin secretion. *Pflugers Arch* 1995;431(2):272–282. [PubMed: 9026789]
26. Braun M, Ramracheya R, Bengtsson M, Zhang Q, Karanauskaite J, Partridge C, Johnson PR, Rorsman P. Voltage-gated ion channels in human pancreatic beta-cells: electrophysiological characterization and role in insulin secretion. *Diabetes* 2008;57(6):1618–1628. [PubMed: 18390794]
27. Meyer-Hermann ME. The electrophysiology of the beta-cell based on single transmembrane protein characteristics. *Biophys J* 2007;93(8):2952–2968. [PubMed: 17573431]
28. Reimann F, Gribble FM. Glucose-sensing in glucagon-like peptide-1-secreting cells. *Diabetes* 2002;51(9):2757–2763. [PubMed: 12196469]
29. O'Malley D, Reimann F, Simpson AK, Gribble FM. Sodium-coupled glucose cotransporters contribute to hypothalamic glucose sensing. *Diabetes* 2006;55(12):3381–3386. [PubMed: 17130483]
30. Izhikevich, EM. *Dynamical Systems in Neuroscience: The Geometry of Excitability and Bursting*. MIT Press; Cambridge, MA: 2007.
31. Göpel SO, Kanno T, Barg S, Eliasson L, Galvanovskis J, Renström E, Rorsman P. Activation of Ca^{2+} -dependent K^{+} channels contributes to rhythmic firing of action potentials in mouse pancreatic β cells. *J Gen Physiol* 1999;114:759–769. [PubMed: 10578013]

32. Zhang M, Houamed K, Kupersmidt S, Roden D, Satin L. Pharmacological properties and functional role of Kslow current in mouse pancreatic β -cells: SK channels contribute to Kslow tail current and modulate insulin secretion. *J Gen Physiol* 2005;126(4):353–363. [PubMed: 16186562]
33. Chay TR, Keizer J. Minimal model for membrane oscillations in the pancreatic β cell. *Biophys J* 1983;42:181–190. [PubMed: 6305437]
34. Kinard TA, de Vries G, Sherman A, Satin LS. Modulation of the bursting properties of single mouse pancreatic β cells by artificial conductances. *Biophys J* 1999;76:1423–1435. [PubMed: 10049324]
35. Bertram R, Smolen P, Sherman A, Mears D, Atwater I, Martin F, Soria B. A role for calcium release-activated current (CRAC) in cholinergic modulation of electrical activity in pancreatic β cells. *Biophys J* 1995;68:2323–2332. [PubMed: 7647236]
36. Chay TR. Effects of extracellular calcium on electrical bursting and intracellular and luminal calcium oscillations in insulin secreting pancreatic beta-cells. *Biophys J* 1997;73:1673–1688. [PubMed: 9284334]
37. Bertram R, Sherman A. A calcium-based phantom bursting model for pancreatic β -cells. *Bull Math Biol* 2004;66:1313–1344. [PubMed: 15294427]
38. Arredouani A, Henquin JC, Gilon P. Contribution of the endoplasmic reticulum to the glucose-induced $[Ca^{2+}]_c$ response in mouse pancreatic islets. *Am J Physiol (Endocrinol Metab)* 2002;282:E982–991. [PubMed: 11934662]
39. Bertram R, Sherman A. Filtering of calcium transients by the endoplasmic reticulum in pancreatic β -cells. *Biophys J* 2004;87(6):3775–3785. [PubMed: 15465863]
40. Magnus G, Keizer J. Model of β -cell mitochondrial calcium handling and electrical activity. II Mitochondrial variables. *Am J Physiol* 1998;274:C1174–C1184. [PubMed: 9575814]
41. Detimary P, Gilon P, Henquin JC. Interplay between cytoplasmic Ca^{2+} and the atp/adp ratio: a feedback control mechanism in mouse pancreatic islets. *Biochem J* 1998;333:269–274. [PubMed: 9657965]
42. Diederichs F. Mathematical simulation of membrane processes and metabolic fluxes of the pancreatic β -cell. *Bull Math Biol* 2006;68:1779–1818. [PubMed: 16832733]
43. Fridlyand LE, Tamarina N, Philipson LH. Modeling of Ca^{2+} flux in pancreatic beta-cells: role of the plasma membrane and intracellular stores. *Am J Physiol (Endocrinol Metab)* 2003;285(1):E138–E154. [PubMed: 12644446]
44. Bertram R, Sherman A, Satin L. Metabolic and electrical oscillations: partners in controlling pulsatile insulin secretion. *Am J Physiol Endocrinol Metab* 2007;293:890–900.
45. Smolen P. A model for glycolytic oscillations based on skeletal muscle phosphofructokinase kinetics. *J Theo Biol* 1995;174:137–148.
46. Bertram R, Satin L, Zhang M, Smolen P, Sherman A. Calcium and glycolysis mediate multiple bursting modes in pancreatic islets. *Biophys J* 2004;87(5):3074–3087. [PubMed: 15347584]
47. Kang H, Jo J, Kim HJ, Choi MY, Rhee SW, Koh DS. Glucose metabolism and oscillatory behavior of pancreatic islets. *Phys Rev E* 2005;72:051905.
48. Bertram R, Rhoads J, Cimbor WP. A phantom bursting mechanism for episodic bursting. *Bull Math Biol* 2008;70(7):1979–93. [PubMed: 18648884]
49. Nunemaker CS, Bertram R, Sherman A, Tsaneva-Atanosova K, Daniel CR, Satin LS. Glucose modulates $[Ca^{2+}]_i$ oscillations in pancreatic islets via ionic and glycolytic mechanisms. *Biophys J* 2006;91:2082–2096. [PubMed: 16815907]
50. Goel P, Sherman A. The geometry of bursting in the dual oscillator model of pancreatic β -cells. *SIAM J on Applied Dynamical Systems* 2009;8(4):1664–1693.
51. Tornheim K. Are metabolic oscillations responsible for normal oscillatory insulin secretion? *Diabetes* 1997;46(9):1375–80. [PubMed: 9287034]
52. Mears D, Zimlik CL. Muscarinic agonists activate Ca^{2+} store-operated and -independent ionic currents in insulin-secreting HIT-T15 cells and mouse pancreatic β -cells. *J Membr Biol* 2004;197(1):59–70. [PubMed: 15014918]
53. Fridlyand LE, Harbeck MC, Roe MW, Philipson LH. Regulation of cAMP dynamics by Ca^{2+} and G protein-coupled receptors in the pancreatic β -cell: a computational approach. *Am J Physiol Cell Physiol* 2007;293(6):C1924–C1933. [PubMed: 17928534]

54. Grodsky G. A threshold distribution hypothesis for packet storage of insulin and its mathematical modeling. *J Clin Invest* 1972;51:2047–2059. [PubMed: 4559946]
55. Mari A, Ferrannini E. Beta-cell function assessment from modelling of oral tests: an effective approach. *Diabetes Obes Metab* 2008;10(Suppl 4):77–87. [PubMed: 18834435]
56. Breda E, Cavaghan MK, Toffolo G, Polonsky KS, Cobelli C. Oral glucose tolerance test minimal model indexes of beta-cell function and insulin sensitivity. *Diabetes* 2001;50(1):150–158. [PubMed: 11147781]
57. Bertuzzi A, Salinari S, Mingrone G. Insulin granule trafficking in beta-cells: mathematical model of glucose-induced insulin secretion. *Am J Physiol Endocrinol Metab* 2007;293(1):E396–E409. [PubMed: 17456637]
58. Pedersen MG, Corradin A, Toffolo GM, Cobelli C. A subcellular model of glucose-stimulated pancreatic insulin secretion. *Philos Transact A Math Phys Eng Sci* 2008;366(1880):3525–3543. [PubMed: 18653438]
59. MacDonald PE, Sewing S, Wang J, Joseph JW, Smukler SR, Sakellaropoulos G, Wang J, Saleh MC, Chan CB, Tsushima RG, Salapatek AM, Wheeler MB. Inhibition of Kv2.1 voltage-dependent K⁺ channels in pancreatic β -cells enhances glucose-dependent insulin secretion. *J Biol Chem* 2002;277(47):44938–44945. [PubMed: 12270920]
60. Antunes CM, Salgado AP, Rosario LM, Santos RM. Differential patterns of glucose-induced electrical activity and intracellular calcium responses in single mouse and rat pancreatic islets. *Diabetes* 2000;49(12):2028–2038. [PubMed: 11118004]
61. Manning Fox JE, Gyulkhandanyan AV, Satin LS, Wheeler MB. Oscillatory membrane potential response to glucose in islet β -cells: a comparison of islet-cell electrical activity in mouse and rat. *Endocrinology* 2006;147(10):4655–4663. [PubMed: 16857746]
62. van Goor F, Li YX, Stojilkovic S. Paradoxical role of large-conductance calcium-activated K⁺ (BK) channels in controlling action potential-driven Ca²⁺ entry in anterior pituitary cells. *J Neurosci* 2001;21:5902–5915. [PubMed: 11487613]
63. Tarasov AI, Girard CA, Ashcroft FM. ATP sensitivity of the ATP-sensitive K⁺ channel in intact and permeabilized pancreatic β -cells. *Diabetes* 2006;55(9):2446–2454. [PubMed: 16936192]
64. Marder E, Prinz AA. Modeling stability in neuron and network function: the role of activity in homeostasis. *BioEssays* 2002;24(12):1145–1154. [PubMed: 12447979]
65. Hsu H, Huang E, Yang XC, Karschin A, Labarca C, Figl A, Ho B, Davidson N, Lester HA. Slow and incomplete inactivations of voltage-gated channels dominate encoding in synthetic neurons. *Biophys J* 1993;65(3):1196–1206. [PubMed: 8241400]
66. Cheung AT, Dayanandan B, Lewis JT, Korbitt GS, Rajotte RV, Bryer-Ash M, Boylan MO, Wolfe MM, Kieffer TJ. Glucose-dependent insulin release from genetically engineered k cells. *Science* 2000;290(5498):1959–1962. [PubMed: 11110661]
67. Wu L, Nicholson W, Wu CY, Xu M, McGaha A, Shiota M, Powers AC. Engineering physiologically regulated insulin secretion in non-beta cells by expressing glucagon-like peptide 1 receptor. *Gene Ther* 2003;10(19):1712–1720. [PubMed: 12923570]
68. Smolen P, Rinzel J, Sherman A. Why pancreatic islets burst but single β cells do not: The heterogeneity hypothesis. *Biophys J* 1993;64:1668–1680. [PubMed: 8369400]
69. Jonkers FC, Henquin JC. Measurements of cytoplasmic ca²⁺ in islet cell clusters show that glucose rapidly recruits beta-cells and gradually increases the individual cell response. *Diabetes* 2001;50(3):540–550. [PubMed: 11246873]
70. Speier S, Gjinojci A, Charollais A, Meda P, Rupnik M. Cx36-mediated coupling reduces beta-cell heterogeneity, confines the stimulating glucose concentration range, and affects insulin release kinetics. *Diabetes* 2007;56(4):1078–1086. [PubMed: 17395748]
71. Leng G, Brown C, Sabatier N, Scott V. Population dynamics in vasopressin cells. *Neuroendocrinology* 2008;88(3):160–172. [PubMed: 18667805]
72. Sherman A, Rinzel J, Keizer J. Emergence of organized bursting in clusters of pancreatic β cells by channel sharing. *Biophys J* 1988;54:411–425. [PubMed: 2850029]
73. Pedersen MG. Phantom bursting is highly sensitive to noise and unlikely to account for slow bursting in β -cells: Considerations in favor of metabolically driven oscillations. *J Theor Biol* 2007;248:391–400. [PubMed: 17604056]

74. Pedersen MG, Bertram R, Sherman A. Intra- and inter-islet synchronization of metabolically driven insulin secretion. *Biophys J* 2005;89:107–119. [PubMed: 15834002]
75. Zhang M, Fendler B, Peercy B, Goel P, Bertram R, Sherman A, Satin LS. Long lasting synchronization of calcium oscillations by cholinergic stimulation in isolated pancreatic islets. *Biophys J* 2008;95:4676–4688. [PubMed: 18708464]

Figure 1. Sketch of mechanisms in the β cell discussed in the review. Abbreviations: mito, mitochondria; ER, endoplasmic reticulum; PFK, phosphofructokinase; GK, glucokinase; G, glucose; G6P, glucose 6-phosphate; FBP, fructose 1,6-bisphosphate; RRP, readily releasable pool; for others see text and equations. Arrows with sharp ends indicate stimulation, arrows with round ends indicate inhibition. The shaded, encircled letters indicate in which figure the associated mechanism: a, Fig. 2a; b, Fig. 2b; c, Fig. 2c; d, Fig. 3a, b; e, Fig. 3c,d,e,f.

Figure 2. Effects of lowering $g_{K(ATP)}$ conductance on cytosolic $[Ca^{2+}]_i$ for a spiker (a), a burster (b) and a burster with ER (c). The boxes in (a) indicate the $g_{K(ATP)}$ values for panels (a)–(c). For the spiker, there is both an increase in spike frequency and a rise in the gross level due to increased mean membrane potential. For the bursters, there is a rise in plateau fraction within the burst regime. Panel (d): mean cytosolic $[Ca^{2+}]_i$ for the models in (a) and (c).

Figure 3.

Panels (a) and (b): Oscillations of $[Ca^{2+}]_i$ and $g_{K(ATP)}$ generated by a model based on Keizer-Magnus model [40], in which Ca^{2+} uptake by the mitochondria inhibits ATP production. Panels (c) and (d): Combined fast and slow oscillations generated by a model in which glycolytic oscillations drive oscillations in mitochondrial ATP production [46]. Panels (e) and (f): Very slow oscillations driven by glycolytic oscillations [46].