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Metabolic and electrical oscillations: partners in controlling pulsatile insulin secretion

Richard Bertram,[1] Arthur Sherman,[2] and Leslie S. Satin[3]

[1]*Department of Mathematics and Programs in Neuroscience and Molecular Biophysics, Florida State University, Tallahassee, Florida;* [2]*Laboratory of Biological Modeling, National Institutes of Health, Bethesda, Maryland; and* [3]*Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, Virginia*

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Bertram R, Sherman A, Satin LS. Metabolic and electrical oscillations: partners in controlling pulsatile insulin secretion. *Am J Physiol Endocrinol Metab* 293: E890–E900, 2007. First published July 31, 2007; doi:10.1152/ajpendo.00359.2007.—Impairment of insulin secretion from the β-cells of the pancreatic islets of Langerhans is central to the development of type 2 diabetes mellitus and has therefore been the subject of much investigation. Great advances have been made in this area, but the mechanisms underlying the pulsatility of insulin secretion remain controversial. The period of these pulses is 4–6 min and reflects oscillations in islet membrane potential and intracellular free Ca^{2+}. Pulsatile blood insulin levels appear to play an important physiological role in insulin action and are lost in patients with type 2 diabetes and their near relatives. We present evidence for a recently developed β-cell model, the "dual oscillator model," in which oscillations in activity are due to both electrical and metabolic mechanisms. This model is capable of explaining much of the available data on islet activity and offers possible resolutions of a number of longstanding issues. The model, however, still lacks direct confirmation and raises new issues. In this article, we highlight both the successes of the model and the challenges that it poses for the field.

pancreatic β-cells; mathematical model; diabetes

IN THE CONSENSUS MODEL for glucose-stimulated insulin secretion (GSIS) from pancreatic β-cells (Fig. 1*A*), glucose enters the cell through GLUT2 glucose transporters and is metabolized to increase the ATP/ADP ratio, which in turn closes ATP-sensitive K^+ (K_{ATP}) channels. This depolarizes the membrane, which opens L-type Ca^{2+} channels and promotes Ca^{2+} influx. The resulting increase in the cytosolic Ca^{2+} concentration evokes exocytosis of insulin granules. The development of this model represented a major achievement that overturned early, more orthodox suggestions that glucose acted through a receptor, like many other signals for hormone secretion. This model has not been reversed by subsequent findings, although it has been extended by consideration of noncalcium amplifying factor(s) of yet undetermined identity (35). Nonetheless, the consensus picture is essentially a static view, as it fails to account for important dynamic features of GSIS, in particular, its pulsatile nature. Oscillations in insulin secretion have been measured in vivo (59, 65), in the perifused pancreas (76), and in isolated islets (4, 27, 46, 70). The oscillations typically have two components; the faster component has a period of tens of seconds (6, 27, 59), and the slower component has a period of 4–6 min (59, 65, 66). This latter component appears to play an important physiological role in insulin action (54) and is lost in patients with type 2 diabetes and their near relatives (53, 60, 64, 87). In this article we discuss a nascent model that includes the consensus mechanism but also offers an integrated account of both the "fast" (tens of seconds) and "slow" (4–6 min) insulin oscillations in secretion and their interactions. We propose that the fast oscillations result from electrical mechanisms, predominantly feedback of cytosolic free calcium on plasma membrane ion channels, and that the slow oscillations result from metabolic, possibly glycolytic, oscillations. This model has been successful in providing parsimonious explanations for a large body of data accumulated over decades, including many phenomena not previously accounted for by any other hypotheses. However, the model also faces a number of critical challenges that must be met before it can be accepted. Although it may still require changes in detail or may even ultimately be rejected, we feel that this new model has the potential to generate new hypotheses and to redirect the thinking of the field as it goes forward.

Since pulsatile insulin secretion has been observed in isolated islets, it is generally accepted that its underlying rhythm is generated endogenously within the β-cells themselves, although it may be modulated by external factors in vivo. Oscillations in glycolysis have been proposed as a key mechanism for pulsatile insulin secretion from β-cells (78). The M-type isoform of the glycolytic enzyme phosphofructokinase (PFK) is known to exhibit oscillatory activity in muscle extracts, as measured by oscillations in the levels of the PFK substrate fructose 6-phosphate (F6P) and product fructose 1,6-bisphosphate (FBP) (81, 82). The period of these oscillations, 5–10 min, is similar to the period of slow insulin oscillations (78), and two human subjects with mutations in PFK-M were found to have impaired insulin oscillations (69). The mechanism for oscillatory activity of this isoform, which is the dominant isoform in islets (89), is the positive feedback of its product FBP on the kinase activity and subsequent depletion of substrate (74, 80, 88). Although direct evidence for glycolytic oscillations in β-cells is presently lacking, there is substantial indirect evidence in support of this hypothesis. This consists primarily of measurements of oscillations in several key, and related, metabolic variables, such as oxygen consumption (9, 40, 46, 61), ATP or the ATP/ADP ratio (1, 39, 57), the mitochondrial inner membrane potential (42), lactate release (16), and NAD(P)H levels (47). The period of the latter oscillations is typically in the 4- to 6-min range, similar to that of slow Ca^{2+} oscillations. The lactate oscillations were slower (16–20 min), but this may reflect limited resolution of the measurements used, as the insulin oscillations were similarly

Address for reprint requests and other correspondence: L. S. Satin, Dept. of Pharmacology and Toxicology, Virginia Commonwealth University, P. O. Box 980524, Richmond, VA 23298 (e-mail: lsatin@vcu.edu).

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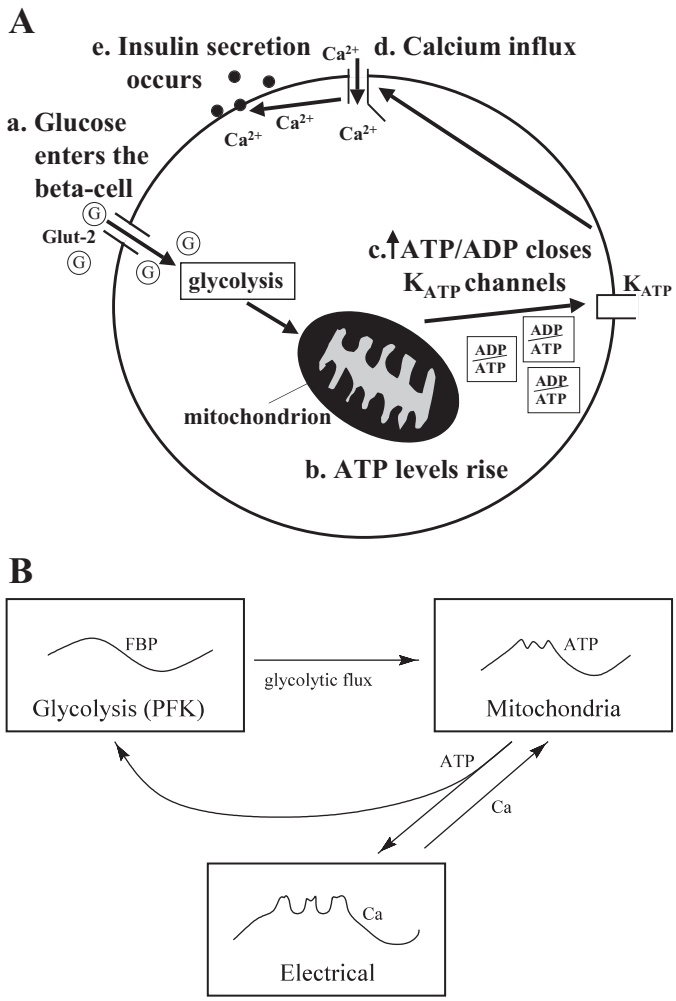

Fig. 1. *A*: consensus model of glucose-stimulated insulin secretion (GSIS). *B*: the combined glycolytic-Ca^{2+} or "dual oscillator model." K_{ATP}, ATP-sensitive K^+; FBP, fructose 1,6-biphosphate; PFK, phosphofructokinase.

slow. Importantly, some laboratories have shown that fuels that are metabolized downstream of PFK, such as glyceraldehyde, α-ketoisocaproic acid (KIC), and methylpyruvate, evoke insulin secretion but do not generate Ca^{2+} oscillations with the 4- to 6-min period that characterizes slow insulin oscillations (some laboratories have found otherwise, as we discuss later). Only when combined with substimulatory or stimulatory concentrations of glucose do these fuels evoke slow Ca^{2+} oscillations (21, 45). Using an oxygen electrode, it was shown (21) that slow oxygen oscillations occur in 10 mM glucose or 5 mM glucose plus 5 mM glyceraldehyde but not in 5 mM glyceraldehyde alone. Similarly, KIC, pyruvate, and methylpyruvate were all unable to induce slow oxygen oscillations (21). These data suggest that the slow rhythm underlying slow oscillations in islet activity is early in the glycolytic pathway, most likely at the level of PFK.

A parallel line of inquiry has focused on Ca^{2+} feedback as the mechanism for insulin oscillations. This is natural because the Ca^{2+} concentration oscillates simultaneously with bursting electrical activity (4, 90), since Ca^{2+} activates at least one type of K^+ ion channel (29, 30) and because Ca^{2+} is known to evoke insulin granule exocytosis (34). In addition, Ca^{2+} influences metabolism in at least three ways. First, the flux of Ca^{2+} across the mitochondrial inner membrane through Ca^{2+} uniporters depolarizes the membrane, reducing the driving force for oxidative phosphorylation (49, 50). Second, mitochondrial Ca^{2+} activates several dehydrogenases, stimulating metabolism (18, 55). Finally, Ca^{2+} is pumped from the cytosol into the endoplasmic reticulum or out of the cell by Ca^{2+} ATPase pumps (23). The ATP utilized by this action must be replenished by fuel metabolism. Thus, there is debate as to whether oscillations in metabolic variables are due to oscillations in glycolysis or due to the feedback effects of Ca^{2+} and more generally as to whether oscillations in Ca^{2+} are primarily driven by oscillations in metabolism or vice versa. It has also been suggested, on the basis of data showing citrate oscillations in isolated mitochondria (48), that oscillations in metabolic variables are independent of glycolysis.

Glucose metabolism influences insulin secretion partly through the effects of ADP and ATP on K_{ATP} channels (2). Thus, if metabolism is oscillatory, by whatever means, it will convey an oscillatory component to insulin secretion through the action of K_{ATP} channels on β-cell electrical activity. Calcium also has a direct effect on the cell's electrical activity through its action on Ca^{2+}-dependent K^+ [K(Ca)] channels (29, 30). Therefore, oscillations in insulin secretion could reflect oscillations in both metabolism and the cytosolic Ca^{2+} concentration.

How can we reconcile evidence for the two likely candidate mechanisms for insulin oscillations, glycolytic oscillations and Ca^{2+} feedback? Recent islet data provide a clue. Figure 2*A* shows islet Ca^{2+} measurements obtained in stimulatory (15 mM) glucose. Slow oscillations (period ~5 min) are evident with much faster oscillations superimposed on the slower plateaus. Such "compound" Ca^{2+} oscillations have been observed by many laboratories (4, 7, 85, 90). Figure 2*B* shows measurements of islet oxygen levels in 10 mM glucose. Again we see large-amplitude slow oscillations (period of 3–4 min) with superimposed low-amplitude fast oscillations, which ap-

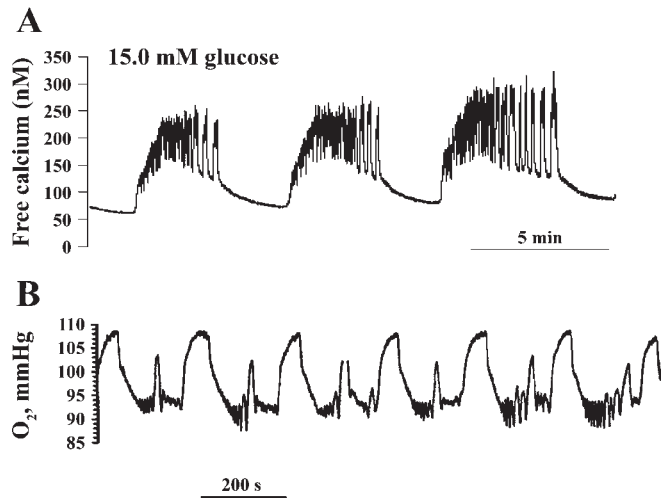

Fig. 2. *A*: compound Ca^{2+} oscillations recorded using fura 2 in a mouse islet in 15 mM glucose. The fast oscillations are superimposed on the plateaus of the slow oscillations. Reprinted with permission from Bertram et. al. (11). *B*: slow oxygen oscillations with superimposed fast "teeth" recorded with an oxygen electrode in a mouse islet in 10 mM glucose. Reprinted with permission from Jung et. al. (37).

pear as "teeth" in the records. Similar compound oscillations have been observed not only in oxygen but also in intraislet glucose and in insulin secretion, as assayed by Zn^{2+} efflux from β-cells, suggesting that compound oscillations are a fundamental property of the islet as a whole (21, 37, 38).

We believe that the slow component of the compound oscillations in Ca^{2+} and O_2 reflect oscillations in glycolysis, whereas the fast component is due to Ca^{2+} feedback onto ion channels and metabolism. This hypothesis has been implemented as a mathematical model, the glycolytic-Ca^{2+} or "dual oscillator model" (11, 12). This model accounts not only for compound oscillations but also for additional islet behaviors that cannot readily be accounted for by either purely glycolytic or purely Ca^{2+}-driven models. In the remainder of this article we demonstrate the explanatory power that is achieved by marrying the glycolytic and electrical mechanisms and point out several challenges that remain for the model.

THE DUAL OSCILLATOR MODEL

The dual oscillator model for β-cell activity consists of three compartments (Fig. 1*B*). The first compartment contains the essential ingredients for glycolytic oscillations: the M-type isoform of the enzyme PFK, its substrate F6P, and its product FBP. Positive feedback of FBP onto PFK increases kinase activity, producing more FBP at the expense of F6P and further increasing PFK activity. This continues until the substrate is largely depleted, causing a crash in PFK activity. Cessation of the kinase activity allows the F6P level to slowly recover using input from the upstream enzyme glucokinase. The rise in F6P reactivates PFK and restarts the cycle. The result is oscillatory PFK activity with resulting oscillatory F6P and FBP levels (80). The primary output of glycolysis, pyruvate, would then be oscillatory due to the oscillatory FBP levels.

In this model, the negative feedback to PFK required for oscillations is the partial depletion of substrate rather than inhibition of PFK by ATP acting at an allosteric site. Support for this mechanism is provided by Tornheim (79), where it is shown that glycolytic oscillations in muscle extracts can occur even when the ATP concentration is nonoscillatory. The inhibitory effect of ATP is included in the model, however, and plays an important modulatory role, as described below.

An important feature of this rhythmic mechanism is that it depends critically on the level of input from glucokinase and thus on the external glucose concentration. If the glucokinase flux rate, J_{GK}, is too low the PFK activity level will not reach the threshold for oscillatory activity, resulting in low and steady glycolysis. If J_{GK} is too high, then the PFK substrate F6P will be too high, such that PFK activity will not fall sufficiently, resulting in high and steady glycolysis in place of the oscillations. Hence, glycolytic oscillations occur only at intermediate levels of J_{GK}. This point has important implications, as we will discuss later.

The second compartment of the model includes key elements of mitochondrial metabolism. The inputs are glycolytic flux and Ca^{2+} from the cytosol, and the output is ATP. Slow oscillations in the glycolytic flux result in oscillations in aerobic ATP production. The ATP produced in the mitochondria is transported into the cytosol through adenine nucleotide transporters, where it affects the plasma membrane potential through its actions on K_{ATP} channels.

Although we have proposed that PFK by itself can drive metabolic oscillations, it also integrates other signals. For example, cytosolic ATP inhibits PFK activity. Thus, increased mitochondrial ATP production tends to reduce glycolytic activity, as in other cells. If the flux through glucokinase is sufficiently high, however, the model suggests that glycolysis can remain active and undergo oscillations in the face of elevated cytosolic ATP. The outcome thus depends on the balance of competing influences on PFK. The typical case in the model is for glycolysis to drive oscillations in mitochondrial activity through the supply of substrate and for the mitochondria to amplify the input from glycolysis rather than shut it down. This control system is appropriate for the β-cell, which is a fuel sensor and uses the metabolic product ATP as a signaling molecule, in contrast to other cells, such as muscle, that consume glucose only to the extent needed to meet their metabolic demands.

The third model compartment accounts for the electrical activity of the β-cell. In the dual oscillator model, bursts in electrical activity are driven by Ca^{2+} feedback onto K(Ca) ion channels and ATP/ADP feedback onto K_{ATP} channels. The endoplasmic reticulum also plays a role as a Ca^{2+} filter that adds a slow component to the cytosolic Ca^{2+} dynamics (14). The result is what we refer to as "phantom bursting." With phantom bursting one can achieve bursting oscillation periods ranging from a few seconds to several minutes (10, 13), and this is what drives the fast oscillations in the dual oscillator model when glycolysis is nonoscillatory. However, with a phantom bursting model alone, compound bursting is typically not produced. Other mechanisms for bursting involving different ionic currents and pumps, and even insulin feedback (15, 24, 26, 72), have been proposed, but our main focus here is not on the pros and cons of which specific ion channel mechanism is in play, as several models for fast ionic oscillations could fill this role. The bursts result in oscillations in the cytosolic Ca^{2+} concentration, which affect mitochondrial ATP production and consumption, as discussed earlier. The dual oscillator model for β-cell activity is described in detail by Bertram and colleagues (11, 12).

The electrical compartment can also affect the glycolytic oscillator. Rises in cytosolic Ca^{2+} due to Ca^{2+} entry reduce ATP because Ca^{2+} must then be pumped out of the cell or into internal stores. The reduction in ATP then disinhibits PFK. Conversely, we have proposed that islet hyperpolarization with diazoxide can terminate metabolic oscillations (12); the reduction of Ca^{2+}-dependent ATP consumption may raise cytosolic ATP sufficiently to shut down PFK. Thus, the observation that blocking Ca^{2+} entry can stop metabolic oscillations does not necessarily imply that metabolism is primarily driven by Ca^{2+} but is compatible with the hypothesis that a primary metabolic oscillator is modulated by Ca^{2+} levels. We have also proposed that this indirect positive feedback of Ca^{2+} on PFK via ATP consumption may contribute to the synchronization of glycolytic oscillations throughout the islet (63). The reciprocal links between the metabolic and glycolytic oscillators are subtle and quantitative, a situation in which having a model is a great aid to understanding the underlying mechanisms.

TYPICAL Ca^{2+} TIME COURSES

The primary success of the dual oscillator model is that it can account for the full range of time courses of Ca^{2+} and metabolic variables observed in glucose-stimulated islets

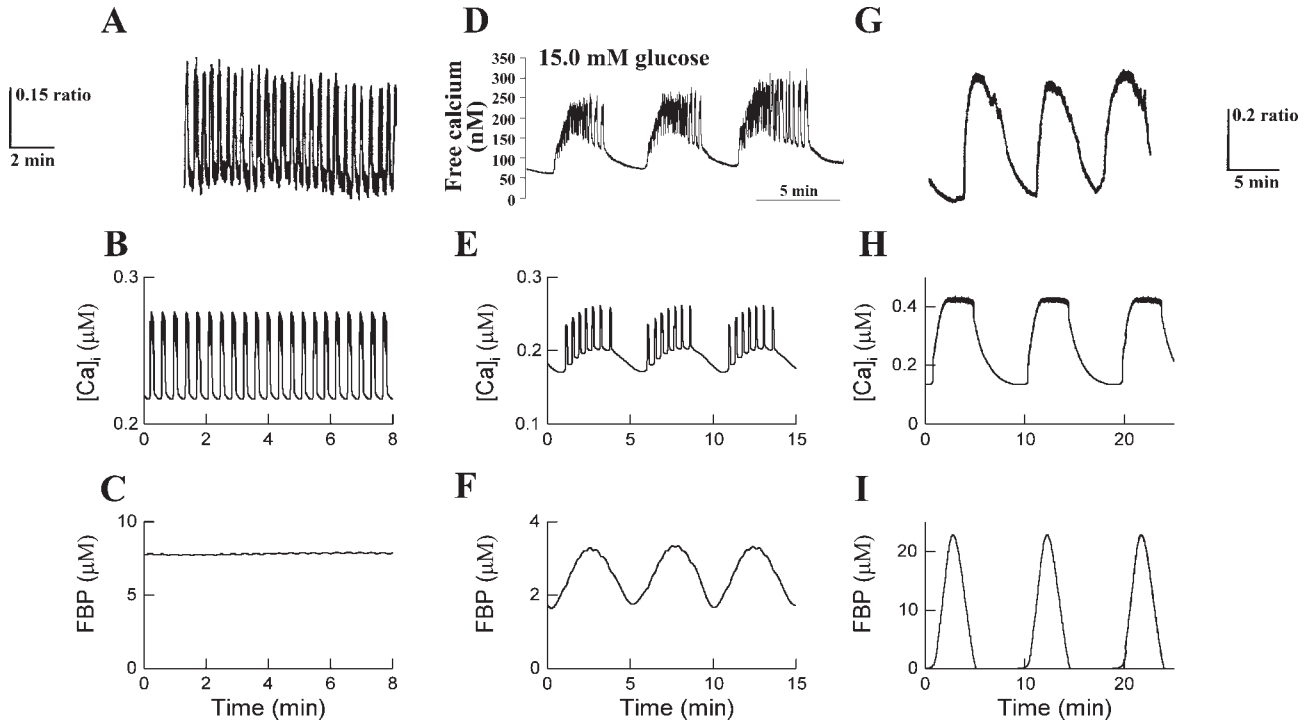

Fig. 3. *A*: fast Ca^{2+} oscillations in 11.1 mM glucose. The vertical scale bar represents the fura 2 ratio. Reprinted with permission from Nunemaker et al. (59). *B*: fast Ca^{2+} oscillations due to fast bursting generated by the dual oscillator model. *C*: glycolysis is nonoscillatory during fast model oscillations. *D*: compound Ca^{2+} oscillations. Reprinted with permission from Bertram et al. (11). *E*: compound Ca^{2+} oscillations generated by the model. *F*: during compound oscillations, glycolysis is oscillatory. *G*: pure slow Ca^{2+} oscillations. Reprinted with permission from Nunemaker et al. (59). *H*: slow Ca^{2+} oscillations generated by the model. *I*: glycolysis is oscillatory during slow oscillations. The various model-generated oscillations in this and other figures were obtained by varying the glucokinase reaction rate and the conductances of the Ca^{2+}-dependent K^+ [K(Ca)] and K_{ATP} currents.

in vitro and in vivo. Fast oscillations are observed frequently in islets, with no underlying slow component. An example is shown in Fig. 3*A*. The dual oscillator model produces this type of pattern (Fig. 3*B*) when glycolysis is nonoscillatory (Fig. 3*C*). The fast oscillations are due to the effects of Ca^{2+} feedback on K^+ channels. Another type of oscillation observed frequently is the compound Ca^{2+} oscillation (Fig. 3*D*). This is produced by the model (Fig. 3*E*) when glycolysis is oscillatory (Fig. 3*F*) and fast electrical oscillations occur simultaneously. Here, the slow glycolytic oscillations combine with the faster, electrically driven Ca^{2+} oscillations to produce the two oscillatory modes evident in the Ca^{2+} time course. This combination of oscillatory mechanisms also produces slow oxygen oscillations with "teeth" (Fig. 4). The slow oscillations in glycolytic flux from glycolysis to the mitochondria, which provide the substrate for mitochondrial respiration, result in oscillations in oxygen consumption by the electron transport chain. The Ca^{2+} feedback onto respiration also affects oxygen

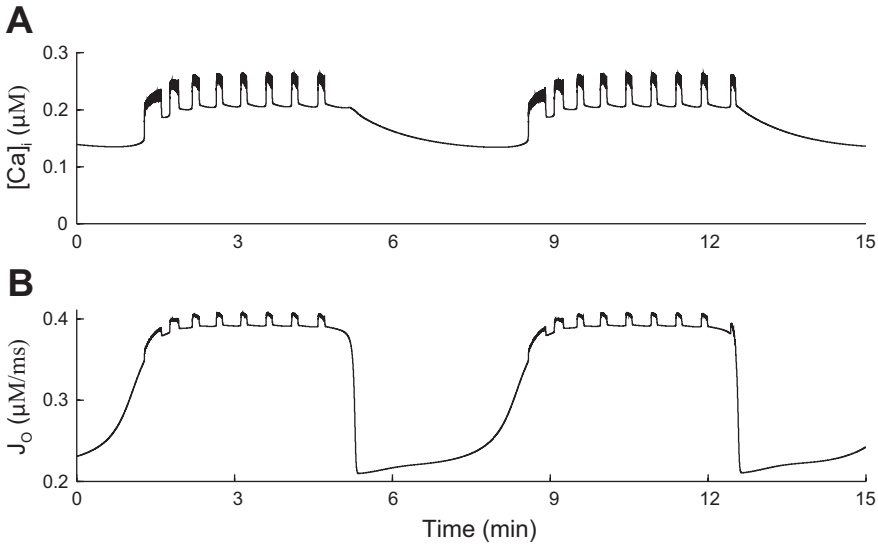

Fig. 4. In the dual oscillator model, compound Ca^{2+} oscillations (*A*) are associated with slow oscillations in oxygen consumption (*B*; J_o) with superimposed teeth.

consumption, resulting in the oxygen teeth. (Note that the model time course is inverted relative to the experimental tracing in Fig. 2 because oxygen flux rather than concentration is plotted.) A third pattern often observed in islets is a purely slow oscillation (Fig. 3*G*). The model produces this behavior (Fig. 3*H*) when glycolysis is oscillatory (Fig. 3*I*) and when the cell is tonically active during the peak of glycolytic activity. Thus, a model that combines glycolytic oscillations with electrical activity-dependent Ca^{2+} oscillations can produce the three types of oscillatory patterns typically observed in islets. In addition, the dual oscillator model can produce teeth in the oxygen time course when in compound mode (Fig. 4; compare with Fig. 2).

The fact that the fast and slow modes of oscillation can occur together, as in the compound pattern, or separately, as in the pure fast and slow patterns, strongly argues that they stem from distinct mechanisms. Even without the example of the compound mode, it seems implausible that a single mechanism could have a time constant that spans two orders of magnitude. The same set of patterns also shows that the metabolic and electrical oscillators, although reciprocally linked as described above and often co-occurring, can also proceed largely independently of each other.

This is underscored by yet another pattern of oscillation that has been observed in membrane potential, calcium, and oxygen (7, 19, 36, 44), one in which fast and slow oscillations coexist, where the fast occurs with varying frequency throughout the recording rather than only on top of the slow plateau phases. The model can produce this "accordion bursting" (Fig. 5), which is accompanied by O_2 oscillations with teeth present at all phases of the oscillation (11), as has been observed experimentally (44). Moreover, the model suggests that the compound and accordion modes are just quantitative variants. Which one is observed depends on how tightly the electrical activity is constrained by the glycolytic oscillator. If K_{ATP} conductance is relatively low or there is a substantial contribution from the nonoscillatory isoform of PFK, electrical oscillations can occur even when the glycolytic oscillator is in its off phase. The ability of the model to account concisely for diverse patterns of activity by quantitative variation results from the semi-independent character of the two oscillators, which can combine in many ways. This is pursued further in the next section.

GLUCOSE SENSING IN FAST AND SLOW ISLETS

In addition to accounting for the three canonical patterns of oscillation observed in mice (fast, slow, and compound), the model has passed a more stringent test in that it can also account for the variations of these patterns as glucose is changed. Early studies (5, 22, 56) showed that the electrical burst duty cycle or plateau fraction, a good indicator of the relative rate of insulin secretion, increases with glucose concentration. These studies focused on islets exhibiting fast oscillations, since at the time these were the primary oscillations reported in vitro. In a recent study of islet Ca^{2+} oscillations, we found that the glucose response of slow islets (islets exhibiting compound or purely slow Ca^{2+} oscillations) is similar to that of fast islets in that an increase in the glucose concentration had no effect on the amplitude of Ca^{2+} oscillations, had little effect on the oscillation frequency, and increased the plateau fraction of the oscillations (58). Surprisingly, we (58) also observed that some islet responses were transformed from fast to slow or compound oscillations when the glucose concentration was increased. This dramatic increase in the oscillation period was accompanied by a large increase in the oscillation amplitude (Fig. 6, *case 2*). We call this a "regime change." The fact that we saw both fast and slow oscillations in the same islet just by changing glucose indicates that these variants are not artifacts of islet preparation but are both part of the natural repertoire of normal islets.

Regime change can be understood using the "sliding threshold" diagrams shown in Fig. 6. 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 causes both the glycolytic and electrical subsystems to cross their thresholds, but not necessarily at the same glucose concentrations. For example, in Fig. 6, *case 1*, 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. An example of a regime change is shown in *case 2*. At 9 mM glucose the EO is on, but the GO is off, so fast Ca^{2+} oscillations predominate due to 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 compound oscillations. A final example is *case 3*. In this islet, subthreshold Ca^{2+} oscillations are produced in 6 mM glucose. We believe that these are due to activation of the GO, whereas 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. This case is of particular interest because it shows that the slow oscillations can occur in the absence of frank calcium oscillations. This is compatible with the hypothesis that the slow oscillations are due to glycolytic oscillations but not with models in which the slow oscillations depend on calcium feedback onto metabolism or ion channels.

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

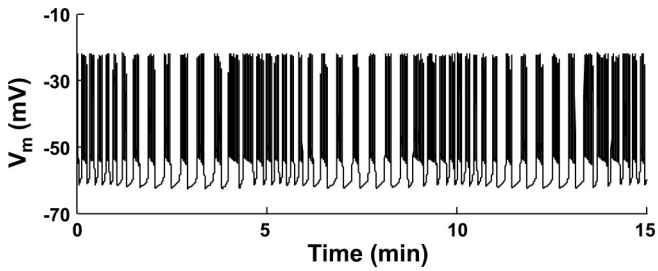

Fig. 5. Accordion bursting produced with the model when the K_{ATP} conductance is not sufficiently large to turn off bursting when the glycolytic oscillator is in its off phase.

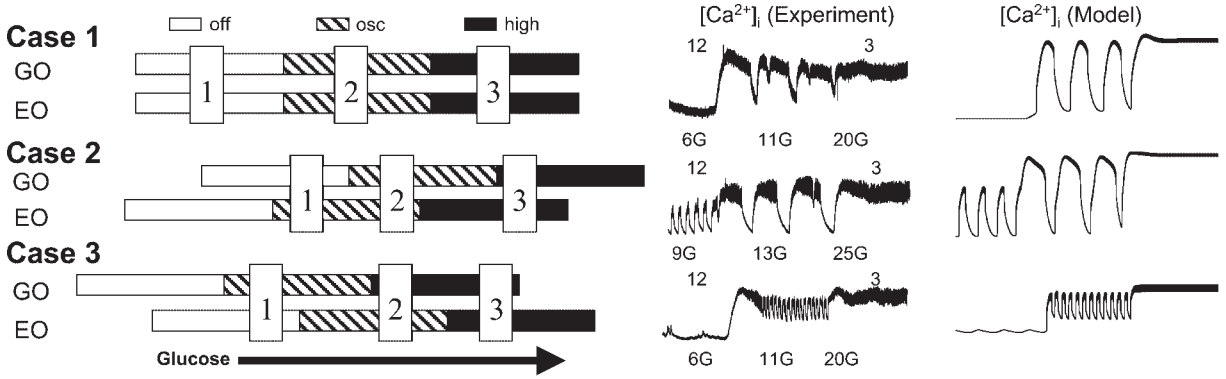

Fig. 6. Schematic model for the diversity of changes in islet activity due to step increases in the glucose concentration. *Left*: sliding threshold representations of the dual oscillator model dynamics. *Right*: measurements of the islet Ca^{2+} concentration and corresponding model simulations. See text for details. GO, glycolytic oscillator; EO, electrical oscillator. Adapted with permission from Nunemaker et al. (58).

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 Fig. 6, *right*. The model also accounts for the glucose-sensing behaviors that occur within both the fast and slow regimes (an increase in plateau fraction without a dramatic change in oscillation period or amplitude) (58).

BIMODAL DISTRIBUTION OF OSCILLATION PERIODS

Further evidence that the fast and slow oscillations stem from distinct mechanisms comes from the distribution of oscillation periods. If a single oscillatory mechanism was capable of producing both fast and slow islet oscillations, then one would expect a relatively uniform distribution of oscillation periods ranging from fast (~15 s) to slow (~6 min), reflecting a single plastic time constant. Instead, a bimodal distribution of Ca^{2+} oscillation periods is observed (59), with peaks in the 15- to 60-s and the 4- to 6-min ranges (Fig. 7). These data argue in favor of two oscillatory mechanisms, one fast and the other slow, as in the dual oscillator model. Interestingly, in this same study, it was observed that islets from the same mouse had similar oscillation periods. Thus, most islets isolated from a "fast mouse" had periods <1 min, whereas most islets from a "slow mouse" had periods of 4–6 min. These in vitro data were reflected in vivo; insulin secretion measured in mice having fast islets was relatively nonpulsatile, as technical limitations in collecting blood samples sufficiently quickly may have rendered oscillations too fast to measure, whereas pulsatile insulin patterns in mice with slower islets were also slow, with periods positively correlated with the periods of the islet calcium oscillations measured in vitro from the same mouse (59). Together, these data suggest that the period of islet oscillations is imprinted on all islets within a mouse. One possible explanation for differences between mice is that glycolytic oscillations are present in islets from slow mice but absent in islets from fast mice.

SINGLE β-CELLS VS. ISLETS

The dual oscillator model also calls for a reconsideration of the role of gap-junctional coupling in islets and the differences between islets and single cells. Simulations suggest that robust synchrony of glycolytic oscillations within islets would require diffusion of metabolites, such as G6P, F6P, or FBP through gap junctions, in addition to the well-known electrical coupling (83). Also, although single β-cells often exhibit slow oscillations similar in period to those in islets, in many cases only fast, irregular activity is seen in membrane potential or calcium. A recent modeling study (62) comparing the effects of channel conductance fluctuations on electrical and glycolytic oscillations found that the electrical oscillations are much more sensitive to fluctuating inputs, perhaps explaining why organized fast bursting is not seen in single cells but regular slow oscillations are. It was further suggested that fast islets are composed primarily of cells that lack the glycolytic oscillator (e.g., have insufficient PFK-M) and become very fast and irregular when isolated, whereas slow islets are composed primarily of cells with adequate PFK-M to support slow oscillations and can do so even when isolated. Finally, the sensitivity to glucose of single β-cells differs from that of islets. The oscillation frequency and active phase duration have been reported to be unchanged once glucose exceeds the threshold for oscillations (32), whereas in islets the slow oscillations exhibit an increase in plateau fraction much like that classically observed in fast islet oscillations (58). Further work is needed to systematize and explain these observations.

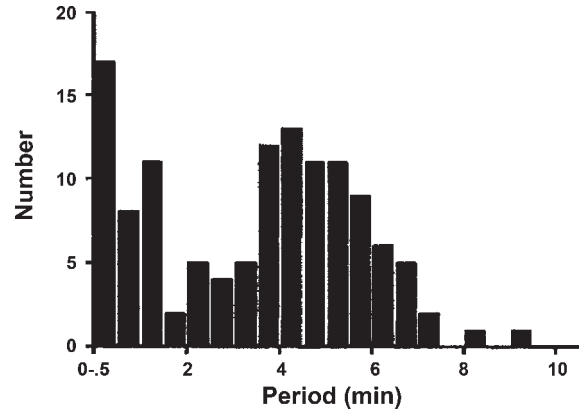

Fig. 7. Bimodal distribution of Ca^{2+} oscillation periods from islets of 21 different mice. Islet periods are binned at 0.5-min intervals. Reprinted with permission from Nunemaker et. al. (59).

CHALLENGES TO THE MODEL

Despite these strengths, there are currently several challenges to the dual oscillator model. The first of these are data showing that islets from sulfonylurea receptor 1 (SUR1) knockout ($SUR1^{-/-}$) mice can still exhibit slow oscillations in membrane potential and Ca^{2+} (25). In the dual oscillator model, K_{ATP} current plays a key role in the transduction of oscillatory metabolism to oscillatory electrical activity and Ca^{2+} oscillations, but in islets from $SUR1^{-/-}$ mice this current is mostly or entirely eliminated (25). If K_{ATP} is eliminated, then what current drives slow oscillations in these islets?

If K_{ATP} is only partially eliminated, then it is possible that the remaining channels compensate by becoming more active. That this may be the case is suggested by data from transgenic Kir6.2[AAA] mice in which ~70% of the β-cells within an islet have nonfunctional K_{ATP} channels (43). Fast Ca^{2+} oscillations have been observed in these islets, suggesting that fast bursting is present at stimulatory glucose levels (71). Moreover, the glucose dose-response curve is shifted by only 1–2 mM to the left, whereas naively one might expect these transgenic islets to spike continuously even in very low glucose. Modeling (not shown) confirms that increasing the open fraction of the remaining K_{ATP} channels threefold can compensate for loss of two-thirds of the K_{ATP} channels. Indeed, the model suggests that one might have to eliminate 90–98% of the channels to completely abolish glucose sensitivity.

This compensation scenario depends on the negative feedback of Ca^{2+} present in normal islets. For example, any rise in cytosolic Ca^{2+} concentration would activate Ca^{2+} pumps, which remove Ca^{2+} from the cytosol at the expense of ATP. The resulting decline in the ATP/ADP ratio could reopen enough of the residual functional K_{ATP} channels to repolarize the cell. This mechanism could work only if the K_{ATP} open fraction is low in wild-type islets, as has been reported previously (20), so that it would be possible for the channel open fraction to increase sufficiently to successfully compensate for the reduction in K_{ATP} conductance in transgenic islets.

A second compensatory mechanism to consider is applicable even if all of the K_{ATP} current is knocked out. The key to this is the Ca^{2+}-activated K^+ current $I_{K,slow}$ that has been measured in normal islets (29, 30) and in islets from $SUR1^{-/-}$ mice (31). Using a computer model, we (13) have shown previously that relatively slow electrical and Ca^{2+} oscillations can be produced through the negative feedback effects of this current. The endoplasmic reticulum (ER) is important here, as it slowly takes up Ca^{2+} during the active phase of bursting and releases it during the silent phase (14). The period of the oscillation is thus determined largely by the slower Ca^{2+} handling properties of the ER. Oscillation periods of several minutes can be achieved, and indeed, without measurements of at least one of the mitochondrial variables it would be difficult to distinguish slow Ca^{2+} oscillations driven by Ca^{2+} activation of $I_{K,slow}$ from slow Ca^{2+} oscillations driven by glycolytic oscillations acting in turn on $I_{K_{ATP}}$. It is therefore possible that the slow oscillations observed in islets from $SUR1^{-/-}$ mice are driven by cyclic Ca^{2+} activation of $I_{K,slow}$. In this scenario, if the ER function is altered by inhibiting the sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA) Ca^{2+} pumps, then the slow oscillations would be expected to increase in frequency (13). A recent study (31) found that this was indeed the case; inhibiting SERCA pumps with cyclopiazonic acid increased the frequency of the slow Ca^{2+} oscillations in islets from $SUR1^{-/-}$ mice. However, it is hard to see how in this scenario compound oscillations could be generated as reported by Düfer et al. (25).

A third possibility is that the slow oscillations in $SUR1^{-/-}$ islets and β-cells are metabolic, as in wild-type, but that the ATP/ADP ratio acts on a target other than K_{ATP}, a channel or pump that is either upregulated or expressed de novo in the knockouts. One possibility is the Na^+-K^+ exchanger, but a recent study (77) suggests that another K^+ channel of unknown identity takes over the role of K_{ATP} in $SUR1^{-/-}$ islets.

This is an unsettled area that should be a priority for future investigation. We would like to point out, however, that the total loss of K_{ATP} poses a challenge to all existing β-cell models, which uniformly predict continuous spiking in low glucose if the cells are deprived of their primary inhibitory current.

Another challenge to the dual oscillator model is data showing that KIC, a fuel that enters metabolism downstream of glycolysis in the citric acid cycle, can evoke slow Ca^{2+} oscillations and compound bursting even in the absence of glucose (33, 51). This is problematic for the model because in this case there is no glycolytic substrate present to initiate and sustain glycolytic oscillations. The model does predict, however, that KIC application in the presence of substimulatory glucose is able to initiate slow oscillations (not shown), consistent with experimental data (51). In other studies it was found that the same concentration of KIC (5 mM) used by Martin et al. (51) was unable to evoke slow oscillations in Ca^{2+} (21, 45) or O_2 (21). Indeed, even when KIC was applied simultaneously with 5 or 10 mM glucose, slow oscillations were not observed. With this conflicting data it is hard to know how to interpret the effects of KIC on islet oscillatory activity.

It is interesting to note that glyceraldehyde, another fuel that enters metabolism downstream of PFK, is capable of elevating the intracellular Ca^{2+} concentration and evoking fast oscillations but does not seem to evoke slow Ca^{2+} oscillations when applied in the absence of glucose (21, 45, 52). However, in the presence of a substimulatory (3–5 mM) concentration of glucose, glyceraldehyde does evoke slow oscillations in Ca^{2+} (21, 45) and O_2 (21). This is consistent with model simulations (not shown). These data suggest that some glucose is necessary to power the glycolytic oscillations that provide the slow component of the Ca^{2+} and O_2 oscillations (21).

CALCIUM OSCILLATIONS AND PULSATILE INSULIN SECRETION

The importance of islet Ca^{2+} oscillations lies in the fact that insulin secretion is pulsatile, and this pulsatility likely reflects oscillatory Ca^{2+}. In one recent study (59), insulin was measured in vivo in mice and exhibited oscillations with periods ranging from 3 to 4.5 min. Calcium measurements were made later in vitro using islets from these same individual mice. It was found that in most cases the periods of the in vitro Ca^{2+} oscillations were close to those of the in vivo insulin oscillations. One example is illustrated in Fig. 8, where Ca^{2+} oscillations with a period of 4.3 min were observed in an isolated islet, whereas the in vivo insulin level oscillated with a period of 4.5 min. These data demonstrate that islet Ca^{2+} oscillations

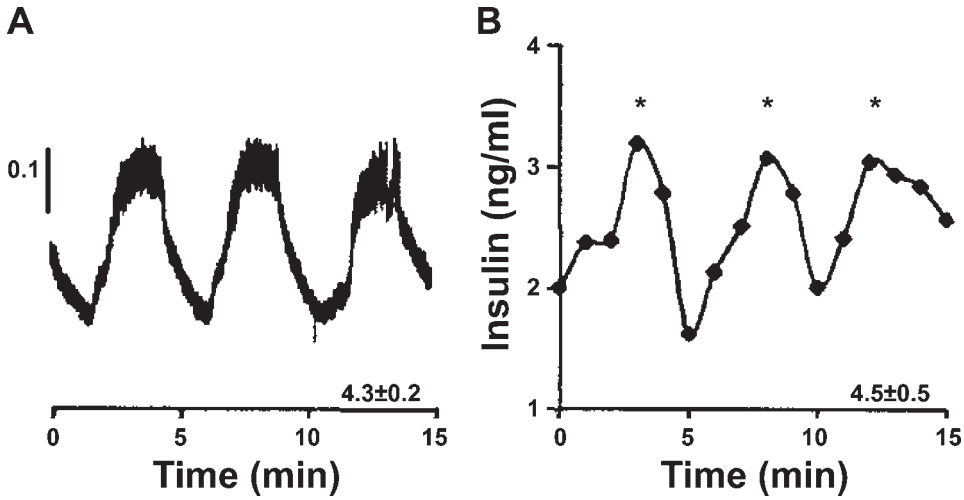

Fig. 8. *A*: Ca^{2+} oscillations with mean period of 4.3 min measured in a mouse islet in 11.1 mM glucose. *B*: in vivo insulin oscillations with mean period of 4.5 min from the same mouse used for islet Ca^{2+} measurements. The blood glucose level was maintained near 13.1 mM. Reprinted with permission from Nunemaker et. al. (59).

have a frequency similar to in vivo insulin oscillations and are likely then the cause of the insulin oscillations.

If islet Ca^{2+} oscillations are responsible for pulsatile in vivo insulin secretion, then the islet oscillations must be synchronized. Otherwise, the various phases and periods of the oscillators (islets) would average out to yield a relatively flat insulin signal. However, in vivo electrical recordings from two separate islets in a mouse (84) showed that the electrical bursting patterns observed were not in phase with each other. This was considered to be in conflict with the hypothesis that pulsatile in vivo insulin secretion is due to coordinated islet activity. However, our model provides a possible explanation for apparently asynchronous electrical activity in the face of synchronous secretion. Figure 9 shows computer simulations for two model islets. Each model islet exhibits compound bursting, but the characteristics of the compound bursting are different in the two islets. As a result, bursts in one islet are out of phase with bursts from the other (Fig. 9*A*). However, the glycolytic oscillations of the two islets (as measured through the FBP variable) are in phase with each other (Fig. 9*B*). Can the total insulin released by the two model islets show pulsatility even though the electrical bursts are out of phase with one another? Figure 9*C* shows that, indeed, the total insulin level is rhythmic with a period equal to that of the glycolytic oscillators. This works because, although the fast bursts lack coordination, the slower burst episodes are coordinated through the synchronized glycolytic oscillators. That is, the model suggests that pulsatile insulin release reflects the coordination of compound Ca^{2+} oscillations within the islet population rather than coordination of the fast components of these oscillations. Potential mechanisms for this coordination include pancreas-liver interactions (63), islet entrainment through intrapancreatic ganglia (75), and a diffusible factor such as insulin itself (3, 41).

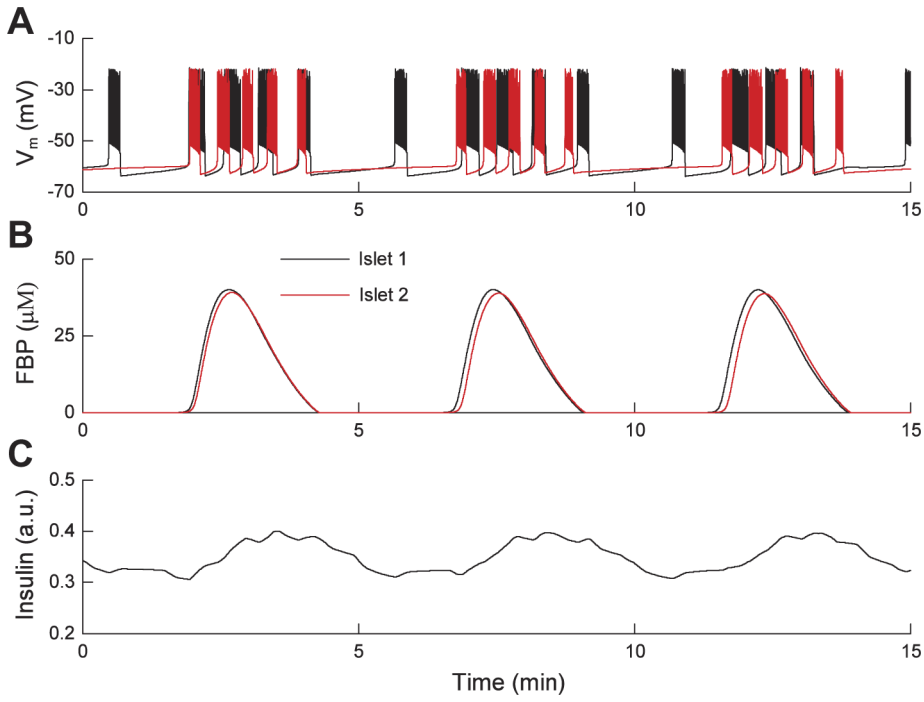

Fig. 9. *A*: 2 model islets in which fast electrical bursts are uncoordinated. *B*: the glycolytic oscillations in the 2 model islets are synchronized. *C*: although the fast bursts are uncoordinated, the slower burst episodes are coordinated. This results in rhythmic insulin secretion with period equal to the period of the glycolytic oscillators. Insulin secretion for each model islet was calculated as described in Pedersen et. al. (63).

WHY COMPOUND OSCILLATIONS?

It has been reported (8) that islets respond to an increase in glucose from one stimulatory level to a higher level by increasing the amplitude of the insulin oscillations rather than the frequency. One mechanism for this is the "amplifying pathway," where an elevated glucose concentration amplifies the effect of Ca^{2+} on insulin secretion (67). We suggest a second, complementary, mechanism. This "metronome hypothesis" postulates a key role for compound oscillations in amplitude modulation of insulin secretion. In the dual oscillator model, the slow component of compound oscillations is provided by glycolytic oscillations. As illustrated in Fig. 9, the period of this component sets the period of the insulin oscillations, and computer simulations with a model glycolytic oscillator show that the period of glycolytic oscillations is only weakly dependent on glucose. The electrical bursting activity provides the fast component of the compound oscillations, and each electrical burst evokes insulin secretion. The plateau fraction of the bursting oscillations increases when the glucose concentration is increased, causing more insulin to be secreted. Since the electrical bursts occur only during the peak of a glycolytic oscillation (Fig. 3), and since the frequency of the glycolytic oscillations is only weakly sensitive to glucose, the effect of increasing glucose is to increase the amount of insulin secreted during each glycolytic peak while having only a small effect on the frequency of the peaks. Thus, compound oscillations provide an ideal substrate for amplitude modulation of insulin secretion. The slow glycolytic component provides the pacing for the insulin metronome, whereas the fast electrical component determines the amplitude, which can be adjusted upward by a glucose-induced increase in the burst plateau fraction.

CONCLUSIONS AND FUTURE DIRECTIONS

By marrying two oscillatory mechanisms, glycolysis and Ca^{2+} feedback, the explanatory power of the dual oscillator model is greatly extended over that of models based on a single oscillatory mechanism. It also provides a natural mechanism for glucose-induced amplitude modulation of insulin secretion. Finally, since Ca^{2+} is elevated only during the peaks of glycolysis in the model, any metabolism-dependent but K_{ATP}-independent mechanism for insulin secretion amplification (35) would be coordinated with elevated Ca^{2+} levels. This ensures that the K_{ATP}-independent signal is in phase with the K_{ATP}-dependent signal, providing maximum impact on insulin secretion.

The dual oscillator model is not, however, without challenges, such as data on K_{ATP} channel mutants and slow oscillations induced by fuels other than glucose. A possible answer is that slow oscillations may be purely ionic. However, no purely ionic model has yet been shown to account for all the phenomena that the dual oscillator model can. Compound and accordion oscillations would be particularly challenging to explain in this way. Another possible scenario is that the slow oscillations are indeed metabolic, but they arise from the mitochondria. There are, in fact, some data demonstrating citrate oscillations in isolated mitochondria (48). However, no quantitative model has yet been proposed to explain how such oscillations arise, but we note that the sliding threshold formulation of Fig. 6 does not stipulate that the metabolic oscillations be glycolytic in origin. In principle, the glycolytic oscillator could be replaced by a mitochondrial slow oscillator or some other slow oscillator that has the right properties but has yet to be identified.

Nonetheless, we are encouraged by the successes of the dual oscillator model, and future experimental observations or improvements to the model may enable it to account for the problematic data discussed above. A recent study (77) suggests that there may be another K^+ channel that can serve as a target for metabolism in addition to K_{ATP}. This is an important issue to resolve both in terms of testing the model and in understanding how glucose sensing can be maintained in mice and humans lacking K_{ATP} channels. At minimum, the dual oscillator model has raised the bar for any successor model. Future measurements of mitochondrial variables such as the mitochondrial membrane potential, NAD(P)H, and O_2 consumption could help to elucidate the respective contributions of glycolysis and mitochondrial metabolism to oscillatory islet activity. Because direct evidence for or against glycolytic oscillations in β-cells is presently lacking, measurements are needed of glucose 6-phosphate, F6P, FBP, or PFK activity in islets in real time.

Understanding the biophysical and biochemical basis of insulin secretion oscillations is highly important, as metabolic defects known to be linked to forms of type 2 diabetes in human patients may account for defective insulin secretion. For example, K_{ATP} channel polymorphisms are among the leading factors implicated in type 2 diabetes in recent genome-wide scans (28, 73) and lead to hyperinsulinism when the channels are insufficiently active and diabetes when the channels are overactive (68). Similarly, glucokinase loss-of-function mutations are associated with maturity-onset diabetes of the young type 2 (86), whereas gain-of-function mutations are associated with hyperinsulinism (17). Less attention has been paid thus far to effects of such mutations on the oscillatory dynamics of insulin secretion. The dual oscillator model provides a new perspective from which to view such mutations, as K_{ATP} channel mutations would shift the threshold of the electrical oscillator (Fig. 6) and glucokinase mutations would shift the thresholds of both the electrical and glycolytic oscillations. If the effects on the electrical and glycolytic thresholds were equal, the result would be a shift in the dose-response curve, but if unequal, there could be a change in the pattern as well. It would be interesting to find this out. Achieving a deeper understanding of β-cell stimulus-secretion coupling through such investigations has the potential to be of great clinical significance in the diagnosis and treatment of people with diabetes.

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