Authors


PhD Richard Bertram
Department of Mathematics, Florida State University, Tallahassee, USA

Arthur Sherman
Laboratory of Biological Modeling, Bethesda, USA

Leslie S. Satin
Department of Pharmacology and Brehm Diabetes Center, Ann Arbor, USA

Editor


Md. Shahidul Islam, M.D., Ph.D.
Department of Clinical Sciences and Education, Södersjukhuset, Stockholm, Sweden

Electrical, Calcium, and Metabolic Oscillations in Pancreatic Islets

Islets of Langerhans · Article ID: 366965 · Chapter ID: 10

[edit] [options]**ABSTRACT**

Oscillations are an integral part of insulin secretion, and are due ultimately to oscillations in the electrical activity of pancreatic β-cells, called bursting. We discuss the underlying mechanisms for bursting oscillations in mouse islets and the parallel oscillations in intracellular calcium and metabolism. We present a unified biophysical model, called the Dual Oscillator Model, in which fast electrical oscillations are due to the feedback of Ca^{2+} onto K^+ ion channels, and the slow component is due to oscillations in glycolysis. The combination of these mechanisms can produce the wide variety of bursting and Ca^{2+} oscillations observed in islets, including fast, slow, compound, and accordion bursting. We close with a description of recent experimental studies that have tested unintuitive predictions of the model and have thereby provided the best evidence to date that oscillations in glycolysis underlie the slow (~5 min) component of electrical, calcium, and metabolic oscillations in mouse islets.

Keywords: bursting, insulin secretion, islet, pulsatility, oscillations

Like many neurons and endocrine cells, pancreatic β-cells are electrically excitable, producing electrical impulses in response to elevations in glucose. The electrical spiking pattern typically comes in the form of bursting, and is most well studied in mouse islets. Bursting is characterized as periodic clusters of impulses followed by silent phases when there is a cessation of impulse firing (Fig. 1). In this chapter we discuss the different types of bursting patterns observed in mouse islets and the underlying mechanisms for these oscillations and parallel oscillations in intracellular Ca^{2+} and metabolism.

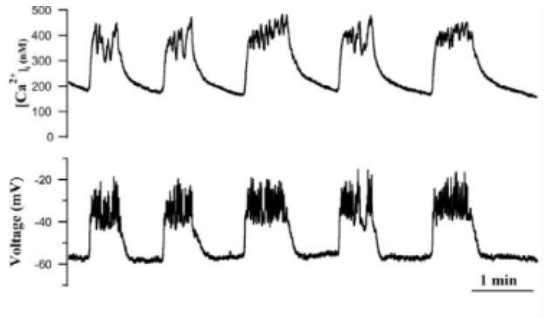

Figure 1: Intracellular free Ca^{2+} concentration measured using fura-2/AM (top) and electrical bursting (bottom) recorded from a mouse islet. Reprinted from Zhang et al. (2003).

Bursting electrical activity is important since it leads to oscillations in the intracellular free Ca^{2+} concentration (Santos et al. 1991; Beauvois et al. 2006), which in turn lead to oscillations in insulin secretion (Gilon et al. 1993). Oscillatory insulin levels have been measured *in vivo* (Lang et al. 1981; Pørksen et al. 1995; Pørksen 2002; Nunemaker et al. 2005), and sampling from the hepatic portal vein in rats, dogs, and humans shows large oscillations with period of 4 to 5 min (Song et al. 2000; Matveyenko et al. 2008). Deconvolution analysis demonstrates that the oscillatory insulin level is due to oscillatory secretion of insulin from islets (Pørksen et al. 1997; Matveyenko et al. 2008), and in humans at least 75% of insulin secretion is in the form of insulin pulses (Pørksen et al. 1997). The amplitude of insulin oscillations in the peripheral blood of human subjects is ~100 times smaller than in the hepatic portal vein (Song et al. 2000). This attenuation is confirmed by findings of hepatic insulin clearance of ~50% in dogs (Polonsky et al. 1983), and ~40-80% in humans (Eaton et al. 1983; Meier et al. 2005). It has also been demonstrated that the hepatic insulin clearance rate itself is oscillatory, corresponding to portal insulin oscillations. That is, during the peak of an insulin oscillation the insulin clearance rate is greater than during the trough (Meier et al. 2005). This illustrates that insulin oscillations are treated differently by the liver than non-pulsatile insulin levels, and thus suggests an important role for oscillations in the hepatic processing of insulin and, presumably, of glucose. Additional supporting evidence for this is provided by a study showing that glucose clearance is facilitated when insulin is pulsatile (Matveyenko et al. 2012). Clinical evidence for the importance of pulsatile insulin comes from studies showing that coherent insulin oscillations are disturbed or lost in patients with type II diabetes and their near relatives (Matthews et al. 1983; Weigle 1987; O'Rahilly et al. 1988; Polonsky et al. 1988).

Oscillations in insulin have also been observed in the perifused pancreas (Stagner et al. 1980), and in isolated islets (Longo et al. 1991; Bergsten and Hellman 1993; Gilon et al. 1993; Beauvois et al. 2006; Ritzel et al. 2006). The oscillations have two distinct periods; the faster oscillations have a period of 2 min or less (Gilon et al. 1993; Bergsten 1995; Bergsten 1998; Nunemaker et al. 2005) while the slower oscillations have greater periods of often 4 min or more (Pørksen et al. 1995; Pørksen 2002; Nunemaker

et al. 2005). In one recent study, insulin measurements were made *in vivo* in mice, and it was shown that some mice had insulin oscillations with period of 1-2 min ("fast mice"), while others exhibited a greater period of 3-5 min ("slow mice"). Interestingly, most of the islets examined *in vitro* from a given mouse had Ca^{2+} oscillations with similar periods. Most islets from "fast mice" had fast Ca^{2+} oscillations, while most of those examined from "slow mice" exhibited either slow or compound Ca^{2+} oscillations (fast oscillations superimposed on slow ones). Thus, it was conjectured that islets within a single animal are imprinted with a relatively uniform oscillation period that is reflected in the insulin profile *in vivo*. As we describe later, the two time scales of electrical bursting can explain the two components of oscillatory insulin secretion and their combinations.

THE ROLE OF CALCIUM FEEDBACK

Ca^{2+} enters β-cells through Ca^{2+} channels during the active phase of a burst, during which it accumulates and activates Ca^{2+}-dependent K^+ channels (Göpel et al. 1999; Goforth et al. 2002). The resulting hyperpolarizing current is in many cases sufficient to terminate the active phase of the burst, and the time required to deactivate the current can set the duration of the silent phase of the burst (Chay and Keizer 1983). Indeed, the first mathematical model for bursting in β-cells was based on this mechanism (Chay and Keizer 1983). The endoplasmic reticulum (ER) plays a major role in shaping the Ca^{2+} dynamics, taking up Ca^{2+} during the active phase of a burst (using the sarco- endoplasmic reticulum Ca^{2+} ATPase or SERCA pump; (Ravier et al. 2011)) when Ca^{2+} influx into the cytosolic compartment is large and releasing Ca^{2+} during the silent phase of the burst. These filtering actions have a significant impact on the time dynamics of the cytosolic Ca^{2+} concentration, and on the period of bursting. The influence of the ER on cytosolic free Ca^{2+} dynamics was convincingly demonstrated using pulses of KCl to effectively voltage clamp the entire islet (Gilon et al. 1999; Arredouani et al. 2002). Using 30-sec pulses, similar to the duration of a fast burst, it was shown that the amplitude of the Ca^{2+} response to depolarization was greater when the ER was drained of Ca^{2+} by pharmacologically blocking ER Ca^{2+} pumps (SERCA). In addition, the slow decline of the cytosolic Ca^{2+} concentration that followed the more rapid declining phase of cytosolic Ca^{2+} was absent when SERCA pumps were blocked. The mechanisms for these effects were determined in mathematical modeling studies (Bertram and Sherman 2004; Bertram and Arceo II 2008), and it was demonstrated that an active form of Ca^{2+}-induced Ca^{2+} release (CICR) is inconsistent with data from (Gilon et al. 1999; Arredouani et al. 2002). CICR did occur in single β-cells in response to cyclic AMP (Ämmälä et al. 1993), but in this case electrical activity and Ca^{2+} oscillations are out of phase (Keizer and De Young 1993; Zhan et al. 2008), which contrasts with the in-phase oscillations observed in glucose-stimulated islets (Santos et al. 1991; Beauvois et al. 2006). These predictions of the model were confirmed recently by measurements for the first time of Ca^{2+} in the ER during cytosplasmic Ca^{2+} oscillations (Higgins et al. 2006).

In addition to the direct effect on Ca^{2+}-activated K^+ channels, intracellular Ca^{2+} has two opposing effects on glucose metabolism in β-cells. Most of the Ca^{2+} that enters the cell is pumped out of the cell or into the ER by Ca^{2+} ATPases, which utilize ATP and thus decrease the intracellular ATP concentration (Detimary et al. 1998). Ca^{2+} that enters mitochondria through the Ca^{2+} uniporter depolarizes the mitochondrial inner membrane potential and thus reduces the driving force for mitochondrial ATP production (Magnus and Keizer 1997; Magnus and Keizer 1998; Krippeit-Drews et al. 2000; Kindmark et al. 2001). Once inside the mitochondria, free Ca^{2+} stimulates pyruvate dehydrogenase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase (Civelek et al. 1996; MacDonald et al. 2003), resulting in increased production of NADH, which can increase mitochondrial ATP production. Thus, Ca^{2+} has two opposing effects on the ATP/ADP ratio; one may dominate under some conditions, while the other action dominates in different conditions.

The ATP/ADP ratio is relevant for islet electrical activity due to the presence of ATP-sensitive K^+ channels, which link the potential of the plasma membrane to the metabolic state of the β-cell (Ashcroft et al. 1984). Variations in the nucleotide ratio result in variation of the fraction of open K(ATP) channels. Thus, oscillations in the intracellular Ca^{2+} concentration can lead to oscillations in the ATP/ADP ratio, which can contribute to bursting through the action of hyperpolarizing K(ATP) current (Keizer and Magnus 1989; Henquin 1990; Smolen and Keizer 1992; Bertram and Sherman 2004).

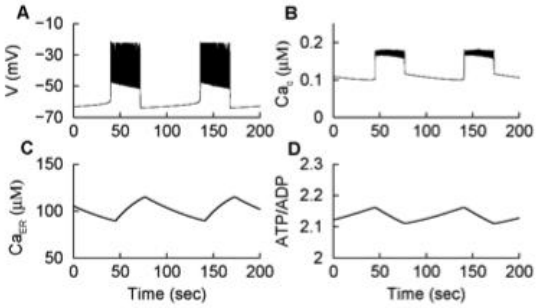

Figure 2: Model simulation of bursting illustrating the dynamics of membrane potential (V), free cytosolic Ca^{2+} concentration (Ca_C), free ER Ca^{2+} concentration (Ca_{ER}), and the ATP/ADP concentration ratio. The model is described in Bertram and Sherman (2004) and the computer code can be downloaded from www.math.fsu.edu/~bertram/software/islet.

Figure 2 uses a mathematical model (Bertram and Sherman 2004) to demonstrate the dynamics of the variables described above. (Other models have been developed, postulating different burst mechanisms and highlighting other biochemical pathways (Fridlyand et al. 2003; Diederichs 2006; Cha et al. 2011).) Two bursts are shown in Fig. 2A and the cytosolic free Ca^{2+} concentration (Ca_C) is shown in Fig. 2B. At the beginning of an active phase Ca_C quickly rises to a plateau that persists throughout the burst. Simultaneously, the ER free Ca^{2+} concentration (Ca_{ER}) slowly increases as SERCA activity begins to fill the ER with Ca^{2+} (Fig. 2C). In contrast, the ATP/ADP ratio during a burst declines (Fig. 2D), since in this model the negative effect of Ca^{2+} on the ATP level dominates the positive effect. Both K(Ca) and K(ATP) currents, concomitantly activated by the increased Ca^{2+} and decreased ATP/ADP, respectively, combine to terminate the burst, after which Ca_C slowly declines. This slow decline reflects the passive release of Ca^{2+} from the ER during the silent phase of the burst, along with the removal of Ca^{2+} from the cell by Ca^{2+} pumps in the plasma membrane. Also, ATP/ADP increases during the silent phase, slowly turning off K(ATP) current. The combined effect of reducing K(Ca) and K(ATP) currents eventually leads to the initiation of a new active phase and the cycle restarts.

METABOLIC OSCILLATIONS

As described above and illustrated in Fig. 2, metabolic oscillations can arise from the effects of Ca^{2+} on the mitochondria and ATP utilization by pumps. In addition, there is considerable evidence for Ca^{2+}-independent metabolic oscillations, reviewed in (Tornheim 1997; Bertram et al. 2007). The leading hypothesis is that glycolysis is oscillatory and is the primary mechanism underlying pulsatile insulin secretion from β-cells (Tornheim 1997). The M-type isoform of the glycolytic enzyme phosphofructokinase-1 (PFK1) is known to exhibit oscillatory activity in muscle extracts, as measured by oscillations in the levels of the PFK1 substrate fructose 6-phosphate (F6P) and the PFK1 product fructose 1,6-bisphosphate (F1,6BP) (Tornheim and Lowenstein 1974; Tornheim et al. 1991). The period of these oscillations, 5-10 min, is similar to the period of slow insulin oscillations (Tornheim 1997). The mechanism for the oscillatory activity of this isoform, which is the dominant PFK1 isoform in islets (Yaney et al. 1995), is the positive feedback of its product F1,6BP on phosphofructokinase activity (Fig. 3) and subsequent depletion of its substrate, F6P (Tornheim 1979; Smolen 1995; Westermark and Lansner 2003). While there is currently no direct evidence for glycolytic oscillations in β-cells, there is substantial evidence for metabolic oscillations. This come mainly from measurements of oscillations in several key metabolic variables, such as oxygen consumption (Longo et al. 1991; Ortsäter et al. 2000; Bergsten et al. 2002; Kennedy et al. 2002), ATP or the ATP/ADP ratio (Nilsson et al. 1996; Ainscow and Rutter 2002; Juntti-Berggren et al. 2003), the mitochondrial inner membrane potential (Kindmark et al. 2001), lactate release (Chou et al. 1992), and NAD(P)H levels (Luciani et al. 2006). Additionally, it has been demonstrated that patients with homozygous PFK1-M deficiency are predisposed to type II diabetes (Ristow et al. 1997), and in a study on humans with an inherited deficiency of PFK1-M it was shown that oscillations in insulin secretion were impaired (Ristow et al. 1999). These data suggest that the origin of insulin secretion oscillations is glycolysis. In the second part of this chapter we discuss additional evidence for glycolytic oscillations, in the context of islet bursting.

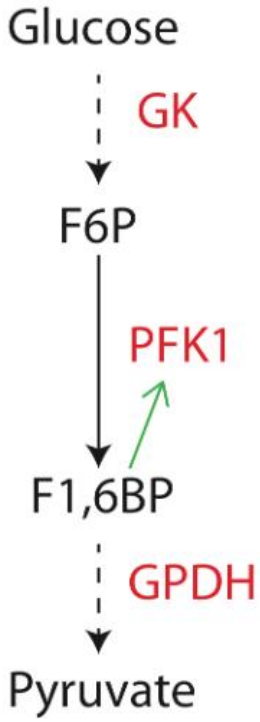

Figure 3: A few steps in glycolysis, focusing on the positive feedback of F1,6BP onto the allosteric enzyme PFK1 (green arrow). Dashed arrows indicate several steps in the glycolytic process, one of which is labeled. GK=glucokinase, F6P=fructose 6-phosphate, F1,6BP=fructose 1,6-bisphosphate, GPDH=glyceraldehyde 3-phosphate dehydrogenase.

There is a long history of modeling of glycolytic oscillations, notably in yeast. Our model has a similar dynamical structure, based on fast positive feedback and slow negative feedback, to some of those models but differs in the identification of sources of feedback. In the models of Sel'kov (Sel'kov 1968) and Goldbeter and Lefever (Goldbeter and Lefever 1972) ATP was considered the substrate, whose depletion provided the negative feedback as F6P does in our model, and ADP was considered the product, which provided the positive feedback, as F1,6BP does in our model.

Such models can be combined with electrical activity to produce many of the patterns described here (Wierschem and Bertram 2004), but the biochemical interpretation is different. In our view, ATP acts rather as a negative modulator, which tends to shut down glycolysis when energy stores are replete, and ADP is a positive modulator, which activates glycolysis when ATP production falls behind metabolic demand. More fundamentally, we argue that β-cells, as metabolic sensors, differ from primary energy-consuming tissues such as muscle in that they need to activate metabolism whenever glucose is present even if the cell has all the ATP it needs. In this view, ATP and ADP are not suitable to serve as essential dynamic variables but do play significant roles as signaling molecules in regulating activity.

THE DUAL OSCILLATOR MODEL FOR ISLET OSCILLATIONS

Recent islet data provide the means to disentangle the influences of Ca^{2+} feedback and glycolysis on islet oscillations. Figure 4A shows compound Ca^{2+} oscillations, recorded from islets in 15 mM glucose. There is a slow component (period ~5 min) with much faster oscillations superimposed on the slower plateaus. These compound oscillations have been frequently observed by a number of research groups (Valdeolmillos et al. 1989; Bergsten et al. 1994; Zhang et al. 2003; Beauvois et al. 2006), and reflect compound bursting oscillations, where fast bursts are clustered together into slower episodes (Henquin et al. 1982; Cook 1983). Figure 4B shows measurements of islet oxygen levels in 10 mM glucose (Jung et al. 1999). Again there are large-amplitude slow oscillations (period of 3-4 min) with superimposed fast oscillations or "teeth". Similar compound oscillations have been observed in intra-islet glucose and in insulin secretion (Jung et al. 2000; Dahlgren et al. 2005), as assayed by Zn^{2+} efflux from β-cells. These data showing compound oscillations in a diversity of cellular variables suggest that compound oscillations are fundamental to islet function.

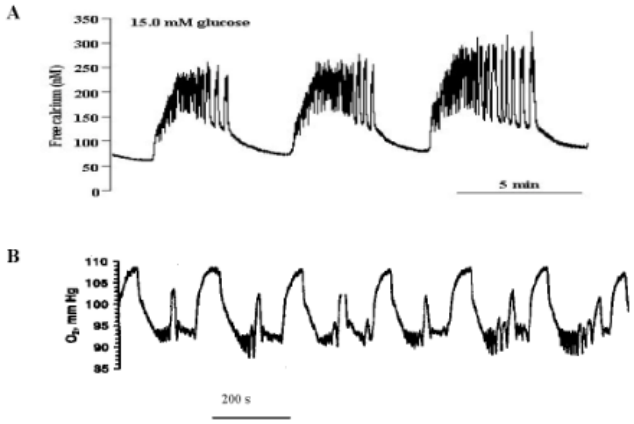

Figure 4: (A) Compound islet Ca2+ oscillations measured using fura-2/AM. The oscillations consist of slow episodes of fast oscillations. Reprinted from Bertram et al. (2004). (B) Slow oxygen oscillations with superimposed fast "teeth". Reprinted from Jung et al. (1999).

We have hypothesized that the slow component of the compound oscillations reflects oscillations in glycolysis, while the fast component is due to Ca^{2+} feedback onto ion channels and metabolism. This hypothesis has been implemented as a mathematical model, which we call the “Dual Oscillator Model” (Bertram et al. 2004; Bertram et al. 2007). The strongest evidence for this model is its ability to account for the wide range of time courses of Ca^{2+} and metabolic variables observed in glucose-stimulated islets both *in vitro* and *in vivo*. The fast oscillations introduced above do not have an underlying slow component. An example is shown in Fig. 5A. The Dual Oscillator Model reproduces this pattern (Fig. 5B) when glycolysis is non-oscillatory (Fig. 5C). The fast oscillations are mainly due to the effects of Ca^{2+} feedback onto K^+ channels as discussed earlier. Compound oscillations (Fig. 5D) are also produced by the model (Fig. 5E) and occur when both glycolysis and electrical activity are oscillatory (Fig. 5F) and become phase-locked. The glycolytic oscillations provide the slow envelope and electrically-driven Ca^{2+} oscillations produce the fast pulses of Ca^{2+} that ride on the slow wave. A variant of compound bursting, not shown in Fig. 5, consists of fast bursting with a slowly-changing plateau fraction. This pattern, which we call “accordion bursting”, has been observed in membrane potential, Ca^{2+}, and oxygen (Henquin et al. 1982; Cook 1983; Bergsten et al. 1994; Kulkarni et al. 2004).

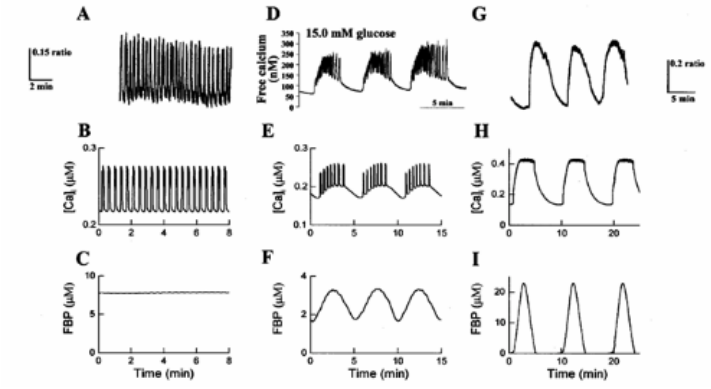

Figure 5: Three types of oscillations typically observed in islets. Top row of panels is from islet measurements of Ca^{2+} using fura-2/AM. Middle row shows simulations of Ca^{2+} oscillations using the Dual Oscillator Model. Bottom row shows simulations of the glycolytic intermediate fructose 1,6-bisphosphate (FBP), indicating that glycolysis is either stationary (C) or oscillatory (F, I). Reprinted from Bertram et al. (2004), Nunemaker et al. (2005), and Bertram et al. (2007).

Compound oscillations in Ca^{2+} are accompanied by slow oscillations in O_2 with "teeth", as in Fig. 4B. The slow oscillations in the flux of metabolites from glycolysis to the mitochondria result in slow oscillations in O_2 consumption by the mitochondrial electron transport chain. The Ca^{2+} feedback onto mitochondrial respiration also affects O_2 consumption, resulting in the faster and smaller O_2 oscillations.

Pure slow oscillations (Fig. 5G) are also reproduced by the model (Fig. 5H) when glycolysis is oscillatory (Fig. 5I) and the cell is tonically active during the peak of glycolytic activity. Thus, a model that combines glycolytic oscillations with Ca^{2+}-dependent oscillations can produce the three types of oscillatory patterns typically observed in islets, as well as faster oscillations in the O_2 time course when in compound mode.

Accordion bursting, like compound bursting, is accompanied by O_2 oscillations with fast teeth, but now present at all phases of the oscillation in both the model (Bertram et al. 2004), and in experiments (Kulkarni et al. 2004). The model thus suggests that the compound and accordion modes are just quantitative variants of the same underlying mechanisms. The former can be converted into the latter by reducing the conductance of the K(ATP) current, limiting its ability to repolarize the islets. It also supports the notion that β-cells have two oscillators that interact but can also occur independently of each other.

GLUCOSE SENSING IN THE DUAL OSCILLATOR FRAMEWORK

The concept of two semi-independent oscillators can be captured in a diagrammatic scheme (Fig. 6) representing how the two sub-systems respond to changes in glucose. Depending on the glucose concentration, glycolysis can be low and steady, oscillatory, or high and steady. Similarly, the electrical activity can be off, oscillatory due to Ca^{2+} feedback, or in a continuous-spiking state. The two oscillators thus have glucose thresholds separating their different activity states. Increasing the glucose concentration can cause both the glycolytic and electrical subsystems to cross their thresholds, but not necessarily at the same glucose concentrations.

The canonical case is for the two oscillators to become activated in parallel. For example, in Case 1 of Fig. 6, when the islet is in 6 mM glucose both the glycolytic oscillator (GO) and electrical oscillator (EO) are in their low activity states. When glucose is raised to 11 mM both oscillators are activated, yielding slow Ca^{2+} oscillations. In this scenario, the electrical burst duty cycle or plateau fraction of the slow oscillation, a good indicator of the relative rate of insulin secretion, increases with glucose concentration, as seen in classical studies of fast bursting (Dean and Mathews 1970; Meissner and Schmelz 1974; Beigelman and Ribalet 1980). The increase in the glucose concentration in this regime has no effect on the amplitude of Ca^{2+} oscillations and little effect on the oscillation frequency (Nunemaker et al. 2006).

However, some islet responses have been observed to be transformed from fast to slow or compound oscillations when the glucose concentration was increased (Nunemaker et al. 2006). This dramatic increase in the oscillation period was accompanied by a large increase in the oscillation amplitude (Fig. 6, Case 2). We interpreted this as a switch from electrical to glycolytic oscillations, and termed this transformation “regime change”. The diagrammatic representation in Fig. 6 indicates that this occurs when the threshold for the GO is shifted to the left of that for the EO. This may occur if glucokinase is relatively active or K(ATP) conductance is relatively low.

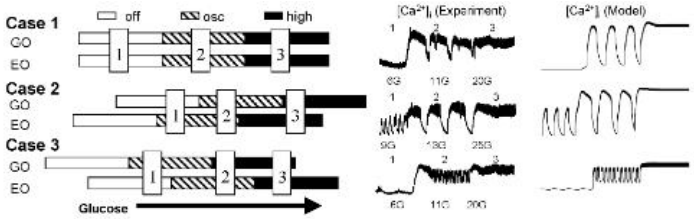

Figure 6: Schematic diagram illustrating the central hypothesis of the Dual Oscillator Model. In this hypothesis, there is an electrical subsystem that may be oscillatory (osc), or in a low (off) or high activity state. There is also a glycolytic subsystem that may be in a low or high stationary state or an oscillatory state. The glucose thresholds for the two subsystems need not be aligned, and different alignments can lead to different sequences of behaviors as the glucose concentration is increased. Reprinted from Nunemaker et al. (2006) and Bertram et al. (2007).

At 9 mM glucose the EO is on, but the GO is off, so only fast Ca^{2+} oscillations result, driven by fast bursting electrical activity. When glucose is increased to 13 mM, the lower threshold for glycolytic oscillations is crossed and the fast Ca^{2+} oscillations combine with glycolytic oscillations to produce much slower and larger-amplitude slow or compound oscillations.

A final example is Case 3. In this islet, subthreshold Ca^{2+} oscillations are produced in 6 mM glucose, which we believe are due to activation of the GO, while the EO is in a low activity (or silent) state. When glucose is increased to 11 mM the lower threshold for electrical oscillations is crossed, initiating a fast oscillatory Ca^{2+} pattern. However, the upper threshold for glycolytic oscillations is also crossed, so the glycolytic oscillations stop. As a result, a fast oscillatory Ca^{2+} pattern is produced, with only a transient underlying slow component.

In all three cases, when glucose is raised to 20 mM or higher the system moves past the upper thresholds for both the GO and the EO, so there are neither electrical bursting oscillations nor glycolytic oscillations, and the islet generates a continuous spiking pattern. The Dual Oscillator Model accounts for each of these regime-change behaviors, as shown in the right column of Fig. 6.

METABOLIC OSCILLATIONS CAN BE RESCUED BY CALCIUM

Given that metabolic oscillations can be driven by either Ca^{2+} feedback onto ATP production/utilization or by an independent mechanism such as glycolytic oscillations, experimental tests have been developed to determine which of these occurs in islets. One such test takes the strategy of

manipulating the islet so that Ca^{2+} oscillations do not occur. Figure 7 shows that when the islet is hyperpolarized with the K(ATP) channel agonist diazoxide (250 μM) the oscillations in Ca^{2+} concentration, as measured by Fura-2 fluorescence, and metabolism, as measured by NAD(P)H autofluorescence, are both terminated (Luciani et al. 2006; Bertram et al. 2007). A similar test was performed by Kennedy and colleagues, except that they used an oxygen-sensing electrode to monitor metabolism (Kennedy et al. 2002). They also found that membrane hyperpolarization terminated metabolic oscillations.

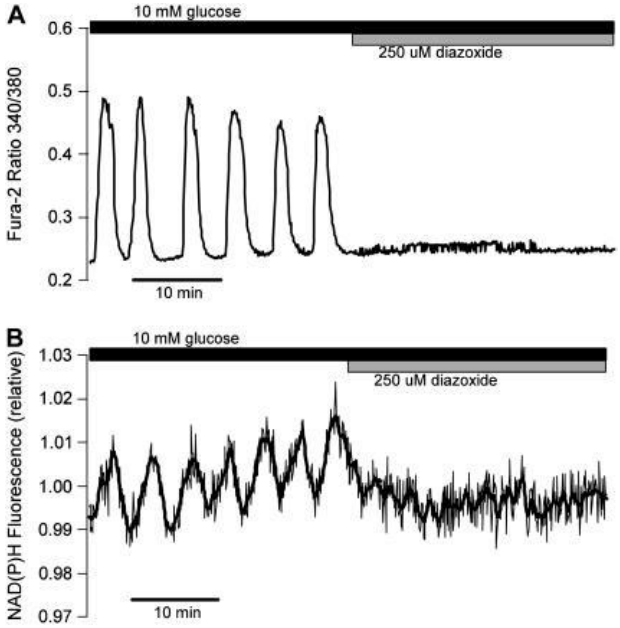

Figure 7: Measurements of (A) fura-2 fluorescence and (B) NAD(P)H autofluorescence demonstrate that islet hyperpolarization with diazoxide can terminate metabolic oscillations. Reprinted from Bertram et al. (2007).

It is tempting to conclude from these data that metabolic oscillations must be driven by Ca^{2+} oscillations, and in the absence of a mathematical model this seems like a logical conclusion. Surprisingly, though, model simulations we carried out using the Dual Oscillator Model showed that under some conditions islet hyperpolarization, as was done in Fig. 7, would in fact terminate metabolic oscillations *even if they were driven by glycolytic oscillations* (Bertram et al. 2007). This is because the decline in Ca^{2+} influx that accompanies hyperpolarization reduces ATP utilization by Ca^{2+} pumps. This results in an increase in the cytosolic ATP concentration, and ATP inhibits the enzyme, PFK1, responsible for glycolytic oscillations.

Thus, cessation of metabolic oscillations by blocking Ca^{2+} oscillations does not imply that Ca^{2+} oscillations are necessary for metabolic oscillations, but the converse is valid. If metabolic oscillations are observed when Ca^{2+} is clamped, it must mean that the metabolic oscillations are not merely a reflection of Ca^{2+} oscillations. Indeed, the Dual Oscillator Model predicts that, in many cases, it should be possible to have metabolic oscillations driven by glycolysis even though the Ca^{2+} concentration is clamped. This requires, however, that the level at which Ca^{2+} is clamped be sufficiently high so that the PFK1 enzyme is not inhibited by the elevated ATP that accompanies cessation of Ca^{2+} pumping. With this in mind, the model was used to design an experiment to truly test whether metabolic oscillations can exist in the absence of Ca^{2+} oscillations (Merrins et al. 2010). In the simulation shown in Fig. 8, a model islet exhibiting compound oscillations in stimulatory glucose was hyperpolarized by increasing the fraction of activated K(ATP) channels (simulating the application of diazoxide). This reduced the cytosolic Ca^{2+} concentration, which in turn increased cytosolic ATP concentration through the reduced activity of Ca^{2+} pumps. Metabolic oscillations, as reflected in the mitochondrial NADH concentration, were thus terminated. The model islet was subsequently depolarized by increasing the Nernst potential for K^+ (simulating the application of KCl). The depolarization activates L-type Ca^{2+} channels in the plasma membrane, raising the level of Ca^{2+} in the cytosol (Fig. 8A). This induced increased activity of the Ca^{2+} pumps, increasing ATP hydrolysis and lowering the ATP level (Fig. 8B). The resultant disinhibition of PFK1 allowed glycolytic oscillations to re-emerge (Fig. 8C). Importantly, these oscillations persisted in the absence of Ca^{2+} oscillations. The combination of diazoxide and KCl effectively clamps the Ca^{2+}: the diazoxide cuts the link between metabolism (i.e., ATP) and the membrane potential by opening the K(ATP) channels, and the KCl inhibits action potential production and the accompanying Ca^{2+} oscillations that would result from spiking-induced Ca^{2+} influx.

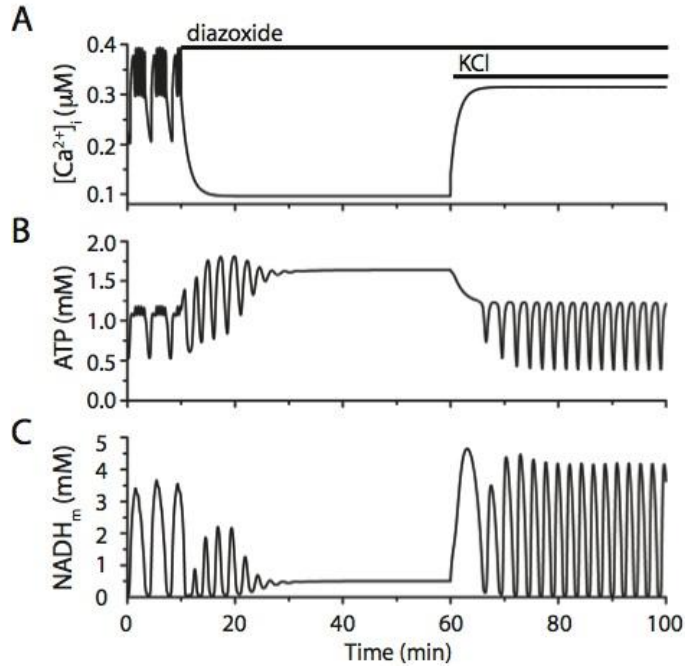

Figure 8: Mathematical simulations demonstrating how increasing the level of intracellular free Ca^{2+} can rescue metabolic oscillations. Simulated application of diazoxide hyperpolarizes the islet, lowering the intracellular Ca^{2+} concentration (A). This greatly reduces the pumping needed to remove Ca^{2+} from the β-cell, thereby decreasing ATP utilization and increasing the ATP concentration in the cytosol (B). The result of this is disinhibition of PFK1, which allows glycolytic oscillations to emerge (C). Reprinted from Merrins et al. (2010).

The prediction that glycolytic oscillations can be rescued by elevating the intracellular Ca^{2+} level was tested experimentally (Merrins et al. 2010). Metabolic oscillations as measured through NAD(P)H autofluorescence were terminated by hyperpolarization (application of 200 μM diazoxide) in about two-thirds of the islets but persisted in the remainder. Subsequent application of KCl increased the level of intracellular Ca^{2+} while prohibiting Ca^{2+} oscillations (Fig. 9A, with different concentrations of KCl). In about one-half of the islets in which Ca^{2+} oscillations had been terminated, metabolic oscillations were restored when the intracellular Ca^{2+} concentration was raised (Fig. 9B). Thus, it was demonstrated that metabolic oscillations can occur in islets in the absence of Ca^{2+} oscillations.

The experiments, however, raised two new questions: Why did slow metabolic oscillations persist in some islets but not in others, and why did raising Ca^{2+} restore oscillations in some islets but not in others? In the latter case, it is possible that oscillations would have been restored if a different KCl concentration had been used, but further mathematical analysis suggests another possibility. The model was found to support yet another type of slow metabolic oscillation that is neither secondary to Ca^{2+} oscillations nor fully independent of Ca^{2+} oscillations. In this regime, neither of the electrical and metabolic oscillators is able to oscillate on its own, but only a reciprocal interaction between the two can result in oscillations (M. Watts, B. Fendler, R. Bertram, and A. Sherman, submitted for publication). It remains to be tested experimentally whether such a regime exists in real islets.

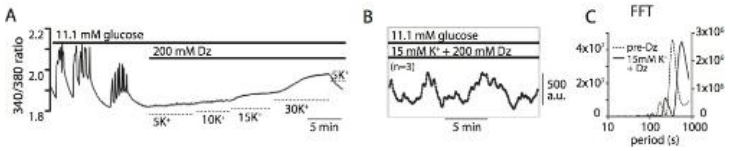

Figure 9: Experimental test of the model prediction made in Fig. 8. (A) Compound Ca^{2+} oscillations are terminated by diazoxide (200 μM). The Ca^{2+} concentration is elevated when KCl is added to the bath, but Ca^{2+} oscillations are not initiated. (B) Oscillations in NAD(P)H autofluorescence can be initiated in the presence of diazoxide when KCl has been added to depolarize the cell and increase the level of Ca^{2+}. (C) Fourier power spectrum of the NAD(P)H fluorescence prior to the addition of diazoxide (dotted) and after diazoxide plus KCl. Large peaks occur at the period of slow metabolic oscillations. Reprinted from Merrins et al. (2010).

MANIPULATING GLYCOLYSIS ALTERS OSCILLATIONS IN A PREDICTABLE WAY

One way to determine if glycolysis is the source of metabolic oscillations is to manipulate it in such a way that glycolytic oscillations, if they exist, are altered in a predictable way. This was done in an indirect way in the experiments described above, through changes in the intracellular Ca^{2+} level, which acts on an inhibitor (ATP) of the key rhythmogenic enzyme PFK1. A second approach is to interfere with the feedback loop that is responsible for the production of putative glycolytic oscillations. This feedback comes from F1,6BP allosterically activating the enzyme that produced it, PFK1. The loop would be broken and the oscillation eliminated if this feedback were removed. In a similar vein, if the feedback were weakened through the actions of another molecule that competes with F1,6BP for the same binding site on PFK1, then the properties of the oscillation (e.g., oscillation frequency and amplitude) would change. A mathematical model of the reaction would allow one to predict the effect of the competitive activator, and if islet Ca^{2+} oscillations were effected in the same way, then this would provide evidence that the Ca^{2+} oscillations are driven by glycolytic oscillations.

This approach was taken in a recent study, which made use of the bifunctional enzyme phosphofructokinase-2/fructobisphosphatase-2, which we call PFK2/FBPase2 or BIF2 (Fig. 10). This enzyme has a kinase on the N-terminal (PFK2) and a phosphatase on the C-terminal (FBPase2). The kinase converts F6P to fructose 2,6-bisphosphate (F2,6BP) and is the sole source of F2,6BP in the cell. The phosphatase end of the enzyme does the opposite. Importantly, F2,6BP is an allosteric activator of PFK1, and is a more potent activator of PFK1 than is F1,6BP (Malisse et al. 1982; Foe et al. 1983; Sener et al. 1984). It is therefore an ideal molecule for weakening the positive feedback of F1,6,BP onto PFK1 and thus changing the properties of putative glycolytic oscillations.

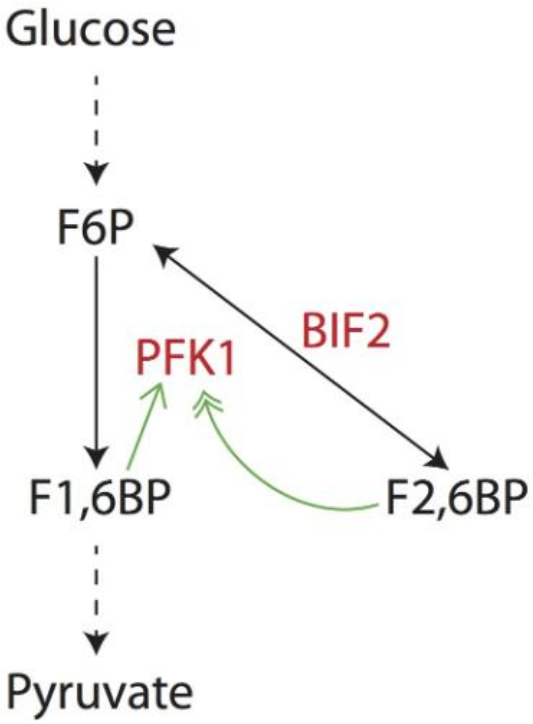

Figure 10: Illustration showing that fructose 2,6-bisphosphate (F2,6BP) activates PFK1 (double green arrow) more strongly than does F1,6BP. The F2,6BP is generated by the enzyme PFK2/FBPase2 (BIF2).

In (Merrins et al. 2012), mutants of the islet isoform of PFK2/FBPase2 (Sakurai et al. 1996; Arden et al. 2008) were expressed in mouse islets using an adenoviral delivery system. Four mutants were examined, but we focus here on only two. One mutant (DD-PFK2) contained only the PFK2 domain, tagged at the N-terminal with a Degradation Domain (DD), which permits transcription and translation, but prevents accumulation of functional protein in the cytosol due to rapid proteasomal degradation. This degradation can be inhibited by a small cell-permeant molecule called Shield1 (Banaszynski et al. 2006). The other mutant (DD-FBPase2) contained only the FBPase2 domain, also tagged at the N-terminal with DD. Adenoviral delivery of the DD-PFK2 mutant in the presence of Shield1 would then result in overproduction of PFK2, and an increase in the concentration of F2,6BP. In the absence of Shield1, functional PFK2 protein would not accumulate, so delivery of DD-PFK2 without Shield1 serves as a control. A similar strategy was used for DD-FBPase2, which when delivered in the presence of Shield1 increases FBPase2 concentration in the cell, resulting in a reduction in the F2,6BP concentration. One advantage of these truncation mutants is that neither activates glucokinase (Merrins et al. 2012), as the full BIF2 molecule is known to do (Langer et al. 2010).

A mathematical model of the allosteric PFK1 reaction was used to predict the effects on glycolytic oscillations of increasing or decreasing the concentration of the competitive allosteric activator F2,6BP. The model predicted that increasing F2,6BP should make oscillations faster and smaller in amplitude, and if F2,6BP was increased too much the oscillations would be terminated. Decreasing the F2,6BP concentration should have the opposite effect, making glycolytic oscillations slower and larger in amplitude. A similar prediction was made using the full Dual Oscillator Model, where now the final readout was the cytosolic Ca^{2+} concentration.

Model predictions were tested using the DD-PFK2 and DD-FBPase2 mutants. When DD-PFK2 was

expressed in mouse islets, the period of the Ca^{2+} oscillations we observed was significantly smaller in the presence of Shield1 than in its absence, and the amplitude of the oscillations was significantly reduced (Fig. 11). That is, when functional PFK2 protein was produced (Shield1 present), which should increase the production of F2,6BP, Ca^{2+} oscillations were faster and smaller compared with islets in which Shield1 was absent. When DD-FBPase2 was expressed, the period of Ca^{2+} oscillations was significantly larger in the presence of Shield1 than in its absence, and the amplitude of the oscillations was significantly increased (Fig. 11). These results match the predictions of the Dual Oscillator Model. Thus, for the first time, it was shown that manipulations that should make Ca^{2+} oscillations faster/slower *if the oscillations are the result of glycolytic oscillations* did indeed make the oscillations faster/slower.

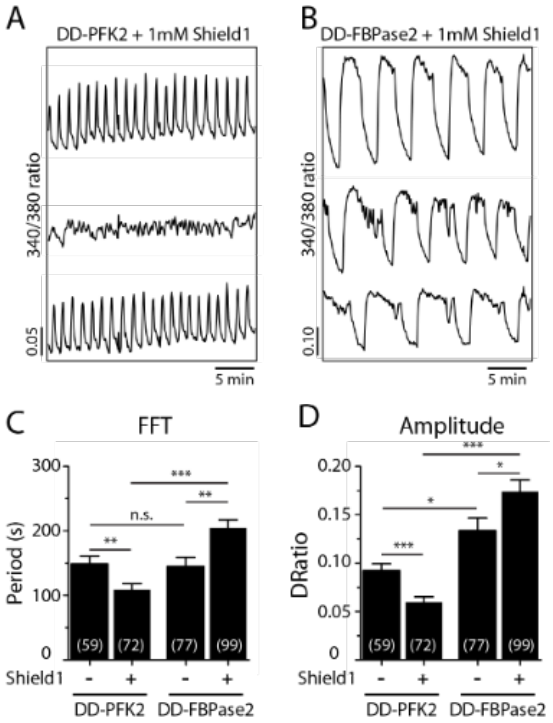

Figure 11: (A) Expression of DD-PFK2 with Shield1 results in the production of F2,6BP which makes Ca^{2+} oscillations smaller and faster (left) compared with islets expressing DD-FBPase2 with Shield1 (right). (B) Fast fourier transform of Ca^{2+} oscillation periods in islets expressing DD-PFK2 or DD-FBPase2 (with or without Shield1). (C) Amplitude of Ca^{2+} oscillations. Reprinted from Merrins et al. (2012).

SUMMARY

The Dual Oscillator Model, developed over a period of time from simpler Ca^{2+}-dependent models of fast bursting to account for slower and more complex patterns of islet oscillatory behavior, has done so successfully, while also clarifying the complex relationship between intracellular Ca^{2+}, β-cell ion channels, and intrinsic oscillations in islet glucose metabolism. The model also clarifies the results of experiments that would be hard to interpret or open to misinterpretation in the absence of a model. In addition to the studies described above, recent work used the DOM to interpret islet electrophysiology experiments, and to understand the role played by gap-junctional coupling between β-cells (Ren et al., American Journal of Physiology, in press). It remains to be determined how functional properties of human islets differ from mouse islets, and whether there are similar mechanisms driving oscillations in islets from the two species. It is also yet to be seen what role the model will have in understanding islet dysfunction in models of diabetes.

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