

## Chapter 12

# Electrical Bursting, Calcium Oscillations, and Synchronization of Pancreatic Islets

Richard Bertram, Arthur Sherman, and Leslie S. Satin

**Abstract** Oscillations are an integral part of insulin secretion and are ultimately due to oscillations in the electrical activity of pancreatic  $\beta$ -cells, called bursting. In this chapter we discuss islet bursting oscillations and a unified biophysical model for this multi-scale behavior. We describe how electrical bursting is related to oscillations in the intracellular  $\text{Ca}^{2+}$  concentration within  $\beta$ -cells and the role played by metabolic oscillations. Finally, we discuss two potential mechanisms for the synchronization of islets within the pancreas. Some degree of synchronization must occur, since distinct oscillations in insulin levels have been observed in hepatic portal blood and in peripheral blood sampling of rats, dogs, and humans. Our central hypothesis, supported by several lines of evidence, is that insulin oscillations are crucial to normal glucose homeostasis. Disturbance of oscillations, either at the level of the individual islet or at the level of islet synchronization, is detrimental and can play a major role in type 2 diabetes.

**Keywords** Bursting · Insulin secretion · Islet · Pulsatility · Oscillations

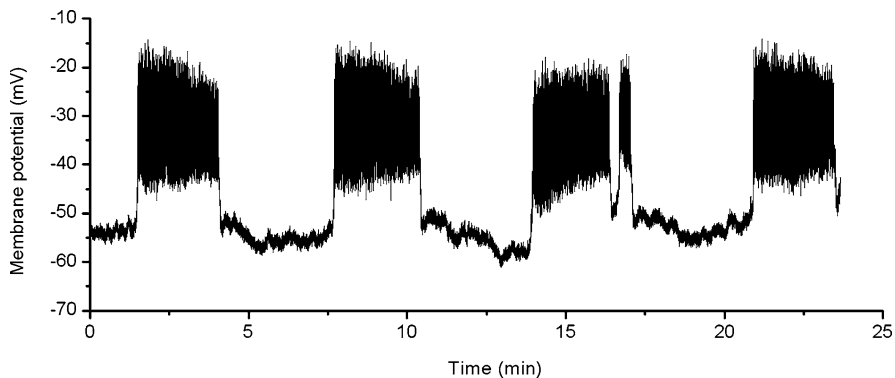
Like nerve and many endocrine cells, pancreatic  $\beta$ -cells are electrically excitable, producing electrical impulses in response to elevations in glucose. The electrical spiking pattern typically comes in the form of bursting, characterized by periodic clusters of impulses followed by silent phases with no activity (Fig. 12.1). In this chapter we discuss the different types of bursting observed in islets, some potential biophysical mechanisms for the bursting, and potential mechanisms for synchronizing activity among a population of uncoupled islets.

Bursting electrical activity is important since it leads to oscillations in the intracellular free  $\text{Ca}^{2+}$  concentration [1, 2], which in turn lead to oscillations in insulin secretion [3]. Oscillatory insulin levels have been measured in vivo [4–7], and sampling from the hepatic portal vein in rats, dogs, and humans shows large oscillations with period of 4–5 minutes [8, 9]. Deconvolution analysis demonstrates that the

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R. Bertram (✉)

Department of Mathematics, Florida State University, Tallahassee, FL 32306, USA  
e-mail: bertram@math.fsu.edu



**Fig. 12.1** Slow electrical bursting recorded from a mouse islet. Provided by J. Ren and L.S. Satin

oscillatory insulin level is due to oscillatory secretion of insulin from islets [8, 10], and in humans at least 75% of insulin secretion is from insulin pulses [10]. In humans, the amplitude of insulin oscillations in the peripheral blood is  $\sim 100$  times smaller than that in the hepatic portal vein [9]. This attenuation is confirmed by findings of hepatic insulin clearance of  $\sim 50\%$  in dogs [11] and  $\sim 40\text{--}80\%$  in humans [12, 13]. It has also been demonstrated that the hepatic insulin clearance rate itself is oscillatory, corresponding to portal insulin oscillations. That is, the insulin clearance rate is greater during the peak of an insulin oscillation than during the trough [13]. 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. In fact, coherent insulin oscillations are disturbed or lost in patients with type 2 diabetes and their near relatives [14–17], and this will most likely affect insulin clearance by the liver [13].

Oscillations in insulin have also been observed in the perfused pancreas [18] and in isolated islets [2, 3, 19–21]. The oscillations have two distinct periods; the faster oscillations have a period of 1–2 minutes [3, 5, 22, 23], while the slower oscillations have a period of 4–6 minutes [4, 5, 7]. In one recent study, insulin measurements were made *in vivo* in mice, and it was shown that some mice exhibit insulin oscillations with period of 3–5 minutes (the “slow mice”), while others exhibit much faster insulin oscillations with period of 1–2 minutes (the “fast mice”). Surprisingly, most of the islets examined *in vitro* from the “fast mice” exhibited fast  $\text{Ca}^{2+}$  oscillations with similar period, while most of those examined from the “slow mice” exhibited either slow or compound  $\text{Ca}^{2+}$  oscillations (fast oscillations clustered together into slow episodes) with similar period [24]. Thus, the islets within a single animal have a relatively uniform oscillation period which is imprinted on the insulin profile *in vivo*. As we describe later, the two components of oscillatory insulin secretion and their combinations can be explained by the two timescales of electrical bursting.

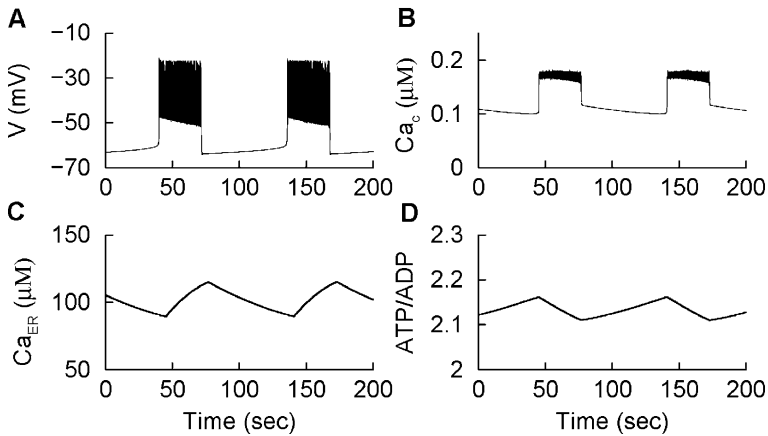
## 12.1 The Role of Calcium Feedback

$\text{Ca}^{2+}$  enters  $\beta$ -cells through  $\text{Ca}^{2+}$  channels during the active phase of a burst during which it accumulates and activates  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  channels [25, 26]. The resulting hyperpolarizing current can itself 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 [27]. The endoplasmic reticulum (ER) plays a major role here, taking up  $\text{Ca}^{2+}$  during the active phase of a burst when  $\text{Ca}^{2+}$  influx into the cytosolic compartment is large and releasing  $\text{Ca}^{2+}$  during the silent phase of the burst. These filtering actions have a significant impact on the time dynamics of the cytosolic  $\text{Ca}^{2+}$  concentration, and thus on the period of bursting. The influence of the ER on cytosolic free  $\text{Ca}^{2+}$  dynamics was convincingly demonstrated using pulses of KCl to effectively voltage clamp the entire islet [28; 29]. Using 30-second pulses, similar to the duration of a medium burst, it was shown that the amplitude of the  $\text{Ca}^{2+}$  response to depolarization was greater when the ER was drained of  $\text{Ca}^{2+}$  by pharmacologically blocking ER  $\text{Ca}^{2+}$  pumps (SERCA). In addition, the slow decline of the cytosolic  $\text{Ca}^{2+}$  concentration, which followed the depolarization in control islets and which follows a burst in free-running islets, was absent when SERCA pumps were blocked. The mechanisms for these effects were investigated in a mathematical modeling study [30]. This study also showed that  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release (CICR) is inconsistent with data from [28, 29]. CICR did occur in single  $\beta$ -cells in response to cyclic AMP, but in this case, electrical activity and  $\text{Ca}^{2+}$  oscillations are out of phase [32, 33], which is in contrast to the in-phase oscillations observed in glucose-stimulated islets [1, 2].

In addition to the direct effect on  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels, intracellular  $\text{Ca}^{2+}$  has two opposing effects on glucose metabolism in  $\beta$ -cells.  $\text{Ca}^{2+}$  enters mitochondria through  $\text{Ca}^{2+}$  uniporters, depolarizing the mitochondrial inner membrane potential and thus reducing the driving force for mitochondrial ATP production [34–37]. Once inside mitochondria, free  $\text{Ca}^{2+}$  stimulates pyruvate dehydrogenase, isocitrate dehydrogenase, and  $\alpha$ -ketoglutarate dehydrogenase [38, 39], resulting in increased production of NADH, which can increase the mitochondrial ATP production. Thus,  $\text{Ca}^{2+}$  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  $\text{K}^+$  channels [40]. Variations in the nucleotide ratio result in variation in the fraction of open  $\text{K}(\text{ATP})$  channels. Thus, oscillations in the intracellular  $\text{Ca}^{2+}$  concentration can lead to oscillations in the ATP/ADP ratio, which can contribute to bursting through the action of the hyperpolarizing  $\text{K}(\text{ATP})$  current [41–44]. However,  $\text{K}(\text{ATP})$  channels are not the whole story, since bursting and  $\text{Ca}^{2+}$  oscillations persist in islets from mice with the sulfonylurea receptor *Surl* gene knocked out or the pore-forming *Kir6.2* gene knocked out [45–47]. Thus it is likely that another channel contributes to bursting, at least in the case of  $\text{K}(\text{ATP})$ -knockout mutant islets.

Figure 12.2 uses a mathematical model [42] to demonstrate the dynamics of the variables described above. (Other models have recently been developed, postulating



**Fig. 12.2** Model simulation of bursting, illustrating the dynamics of membrane potential ( $V$ ), free cytosolic  $\text{Ca}^{2+}$  concentration ( $\text{Ca}_c$ ), free ER  $\text{Ca}^{2+}$  concentration ( $\text{Ca}_{\text{ER}}$ ), and the ATP/ADP concentration ratio. The model is described in [42] and the computer code can be downloaded from [www.math.fsu.edu/~bertram/software/islet](http://www.math.fsu.edu/~bertram/software/islet)

different burst mechanisms and highlighting other biochemical pathways [48, 49].) Two bursts are shown in Fig. 12.2A and the cytosolic free  $\text{Ca}^{2+}$  concentration ( $\text{Ca}_c$ ) is shown in Fig. 12.2B. At the beginning of an active phase,  $\text{Ca}_c$  quickly rises to a plateau that persists throughout the burst. Simultaneously, the ER free  $\text{Ca}^{2+}$  concentration ( $\text{Ca}_{\text{ER}}$ ) slowly increases as SERCA activity begins to fill the ER with  $\text{Ca}^{2+}$  (Fig. 12.2C). In contrast, the ATP/ADP ratio during a burst declines (Fig. 12.2D), since in this model the negative effect of  $\text{Ca}^{2+}$  on ATP production dominates the positive effect. Both  $\text{K}(\text{Ca})$  and  $\text{K}(\text{ATP})$  currents, concomitantly activated by the phase of increased  $\text{Ca}^{2+}$  and decreased ATP/ADP, respectively, combine to eventually terminate the burst, after which  $\text{Ca}_c$  slowly declines. This slow decline reflects the release of  $\text{Ca}^{2+}$  from the ER during the silent phase of the burst along with the removal of  $\text{Ca}^{2+}$  from the cell by  $\text{Ca}^{2+}$  pumps in the plasma membrane. Also, ATP/ADP increases during the silent phase, slowly turning off the  $\text{K}(\text{ATP})$  current. The combined effect of reducing  $\text{K}(\text{Ca})$  and  $\text{K}(\text{ATP})$  currents eventually leads to the initiation of a new active phase and the cycle restarts.

## 12.2 Metabolic Oscillations

As described above and illustrated in Fig. 12.2, there will be metabolic oscillations due to the effects of  $\text{Ca}^{2+}$  on the mitochondria. In addition, there is considerable evidence for  $\text{Ca}^{2+}$ -independent metabolic oscillations, reviewed in [50, 51]. One hypothesis is that glycolysis is oscillatory and is the primary mechanism underlying pulsatile insulin secretion from  $\beta$ -cells [50]. The M-type isoform of the glycolytic enzyme phosphofructokinase 1 (PFK-1) is known to exhibit oscillatory activity

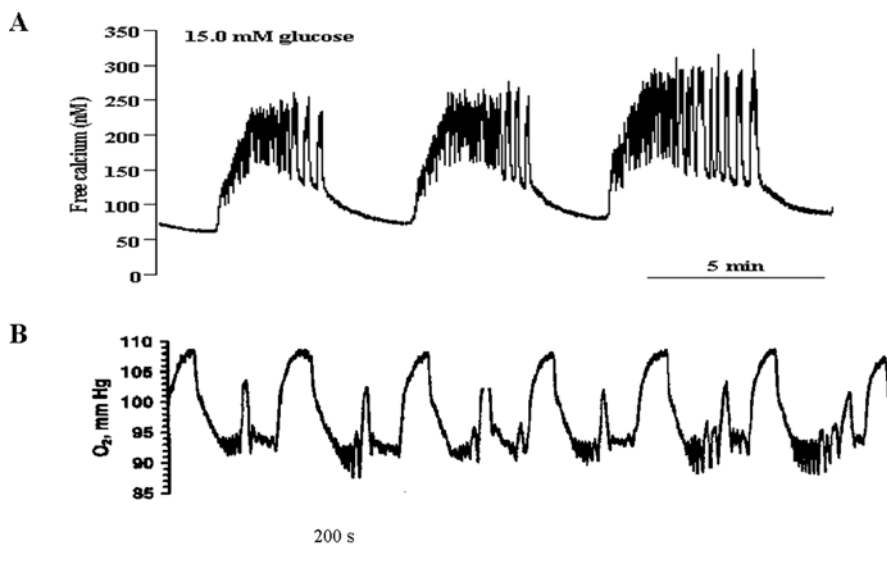
in muscle extracts, as measured by oscillations in the levels of the PFK-1 substrate fructose 6-phosphate (F6P) and product fructose 1,6-bisphosphate (FBP) [52, 53]. The period of these oscillations, 5–10 minutes, is similar to the period of slow insulin oscillations [50]. The mechanism for the oscillatory activity of this isoform, which is the dominant PFK-1 isoform in islets [54], is the positive feedback of its product FBP on phosphofructokinase activity and subsequent depletion of its substrate F6P [55–57]. While there is currently no direct evidence for glycolytic oscillations in  $\beta$ -cells, there is substantial indirect evidence for it. This comes mainly from measurements of oscillations in several key metabolic variables, such as oxygen consumption [19, 58–60], ATP or ATP/ADP ratio [61–63], mitochondrial inner membrane potential [34], lactate release [64], and NAD(P)H levels [65]. Additionally, it has been demonstrated that patients with homozygous PFK-1-M deficiency are predisposed to type 2 diabetes [66], and in a study on humans with an inherited deficiency of PFK-1-M it was shown that oscillations in insulin secretion were impaired [67]. An alternate hypothesis for  $\text{Ca}^{2+}$ -independent metabolic oscillations is that the oscillations are inherent in the citric acid cycle, based on data showing citrate oscillations in isolated mitochondria [38].

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 [68] and Goldbeter and Lefever [69], 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 FBP does in our model.

Such models can also combine with electrical activity to produce many of the patterns described here [70], 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 acts as a positive modulator, which activates glycolysis when ATP production falls behind metabolic demand. More fundamentally, we argue that  $\beta$ -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 in regulating activity.

### 12.3 The Dual Oscillator Model for Islet Oscillations

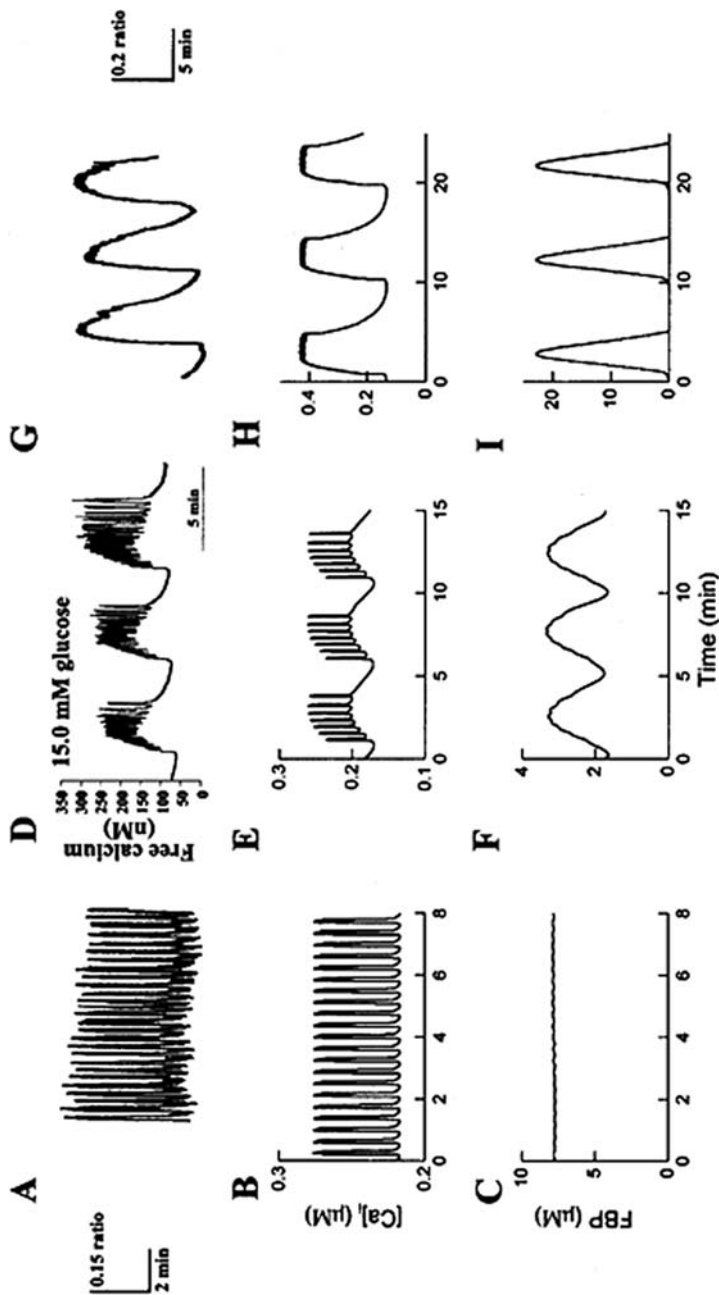
Recent islet data provide the means to disentangle the influences of  $\text{Ca}^{2+}$  feedback and glycolysis on islet oscillations. Figure 12.3A shows “compound”  $\text{Ca}^{2+}$  oscillations, recorded from islets in 15 mM glucose. There is a slow component (period ~5 minutes) with much faster oscillations superimposed on the slower plateaus. These compound oscillations have been frequently observed by a number of research groups [2, 71–73] and reflect compound bursting oscillations, where fast bursts are



**Fig. 12.3** **A** Compound islet  $\text{Ca}^{2+}$  oscillations measured using fura-2/AM. The oscillations consist of slow episodes of fast oscillations. Reprinted with permission from [79]. **B** Slow oxygen oscillations with superimposed fast “teeth.” Reprinted with permission from [76]

clustered together into slower episodes [74, 75]. Figure 12.3B shows measurements of islet oxygen levels in 10 mM glucose [76]. Again there are large-amplitude slow oscillations (period of 3–4 minutes) with superimposed fast oscillations or “teeth.” Similar compound oscillations have been observed in intra-islet glucose and in insulin secretion [77, 78], as assayed by  $\text{Zn}^{2+}$  efflux from  $\beta$ -cells. These data showing compound oscillations in a diversity of cellular variables suggest that compound oscillations are fundamental to islet function.

We have hypothesized that the slow component of the compound oscillations is due to oscillations in glycolysis, while the fast component is due to  $\text{Ca}^{2+}$  feedback onto ion channels and metabolism. This hypothesis has been implemented as a mathematical model, which we call the “dual oscillator model” [79, 80]. The strongest evidence for this model is its ability to account for the wide range of time courses of  $\text{Ca}^{2+}$  and metabolic variables observed in glucose-stimulated islets in vitro and in vivo. One behavior frequently observed in islets is fast oscillations, which do not have an underlying slow component. An example is shown in Fig. 12.4A. The dual oscillator model reproduces this type of pattern (Fig. 12.4B) when glycolysis is non-oscillatory (Fig. 12.4C). The fast oscillations are mainly due to the effects of  $\text{Ca}^{2+}$  feedback onto  $\text{K}^+$  channels as discussed earlier. Compound oscillations (Fig. 12.4D) are also produced by the model (Fig. 12.4E) and occur when both glycolysis and electrical activity are oscillatory (Fig. 12.4F) and become phase locked. The glycolytic oscillations provide the slow envelope and electrically driven  $\text{Ca}^{2+}$  oscillations produce the fast pulses of  $\text{Ca}^{2+}$  that ride on the slow wave. Note



**Fig. 12.4** Three types of oscillations typically observed in islets. *Top row* of panels is from islet measurements of  $\text{Ca}^{2+}$  using fura-2/AM. *Middle row* shows simulations of  $\text{Ca}^{2+}$  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 with permission from [24, 51, 79]

that this pattern, while resembling the bursting of Fig. 12.2 on a slower timescale, is fundamentally different in that the fast bursts are sometimes observed to occur during the valleys of the glycolytic envelope, albeit with lower plateau fraction, and thus are modulated by rather than strictly dependent on the surge in FBP. This pattern (“accordion bursting”) has been observed in membrane potential,  $\text{Ca}^{2+}$ , and oxygen [72, 74, 75, 81].

Compound oscillations also produce slow  $\text{O}_2$  oscillations with “teeth,” as in Fig. 12.3B. The slow oscillations in the flux of metabolites from glycolysis to the mitochondria result in oscillations in  $\text{O}_2$  consumption by the mitochondrial electron transport chain. The  $\text{Ca}^{2+}$  feedback onto mitochondrial respiration also affects  $\text{O}_2$  consumption, resulting in the faster and smaller  $\text{O}_2$  teeth. A third pattern often observed in islets is a purely slow oscillation (Fig. 12.4G). The model reproduces this behavior (Fig. 12.4H) when glycolysis is oscillatory (Fig. 12.4I) and when the cell is tonically active during the peak of glycolytic activity. Thus, a model that combines glycolytic oscillations with  $\text{Ca}^{2+}$ -dependent oscillations can produce the three types of oscillatory patterns typically observed in islets, as well as faster oscillations in the  $\text{O}_2$  time course when in compound mode.

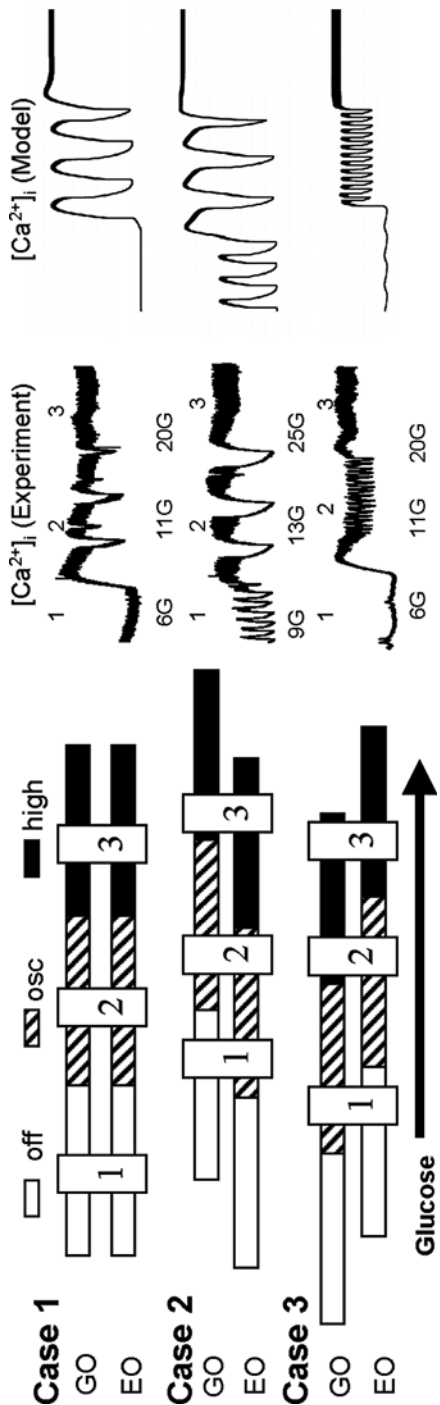
Accordion bursting, like compound bursting, is accompanied by  $\text{O}_2$  oscillations with fast teeth, but now is present at all phases of the oscillation in both the model [79] and in experiments [81]. The model thus suggests that the compound and accordion modes are just quantitative variants of the same underlying mechanisms. The former can be converted into the latter by reducing the conductance of the  $\text{K(ATP)}$  current, limiting its ability to repolarize the islets. It also supports the notion that  $\beta$ -cells have two oscillators that interact but can also occur independently of each other.

## 12.4 Glucose Sensing in the Dual Oscillator Framework

The concept of two semi-independent oscillators can be captured in a diagrammatic scheme (Fig. 12.5) representing how the two subsystems 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  $\text{Ca}^{2+}$  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 the 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. 12.5, when the islet is in 6 mM glucose, both the glycolytic oscillator (GO) and the electrical oscillator (EO) are in their low activity states. When glucose is raised to 11 mM, both oscillators are activated, yielding slow  $\text{Ca}^{2+}$  oscillations. In this scenario, the electrical burst duty cycle or the 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





**Fig. 12.5** 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 with permission from [51, 85]

[82–84]. The increase in the glucose concentration in this regime has no effect on the amplitude of  $\text{Ca}^{2+}$  oscillations and has little effect on the oscillation frequency [85].

However, some islet responses have been observed to be transformed from fast to slow or compound oscillations when the glucose concentration was increased [85]. This dramatic increase in the oscillation period was accompanied by a large increase in the oscillation amplitude (Fig. 12.5, Case 2). We interpreted this as a switch from electrical to glycolytic oscillations and termed this transformation “regime change.” The diagrammatic representation in Fig. 12.5 indicates that this occurs when the threshold for the GO is shifted to the right of that for the EO. This may occur if glucokinase is relatively active or  $\text{K(ATP)}$  conductance is relatively low.

At 9 mM glucose, the EO is on, but the GO is off, so fast  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  oscillations combine with glycolytic oscillations to produce much slower and larger amplitude compound oscillations.

A final example is Case 3. In this islet, subthreshold  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  pattern. However, the upper threshold for glycolytic oscillations is also crossed, so the glycolytic oscillations stop. As a result, a fast oscillatory  $\text{Ca}^{2+}$  pattern is produced, with only a transient underlying slow component. This form of regime change is of particular interest since it suggests that the slow oscillations could occur without large-amplitude oscillations in  $\text{Ca}^{2+}$ . This would argue against any model in which the slow oscillations are dependent on  $\text{Ca}^{2+}$  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 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. 12.5.

## 12.5 Functional Role for Compound Oscillations

Islets respond to increased glucose with increased amplitude of the insulin oscillations, while frequency remains relatively fixed [21]. This can be explained in part by the “amplifying pathway,” in which an elevated glucose concentration amplifies the effect of  $\text{Ca}^{2+}$  on insulin secretion at a step distal to changes in  $\text{Ca}^{2+}$  [86]. A complementary mechanism, which we call the “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. The period of this component sets the period of

the insulin oscillations, and computer simulations using a model glycolytic oscillator show that the period of glycolytic oscillations is only weakly dependent on glucose, except very close to threshold. 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, resulting in more insulin secretion. Since the electrical bursts occur only during the peak of a glycolytic oscillation (Fig. 12.4), 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 encode the stimulatory glucose level through amplitude modulation, as is the case in experimental studies. We thus suggest that the slow glycolytic component sets the timing of the insulin metronome, while the glucose-dependent plateau fraction of the fast electrical component determines the amplitude.

## 12.6 Islet Synchronization

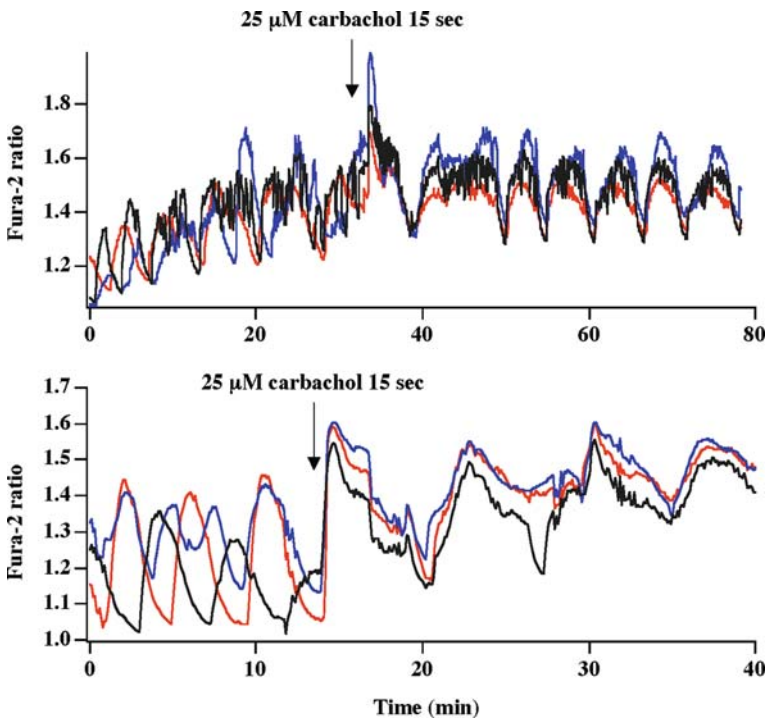
Islet  $\text{Ca}^{2+}$  oscillations appear to be the driving mechanism behind pulsatile insulin. In one recent study, *in vivo* insulin oscillations were recorded in mice with periods of 3–5 minutes [5]. *In vitro* recordings of islets from the same mice showed similar periods as the *in vivo* insulin oscillations. The similarity of the frequencies further supports the hypothesis that the islet  $\text{Ca}^{2+}$  oscillations drive the whole-body insulin oscillations.

This then raises the question of how the oscillations synchronize from islet to islet within the intact pancreas. If the individual islet oscillators were out of phase and had widely discrepant frequencies, the net output would average out to a relatively flat insulin signal. It has been suggested that this synchronization is achieved through the actions of intrapancreatic ganglia [87–93]. The ganglia nerves form a connected network within the pancreas of rat, cat, rabbit, guinea pig, and mouse [90, 94–96], and are shown to be electrically excitable when autonomic nerve trunks are stimulated in the cat [90]. The fibers are primarily cholinergic [87], islets contain ample amounts of choline acetyltransferase and acetylcholinesterase [97], and  $\beta$ -cells express  $\text{M}_1$ - and  $\text{M}_3$ -type muscarinic receptors [98]. Finally, it has been shown that *in vitro* and *in vivo* vagal stimulations promote glucose-dependent insulin release from the pancreas [99–102]. It is thus plausible that cholinergic pulsing from the intrapancreatic ganglia to the subset of innervated islets entrains the islets, synchronizing their oscillations. If enough islets are synchronized in this manner, then the plasma insulin level will exhibit a coherent oscillation, as has been measured in many mammals, including man [103, 104].

The hypothesis that intrapancreatic ganglia act to synchronize endogenous islet oscillators is difficult to test *in vivo*, and indeed the hypothesis is largely untested. However, recent *in vitro* work has demonstrated the ability of a muscarinic agonist

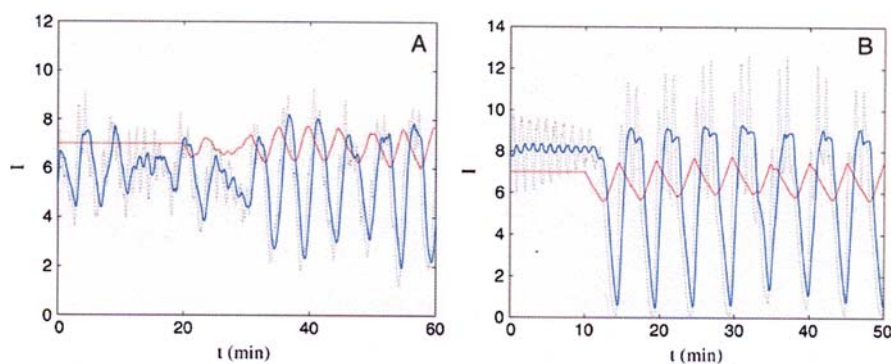
to transiently synchronize a group of individual islets. In this study [91], three to six islets were included in an experimental chamber and intracellular  $\text{Ca}^{2+}$  levels in the islets within the chamber were monitored using the fluorescent dye fura-2/AM. The islets were uncoupled and in the presence of stimulatory glucose (11.1 mM) oscillated with different frequencies and were out of phase with one another. A single 15-second pulse of the muscarinic agonist carbachol was then applied to the bathing solution. In most cases, this brief pulse of agonist resulted in the transient synchronization of the islets (Fig. 12.6). The two panels of Fig. 12.6 show the synchronization for two trials, each containing three islets. The synchronization was transient, but in some cases lasted as long as experimental measurements were made (ca. 40 minutes). This transient synchronization was reproduced in computer simulations of the dual oscillator model, and a mechanism was postulated [91]. Thus, it appears that cholinergic stimulation can synchronize islets and could therefore be responsible for islet synchronization *in vivo*.

An alternate mechanism for islet synchronization has been suggested [4, 105–107]. According to this hypothesis, it is the interaction between pancreatic islets



**Fig. 12.6** A 15-second pulse of the muscarinic agonist carbachol (25  $\mu\text{M}$ ) synchronizes  $\text{Ca}^{2+}$  oscillations in islets maintained in 11.1 mM glucose. The two panels correspond to different groups of islets. Within each panel, different colors correspond to different islets. Reprinted with permission from [91]

and the liver that is responsible for islet synchronization *in vivo*. That is, the insulin secreted by islets acts on the liver, resulting in a reduction in the plasma glucose concentration. This change in the glucose level is then sensed by the entire islet population, providing global coupling among islets. It is plausible that this global coupling can, over time, lead to islet synchronization, but again the mechanism (which is very difficult to test) has not been tested experimentally. A recent mathematical modeling study investigated whether such a feedback system would lead to islet synchronization when the dynamics of the individual islets is described by the dual oscillator model and when the action of the liver is described by a simple equation that lowers the glucose level when the mean insulin level is elevated [105]. Figure 12.7A shows simulation results obtained with 20 heterogeneous model islets (islets have different endogenous oscillation frequencies in the model). The dashed curve is the mean level of the insulin secretion from the 20 islets, while the blue curve is this mean smoothed using a 1-minute moving average. The red curve is the extracellular glucose concentration, which is affected by the model “liver.” For  $t < 20$  minutes, the glucose level is held constant. After 20 minutes, the glucose concentration is allowed to vary according to feedback from the liver in response to the mean insulin level. A clear small oscillation in the glucose concentration results (red curve). Simultaneously, the model islets become largely synchronized. This synchronization can be seen in the blue curve (smoothed insulin level), which exhibited small oscillations for  $t < 20$  minutes, but much larger oscillations for  $t > 20$  minutes. Thus, the dynamic islet–liver interaction leads to a coherent insulin oscillation. This effect would be more dramatic if more islets were used in the simulation, since then the oscillations for  $t > 20$  minutes would be larger relative to those for  $t < 20$  minutes when there are more islets that can synchronize. Figure 12.7B demonstrates that



**Fig. 12.7** Mathematical simulation showing that the interaction between a population of model islets and the liver can lead to islet synchronization and large insulin oscillations. Both panels show results for a population of 20 heterogeneous model islets. The glucose concentration (red) is held constant until  $t = 20$  minutes, after which it varies according to the mean insulin level (dashed black curve). A smoothed version of the mean insulin level (blue) is also shown. (A) Twenty oscillatory islets with different periods. (B) Twenty non-oscillatory islets that begin to oscillate once the “liver” is turned on. Reprinted with permission from [105]

even if the model islets are not oscillating initially, they can be induced to oscillate in phase once feedback from the liver is activated. Once again, interaction between the islets and the liver leads to a coherent insulin oscillation due to islet synchronization. A more recent modeling study, using simpler representations of islets and the liver, found results similar to those shown in Fig. 12.7A when insulin secretion was driven by the product of the glycolytic oscillator [108].

Which of the two mechanisms described above contributes to islet synchronization in vivo is not yet known. Indeed, it is possible that other mechanisms may serve this function. It is also possible that both mechanisms act together to synchronize the islets and that additional synchronizing factors such as ATP acting on purinergic receptors [109, 110] contribute to islet synchronization. Additional experiments must be performed to solve the mystery of islet synchronization in the intact pancreas.

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