Review

Dynamical complexity and temporal plasticity in pancreatic β-cells

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We discuss some of the biological and mathematical issues involved in understanding and modelling the bursting electrical activity in pancreatic βcells. These issues include single-cell versus islet behaviour, parameter heterogeneity, channel noise, the effects of hormones, neurotransmitters, and ions, and multiple slow biophysical processes. Some of the key experimental and modelling studies are described, and some of the major open questions are discussed.

1. Introduction

The endocrine pancreas is a key regulator of glucose homeostasis. The pancreatic βcells secrete insulin in response to an elevation in the blood glucose concentration, as after a meal. The endocrine pancreas is also the target of sympathetic and parasympathetic nerves, through which the hypothalamus exerts control over blood insulin levels (Woods and Porte 1974). Autoimmune destruction of the βcells results in type-I or early-onset diabetes, while impaired insulin secretion contributes to type-II or late-onset diabetes. Motivated largely by the prevalence of diabetes in modern society, βcells have been the focus of a great deal of scientific study, both experimental and computational. In this report, we provide an overview of the complex behaviour of βcells, and describe some of the contributions made by mathematical modelling studies of βcells.

2. Behaviour of single β-cells

Pancreatic βcells are clustered into islets of Langerhans. These are roughly spherical structures of radius 50–250 μm distributed throughout the pancreas. An islet contains 10^3–10^4 cells, 80%–90% of which are insulin-secreting βcells while the majority of the remaining cells secrete the hormone glucagon. Islet cells are electrically excitable, and their electrical activity is largely synchronized via gap junctional coupling (Smolen *et al* 1993; Mears *et al* 1995). This islet structure makes it difficult to use standard voltage-clamp techniques to determine properties of individual cells, such as the type and distribution of ion channels. In addition, it is not clear which properties of islet electrical behaviour are inherent to individual βcells and which are emergent features of the electrical coupling. For these reasons, βcells are often removed from the islet and studied in isolation. In this section we describe the behaviour of such isolated βcells, as well as small clusters of βcells that often form when the cells are cultured.

The electrical behaviour observed in single βcells falls roughly into four classes. Two reports describe cells that generate bursts of electrical impulses with a burst period of several minutes (Smith *et al* 1990; Larsson *et al* 1996). This "slow bursting" is quite different from that typically observed in intact islets, which we call "medium bursting", where the burst period ranges between 10–30 s. Besides the obvious difference in burst period, slow single-cell bursting differs from medium islet bursting in other ways that will be discussed later.

Single-cell behaviour was roughly classified into three patterns in a recent systematic study of isolated βcells (Kinard *et al* 1999). A "class 1" cell exhibits repetitive fast spikes that are not clustered into bursts. Of the 52 βcells studied, 33% fell into this category. A "class 2" cell exhibits fast bursts of spikes, with a burst period of less than 5 s (52% of the population). Finally, a "class 3" cell exhibits periodic plateau depolarizations with no clear

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spikes (15% of the population). The period of these plateau oscillations was 10–20 s, similar to the period of medium bursting in islets (although spikes are clearly present in islet bursts). In this study, the slow bursting pattern reported by Smith *et al* (1990) and Larsson *et al* (1996) was observed in small cell clusters, but not in single cells.

3. Behaviour of intact islets

Bursting electrical activity was first reported in *in vitro* islet studies by Dean and Mathews (1970), and has since been routinely observed and well characterized by many labs. Burst period typically ranges from 10–30 s, depending on the bath glucose concentration, though periods longer than 1 min are sometimes seen. At low glucose concentrations the islet is in a silent hyperpolarized state. At higher concentrations the islet bursts with a relatively long period, due to a long silent phase. At higher glucose concentrations the burst period decreases as the silent phase decreases, and at still higher concentrations the burst period increases again due to a long active or spiking phase. If the glucose concentration is increased further the islet spikes continuously (Beigelman *et al* 1977).

The electrical activity of βcells is reflected in the concentration of Ca^{2+} in the cytosol, $[Ca^{2+}]_i$. Thus, during a typical bursting oscillation $[Ca^{2+}]_i$ oscillates in phase with the electrical activity (Santos *et al* 1991). During the active phase Ca^{2+} enters the cell through voltage-dependent L-type Ca^{2+} channels (Hopkins *et al* 1991), so that $[Ca^{2+}]_i$ is elevated. During the silent phase, Ca^{2+} is removed from the cytosol by Ca^{2+} pumps, lowering $[Ca^{2+}]_i$. Imaging techniques are used to monitor $[Ca^{2+}]_i$ in both single βcells and intact islets and have shown that bursting is synchronized throughout the islet (Santos *et al* 1991). Slow $[Ca^{2+}]_i$ oscillations, with periods of 2 min or longer, have been seen in islets (Valdeolmillos *et al* 1989). Indeed, medium-frequency $[Ca^{2+}]_i$ oscillations have been observed superimposed on a low-frequency $[Ca^{2+}]_i$ oscillation (Valdeolmillos *et al* 1989). It has recently been suggested that this hybrid is due to different regions of the islet bursting at different frequencies (Liu *et al* 1998).

Slow oscillations in $[Ca^{2+}]_i$ can also be induced in islets by the amino acid leucine or its metabolite ketoisocaproate (Martin *et al* 1995). This is an important finding since islets *in vivo* are exposed to a cocktail of amino acids, including leucine. Indeed, in one study in which islets were exposed to a solution containing typical plasma amino acid concentrations, islets exhibiting medium-frequency $[Ca^{2+}]_i$ oscillations began to oscillate with a much lower frequency (Martin and Soria 1995). On the other hand, bath application of the hormone glucagon, a physiological potentiator of insulin secretion, which increases cytosolic cAMP concentration in βcells, has been reported to convert slow bursting into medium bursting in islets (Liu *et al* 1998); this was seen in islets from obese diabetic (*ob*/*ob*) mice, which may tend to be slower than "normal" mice anyway. Recent *in vivo* islet recordings showed islets bursting with periods ranging from less than a minute to several minutes (Sánchez-Andrés *et al* 1995; Valdeolmillos *et al* 1996). Note that in those experiments the islets were subject not to a constant glucose level imposed by the experimentalist, but to a physiologically regulated glucose level in response to an injected bolus of glucose. Thus, it is difficult to say what the "typical" behaviour of islets is, but it is clear that their behaviour is very plastic depending on experimental conditions.

Medium bursting in islets can also be converted into fast bursting. Acetylcholine (ACh), the neurotransmitter secreted by parasympathetic nerves, increases insulin secretion by increasing the frequency of bursting and depolarizing the silent phase (Cook *et al* 1981; Henquin *et al* 1988; Bordin *et al* 1995). Although this fast bursting appears to be similar to the fast bursting observed in single βcells (class 2), it is not known whether this is coincidental or reflects a common mechanism.

4. Noise and heterogeneity

Electrical recordings of single βcells often exhibit a great deal of noise, presumably due to the opening and closing of large-conductance ion channels such as Ca^{2+}-activated K^+ channels [K(Ca) channels] or ATP-inactivated K^+ channels [K(ATP) channels] (Rorsman and Trube 1986; Kinard *et al* 1999). In contrast, islet recordings show little channel noise. It has been suggested that channel noise is minimized in islets because channel currents are shared by the islet cell population due to gap junctional coupling. Thus, any organized bursting behaviour in single cells could be disrupted by spontaneous channel openings, while channel noise would have little effect on bursting in islets (Atwater *et al* 1983). Indeed, this is one hypothesis why medium bursting is so often observed in islets but not in single cells. Motivated by this hypothesis, numerical simulations were performed with model β cells and electrically coupled βcell clusters, in which bursting was intrinsic to each cell (Chay and Kang 1988; Sherman *et al* 1988). These simulations demonstrated that with clusters of more than 50 cells the effects of channel noise were sufficiently attenuated so that organized bursting behaviour could be expressed by the cell cluster (figure 1). Thus, it appears that gap junctional coupling of βcells both synchronizes bursting activity among coupled cells and attenuates channel noise so that bursting may be expressed.

However, the first published figures of slow bursting in single cells, Smith *et al* (1990) were strikingly free of noise, possibly owing to improved experimental technique. An alternative hypothesis for why single cells do not generally exhibit medium bursting as islets do arose from the observation in βcell models that cells burst only within a narrow parameter window. Outside of this window the model cell is either silent or it spikes continuously. In the simplest cases, one can parametrize the behaviour of a model cell with a single parameter μ (here μ is an abstract parameter, but it can be thought of as a glucose-sensing parameter). When $\mu < \mu_1$ the cell is silent; when $\mu_1 < \mu < \mu_2$ the cell bursts; and when $\mu > \mu_2$ the cell spikes continuously. According to the "heterogeneity hypothesis", single cells rarely burst because the biophysical parameters of the cells rarely fall into the narrow parameter window for bursting (Smolen *et al* 1993). However, when many cells are coupled together, the cluster behaves approximately like a single cell with the mean value of the parameter μ Pernarowski (1998, 1999) has studied this approximation by perturbation methods. Since many isolated cells have $\mu < \mu_1$ (silent cells) and many have $\mu > \mu_2$ (spiking cells), the mean value of μ is likely to fall within the medium bursting window (figure 2).

The heterogeneous nature of single βcells was detailed in the study of Kinard *et al* (1999). They found that approximately half of the 52 cells studied exhibited fast bursting when exposed to stimulatory concentrations of glucose, the other half exhibited other fast oscillatory patterns. A key feature of this study was the use of a "dynamic clamp", a technique in which one uses the membrane potential of the cell to compute artificial ionic currents and then applies these currents to the cell. Significantly, it was shown that if the correct mix of K^+ and Ca^{2+} current is added to a

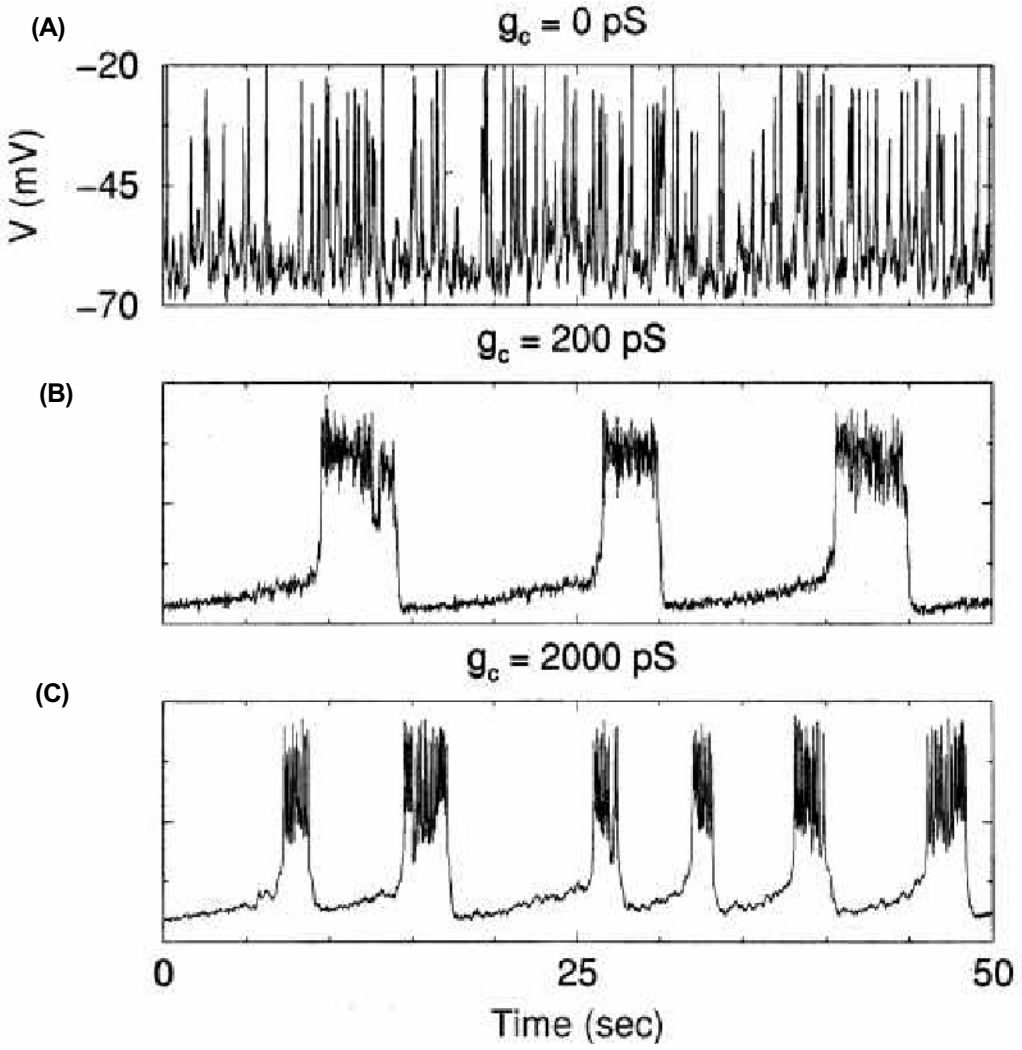

Figure 1. Illustration of channel sharing hypothesis. Simulations of a 5 × 5 × 5 cube of model β-cells with stochastic K(Ca) channels (from Sherman 1996, figure 3). Membrane potential (V) from the middle cell in the cluster is shown. (**A**) When the cells are uncoupled the spike behaviour is erratic. (**B**) At a physiological coupling counductance the effects of channel noise are attenuated and synchronized bursting is expressed. (**C**) Increasing the coupling counductance increases the burst frequency.

fast bursting (class 2) cell, then the burst pattern becomes slower and more similar to that observed in islets. This suggests that if the biophysical parameters of single cells were properly adjusted, then single cell behaviour would be similar to islet behaviour, in support of the heterogeneity hypothesis.

5. Other effects of coupling

In addition to suppressing channel noise, overcoming parameter heterogeneity, and synchronizing electrical activity, coupling has other more subtle effects on the electrical behaviour of islet cells. These coupling effects were uncovered primarily through mathematical modelling and analysis, and serve as predictions that may be tested in the laboratory.

As illustrated in figure 2, coupling tends to synchronize bursts among cells in a cluster, but the spikes within a burst are not synchronized and differ in amplitude except at very large coupling strength. A systematic analysis of the effects of coupling of two identical model βcells (Sherman 1994) showed that at low coupling strengths the spikes generated by the coupled cells are 180° out of phase (an anti-phase oscillation). As the coupling strength is progressively increased, the bursting oscillations go through several bifurcations: first the anti-phase oscillation loses stability and is replaced by quasi-periodic spiking within a burst; next spiking within a burst becomes asymmetric, where the spike amplitudes differ between the cells; finally, when the coupling is very strong the spikes within each burst become synchronized. Chaotic oscillations can arise in the transition between quasi-periodic and asymmetric (deVries *et al* 1998), but this has not been analysed in detail. The burst period of all the out-of-phase oscillations is greater than that of synchronized oscillations since the inhibition of the neighbour cell reduces the spike amplitude. This shifts the homoclinic termination of the burst (HM in figure 5B) to the right and extends the range of values that the slow variable must traverse, increasing the burst period. The quasi-periodic and asymmetric oscillations are more prevalent in clusters of more than two cells (Rinzel *et al* 1992; Smolen *et al* 1993).

The emergence of out-of-phase solutions when cells are coupled provides another mechanism for islets to burst

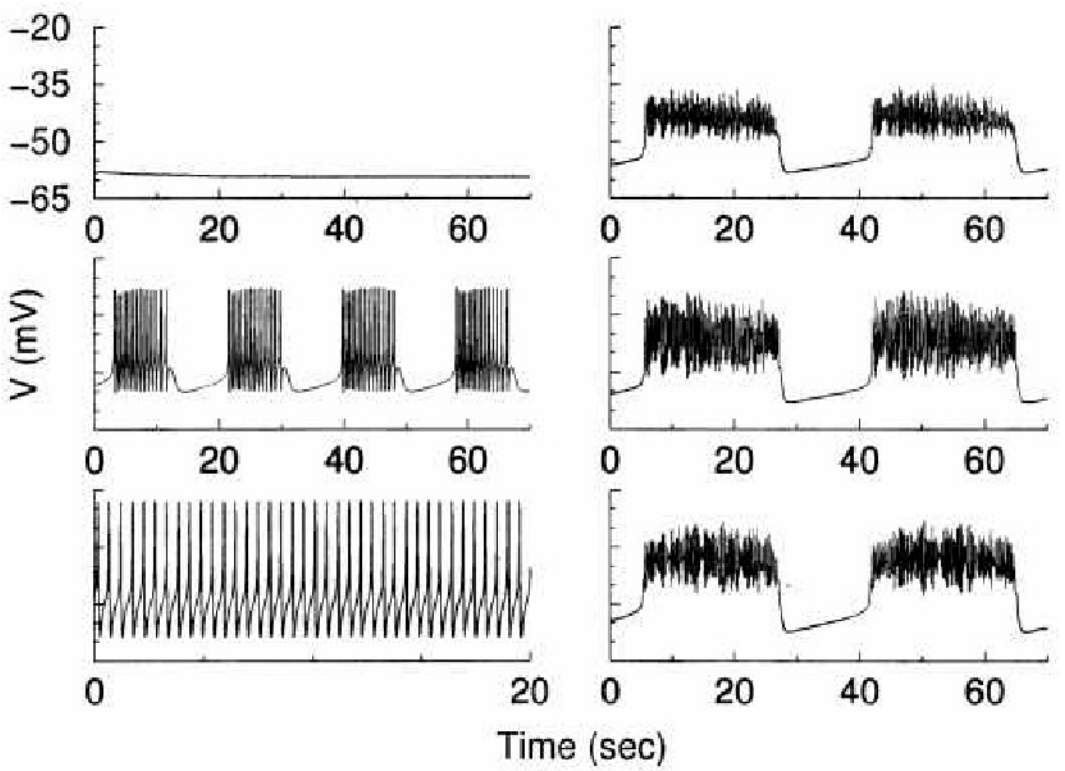

Figure 2. Illustration of heterogeneity hypothesis. Simulations of a 5 × 5 × 5 cube of model β-cells with heterogeneous parameter values (redrawn from Smolen *et al* 1993, figure 2). *Left panels*: Uncoupled cells, only one of which exhibits bursting. *Right panels*: Same cells, but electrically coupled.

even when none of the individual cells can burst when uncoupled. As described in the section, phase resetting, bursting depends on bistability in the fast spiking subsystem. It is easy to eliminate bistability in this class of models by modest parameter variation (e.g. slowing down the voltage-dependent K^+ channels or increasing Ca^{2+} conductance) that increases spike amplitude. This causes the homoclinic orbit HM in figure 5B to move towards the left knee of the Z-curve. When it reaches the knee, the result is a degenerate homoclinic orbit or SNIC (saddle node on an invariant circle) which persists for a significant range of parameter values. Cells in this configuration can beat or remain silent, but cannot burst with a single slow variable (Rinzel 1987; Bertram *et al* 1995a). If two such cells are coupled, however, an out-of-phase solution with smaller amplitude may arise, which has a non-degenerate homoclinic termination on the middle branch of the Z-curve (see figure 10, Sherman 1994). For a small range of coupling strengths, the ensemble will be able to burst. This phenomenon is not very robust, but can be made more robust if noise is introduced into the system (deVries G and Sherman A, manuscript in preparation). Here we illustrate that this phenomenon is also more robust when many cells are coupled.

Figures 3 and 4 show a chain of 201 cells with parameters chosen to eliminate bistability. In the first case, the slow variable is frozen so that only spiking is possible. The initial conditions are identical, except for a few cells in the center. The peripheral cells go into a synchronized large-amplitude beating oscillation, but as the symmetry-breaking wave spreads outward, a non-periodic, possibly quasi-periodic or chaotic, oscillation takes over. The fine structure shows short-range waves of excitation and repolarization going both left and right. Bi-lateral symmetry of the initial conditions keeps the disorder symmetric for aesthetic reasons, but is not a required feature.

In the second case (figure 4), the slow variable is unfrozen, and bursts with non-periodic spiking develop. The long chain permits the ensemble to sustain repetitive bursting even though no individual cell is able to burst. The coupling strength here is more than twice as large as that which will permit such emergent bursting in pairs of cells (Sherman 1994) and can be increased still further. However, the wavelength of the spike waves increases as the coupling strength g_c increases and, for sufficiently large coupling, the chain will exhibit synchronized, periodic beating.

Similar spatial patterns can be generated in two- or three-dimensional clusters of model β cells; occasionally in two dimensions one can see spiral waves spontaneously developing. Examples can be found at: http://mrb.niddk.nih.gov/sherman.

The bursts and spikes in figure 4 are much more irregular than in islets, but the simulations demonstrate that an islet built only out of beating cells (class 1 in the terminology of Kinard *et al* 1999) can burst. One expects that including a mix of other classes would improve the robustness and character of the bursting, but further study, using the newer data on what isolated cells do, is called for.

6. Phase resetting

In most mathematical models of β cells, bursting is produced as the result of bistability in the "fast subsystem", the set of variables that change rapidly and generate voltage spikes. In these models there is a single "slow variable", whose slow activity-dependent variation is responsible for driving the bursting oscillation (figure 5A):

$$\text{Fast subsystem: } \frac{d\vec{F}}{dt} = f(\vec{F}, s)\,.$$

$$\text{Slow variable: } \frac{ds}{dt} = \varepsilon g(\vec{F}, s),\ \varepsilon \ll 1\ ,$$

where $\vec{F}$ is a vector with two or more components, including voltage V, and the functions f and g reflect the ionic currents or other processes, such as metabolism and pumps, included in the model. For some values of the slow variable, s, the fast subsystem has a stable hyperpolarized equilibrium, and for others the fast subsystem has a stable periodic solution. These two states correspond to a silent cell and a spiking cell, respectively. For still other values of s the stable stationary and periodic solutions coexist, so there is a range of values of the slow variable where the fast subsystem is bistable.

The bifurcation structure of the fast subsystem of a generic β cell model is shown in figure 5B. The equilibrium solutions form the z-shaped "slow manifold" (SM). The solutions lose stability at a Hopf bifurcation (HP) and regain stability at a saddle node bifurcation (SN). The branch of periodic spiking solutions is born at the Hopf bifurcation and terminates at an infinite-period homoclinic bifurcation (HM). There is bistability in the fast subsystem for s between s_l and s_h.

The dynamics of bursting become evident when the bursting trajectory and the slow variable nullcline are superimposed onto the fast subsystem bifurcation diagram (figure 5B). When the phase point is on the stable bottom branch of the SM (the silent phase of bursting) it moves to the left, since it is located below the s-nullcline. When the SN is reached, the phase point is attracted to the periodic branch (the active phase of bursting) and moves to the right, since it is now located above the s-nullcline. The active phase ends when the HM is reached, and the cycle starts over.

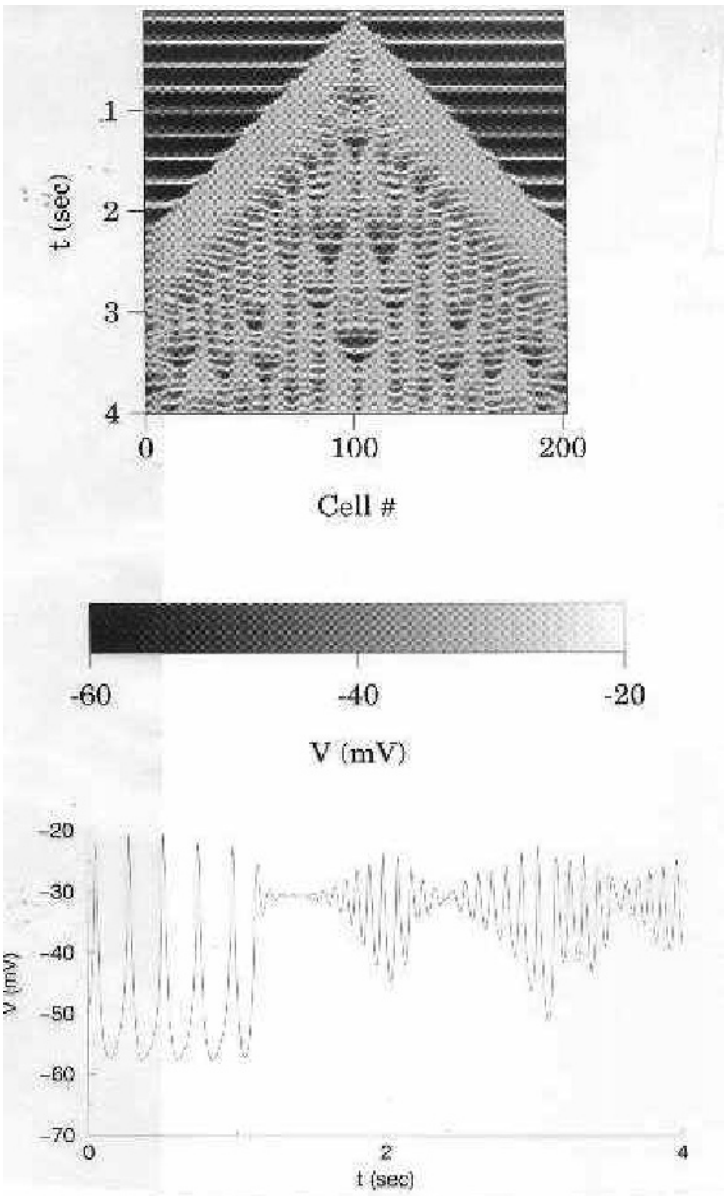

Figure 4. Same as figure 3, but with slow variable unfrozen (τ_s = 35 s) and run for longer time. *Top*: Space–time diagram. *Bottom*: Timecourse of the center cell (# 101).

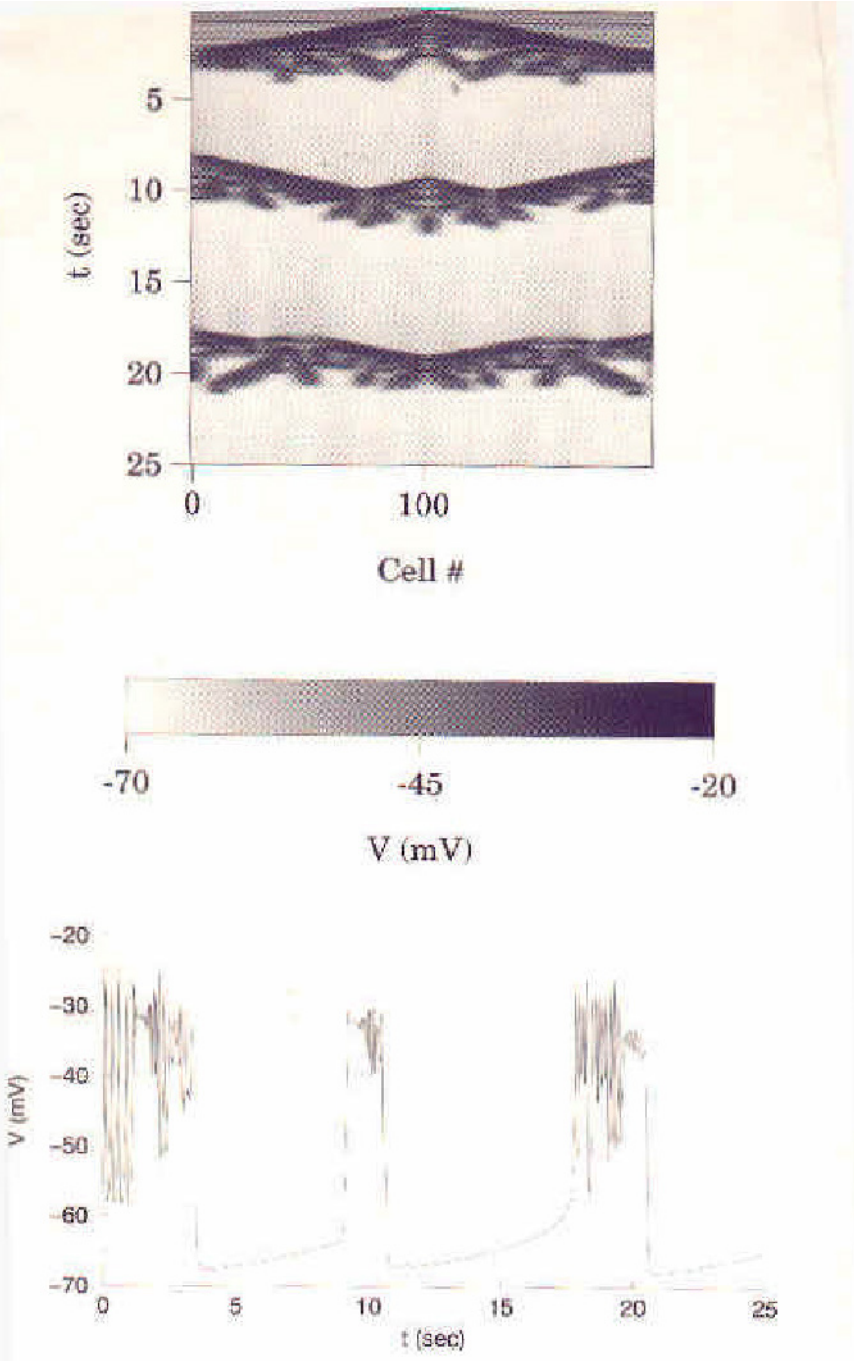

Figure 4. Same as figure 3, but with slow variable unfrozen ($\tau_s = 25$ s) and run for longer time. *Top:* Space–time diagram. *Bottom:* Timecourse of the center cell (# 101).

In this scenario, the bursting oscillation can be reset by a brief voltage perturbation that moves the phase point across the *s*-nullcline from the silent state to the active state, or vice versa. If the perturbation is applied during the silent phase of bursting, this results in a short subsequent active phase, after which the durations of the two phases return to normal. Similarly, if the system is perturbed out of the active phase, the subsequent silent phase is abnormally short.

Unfortunately, this simple behaviour has not been observed in experimental studies. In single cells that exhibit slow bursting or slow oscillations in cytosolic Ca^{2+}, both short and long voltage perturbations (produced by voltage clamping the cell) were unable to reset the bursting (Smith *et al* 1990; Larsson *et al* 1996). This suggests that the dynamics of slow bursting in single cells may not involve bistability of the fast subsystem. This property has not yet been systematically studied, and there may be loopholes. For example, the single cells may really have been clusters, which could not be reset by injecting current into a single cell. If this result holds up under further scrutiny, however, the impact for βcell modelers would be great – it would necessitate a rethinking of the underlying dynamic structure of the bursting oscillation, at least in the case of slow bursting.

Resetting experiments are considerably more difficult to perform in islets, where the voltage perturbation must be applied to all cells simultaneously. Indeed, there has been only one study of resetting in islets, using either a short pulse in extracellular K^+ or a

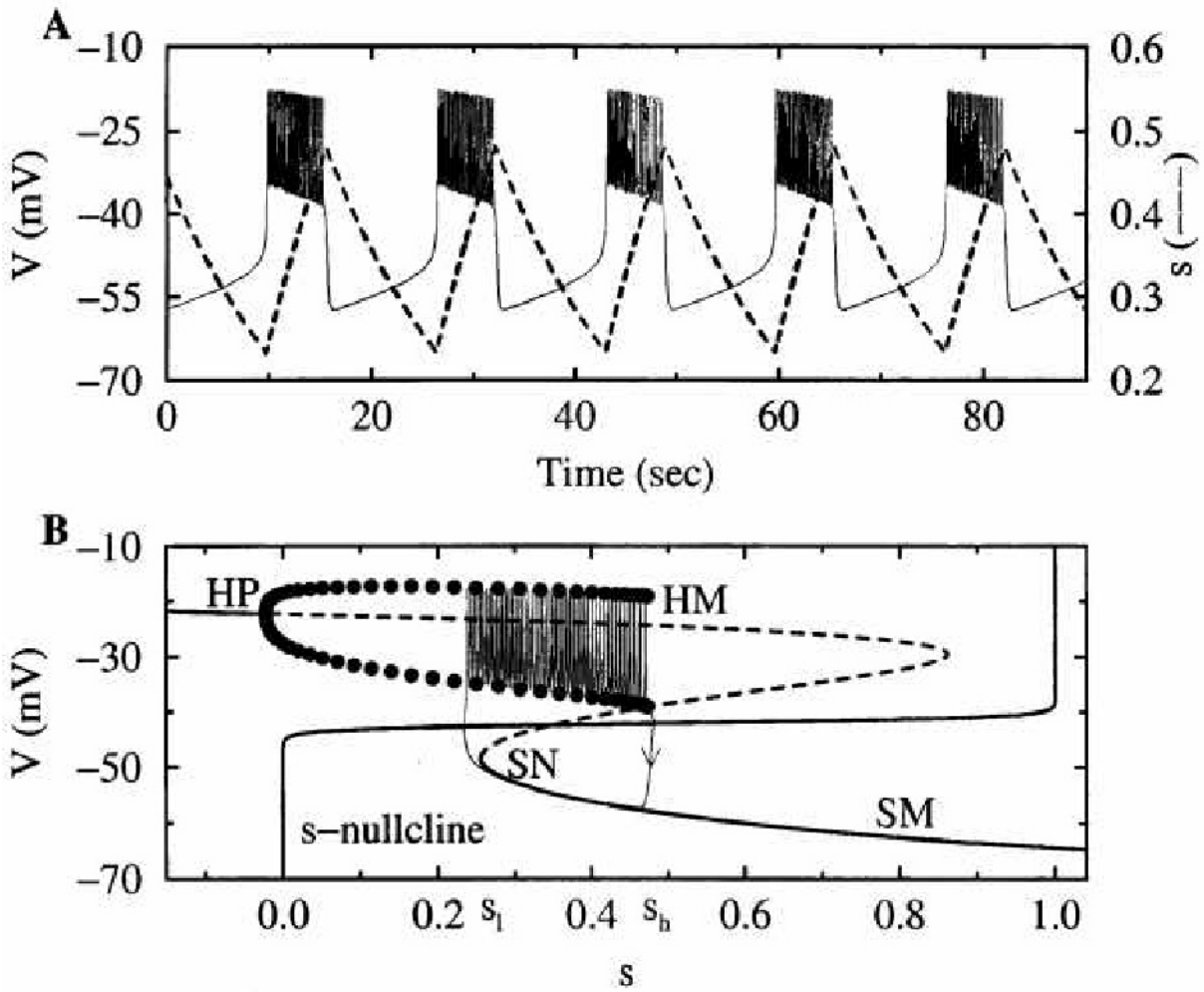

Figure 5. **(A)** Typical bursting oscillation (solid) generated by a β-cell model with a single slow variable. The slow activity-dependent oscillation in the slow variable (dashed) drives the bursting. (**B**) Fast-slow analysis of the bursting oscillation in **A**. The bursting trajectory and the *s*-nullcline are superimposed onto the fast subsystem bifurcation diagram. SM, slow manifold; HP, Hopf bifurcation; HM, homoclinic bifurcation; SN, saddle-node bifurcation; solid, stable; dashed, unstable.

suction electrode to apply a voltage perturbation (Cook *et al* 1981). In both cases, the medium islet bursting was prematurely reset from the silent phase to the active phase of bursting. However, the active phase immediately following the perturbation had a normal duration, not the short duration predicted by β cell models. Using the suction electrode, the islet was also reset from the active phase to the silent phase, but once again the silent phase immediately following the perturbation had a normal duration, contrary to the β cell models. The finding that bursting in the islet resets in a phase-independent manner suggests that for medium islet bursting there is bistability in the fast subsystem, but the slow subsystem dynamics are more complex than in the standard models. Cook *et al* (1981) suggested that the silent and active phases are controlled by separate slow variables. Modelling showed that if the variable responsible for setting the length of the active phase returns to its starting value at the beginning of the silent phase (and vice versa), the active phase (silent phase) duration is the same regardless of how the active phase (silent phase) is initiated (Smolen and Sherman 1994).

7. Islet response to glucose, acetylcholine, and high calcium

The bursting behaviour of islets that we have discussed so far is a steady-state response to a stimulatory concentration of extracellular glucose. A consistent finding is that when a stimulatory concentration of glucose is added to a bath containing little or no glucose there is a transient period of continuous spiking lasting a minute or more. It is only after the termination of this transient activity that the medium bursting pattern appears. This transient phase of continuous spiking, called "phase 1" or "the biphasic", is poorly understood. It has recently been suggested that the first phase may be due to a depolarizing calcium release activated current (I_{CRAC}) that is activated when the endoplasmic reticulum (ER) is depleted of Ca^{2+} (Bertram *et al* 1995b), as would be the case when the cell is hyperpolarized in low glucose. The first phase ends when enough Ca^{2+} has entered the cell through voltage-dependent Ca^{2+} channels to fill the ER. According to this hypothesis, the longer the islet is in low glucose, the longer the duration of the first phase upon glucose application. This prediction was tested experimentally, as were the predictions that maneuvers that increase (decrease) the ER Ca^{2+} concentration would decrease (increase) the duration of the first phase (Mears *et al* 1997). In all but one case, the experimental data were consistent with predictions, supporting the role of ER Ca^{2+} and I_{CRAC} in the biphasic response to glucose. More generally, these studies suggest that the first phase is due to a slow variable other than that responsible for driving bursting, and this slow variable activates a depolarizing current that is turned on when the cell is hyperpolarized.

Another important feature of islet electrical activity is the response to muscarinic agonists such as acetylcholine (ACh) or carbachol. The activity of the endocrine pancreas is regulated by the hypothalamus through both sympathetic and parasympathetic nerves (Woods and Porte 1974). Catecholamines, the neurotransmitters released by sympathetic nerves, eliminate insulin secretion entirely, while β cell bursting persists at a reduced frequency (Ashcroft and Rorsman 1989; Cook and Perara 1982). Acetylcholine, the neurotransmitter released by parasympathetic nerves, potentiates glucose-induced insulin secretion by increasing the frequency of β cell bursting (Henquin *et al* 1988; Santos and Rojas 1989; Bordin *et al* 1995). This "muscarinic bursting" has a period of a few seconds and a relatively depolarized silent phase (Cook *et al* 1981), resulting in an elevated cytosolic Ca^{2+} concentration (Bertram *et al* 1995b), which leads to an enhancement in insulin secretion. Two mechanisms have been suggested for the muscarinic effects. One hypothesis is that ACh directly activates a Na^{+} current (I_{Ach}), depolarizing the β cell and increasing the burst frequency (Miura *et al* 1996). Another hypothesis is that ACh depolarizes the cell through activation of a CRAC current (Bertram *et al* 1995b). That is, ACh binds to muscarinic receptors in the plasma membrane which activate phospholipase C, leading to the production of inositol 1,4,5-trisphosphate (IP_3). This second messenger in turn binds to IP_3 receptors in the ER membrane that allow Ca^{2+} to flow downstream out of the ER. The consequent increase in cytosolic Ca^{2+} transiently *hyperpolarizes* the β cell through activation of Ca^{2+}-activated K^{+} current [$I_{K(Ca)}$]. However, as Ca^{2+} pumps in the plasma membrane remove Ca^{2+} from the cell, the $I_{K(Ca)}$ is deactivated. Simultaneously, the depolarizing I_{CRAC} increases in strength as the ER empties, so that the cell is ultimately depolarized and fast muscarinic bursting is produced.

It may be the case that muscarinic bursting involves both mechanisms described above. Thus, ACh binding to muscarinic receptors directly activates I_{Ach} and indirectly activates I_{CRAC} by dumping the ER Ca^{2+} stores. If I_{Ach} is activated first, because activation of I_{CRAC} involves several intermediate steps, this could explain the typical triphasic response to ACh: a spiking phase, followed by a silent hyperpolarized phase, followed by muscarinic bursting. A mathematical model incorporating both fast activation of I_{Ach} and slower activation of I_{CRAC} reproduces this triphasic response (figure 6). The initial spiking phase following ACh application is due to I_{Ach}, the following silent phase is due to activation of $I_{K(Ca)}$, and the final muscarinic bursting is due to depolarization from I_{CRAC}.

The external Ca^{2+} concentration used in most *in vitro* islet studies is approximately 2· 5 mM. When the external Ca^{2+} concentration is elevated above that level, the medium bursting produced by islets is transformed into a slower bursting pattern and the amplitude of the accompanying Ca^{2+} oscillations is greatly increased (Henquin 1990; Gilon and Henquin 1992). Furthermore, when the external Ca^{2+} concentration is elevated in the presence of glucose sufficiently high to induce continuous firing, the pattern is transformed back into bursting (Gilon and Henquin 1992). One might expect raising the external Ca^{2+} concentration to increase burst frequency because it would increase the Ca^{2+} current. However, Ca^{2+} also could have inhibitory effects through inactivation of Ca^{2+} current and

activation of an $I_{K(Ca)}$ current. A recent model accounts for some of the effects of high external Ca^{2+} in this way because the hyperpolarizing effects outweigh the extra depolarizing Ca^{2+} current (Chay 1997). This model depends on a voltage-dependent inward current, which does not carry Ca^{2+}. Such a current has not been observed experimentally, however, so the question of how external Ca^{2+} affects bursting is still open.

8. Identity of the slow variables

The identity of the slow variable or variables driving fast, medium, and slow bursting is a matter of great interest to many βcell researchers. Is there one slow variable with a highly plastic time constant? If so, why is the time constant such that medium bursting is often observed in islets but never in single cells? Is there more than one slow variable? If so, how do the slow variables interact to preferentially produce medium bursting in islets? Why have researchers been unable to identify a biophysical process with a time constant appropriate for medium bursting?

The first βcell model was based on the hypothesis that the cytosolic Ca^{2+} concentration is the slow variable, influencing the membrane through a Ca^{2+}-activated K^+ current (Atwater *et al* 1980; Chay and Keizer 1983). This mechanism appeared to be ruled out by Ca^{2+} imaging data (Santos *et al* 1991) showing that the cytosolic Ca^{2+} reaches a plateau early in the active phase of a burst, rather than rising slowly throughout the active phase as expected of a slow variable driving medium bursting (figure 5A). The question is not yet fully resolved, as other Ca^{2+} imaging data show timecourses that are more rounded, rather than square (Henquin 1990; Gilon and Henquin 1992). Note also that it is possible for cytosolic Ca^{2+} acting through $I_{K(Ca)}$ to drive the fast bursting found in single cells, and it has been proposed that this is responsible for driving fast muscarinic bursting (Bertram *et al* 1995b).

With the discovery that glucose affects the βcell electrical activity by increasing the ratio of ATP to ADP in the cell, inactivating ATP-sensitive K^+ channels (Ashcroft *et al* 1984), it was natural to suspect that the ATP/ADP ratio is the slow variable driving medium bursting. Indeed, several mathematical models were developed in which the cytosolic Ca^{2+} concentration influences the ATP/ADP ratio on a slow time scale so as to produce a slow activity-dependent oscillation. In these models, the ATP/ADP ratio increases during the silent phase when the Ca^{2+} concentration is low, and decreases during the active phase when the Ca^{2+} concentration is elevated (Keizer and Magnus 1989; Smolen and Keizer 1992). This oscillation in the ATP/ADP ratio generates bursting by decreasing the K(ATP) current [$I_{K(ATP)}$] dur

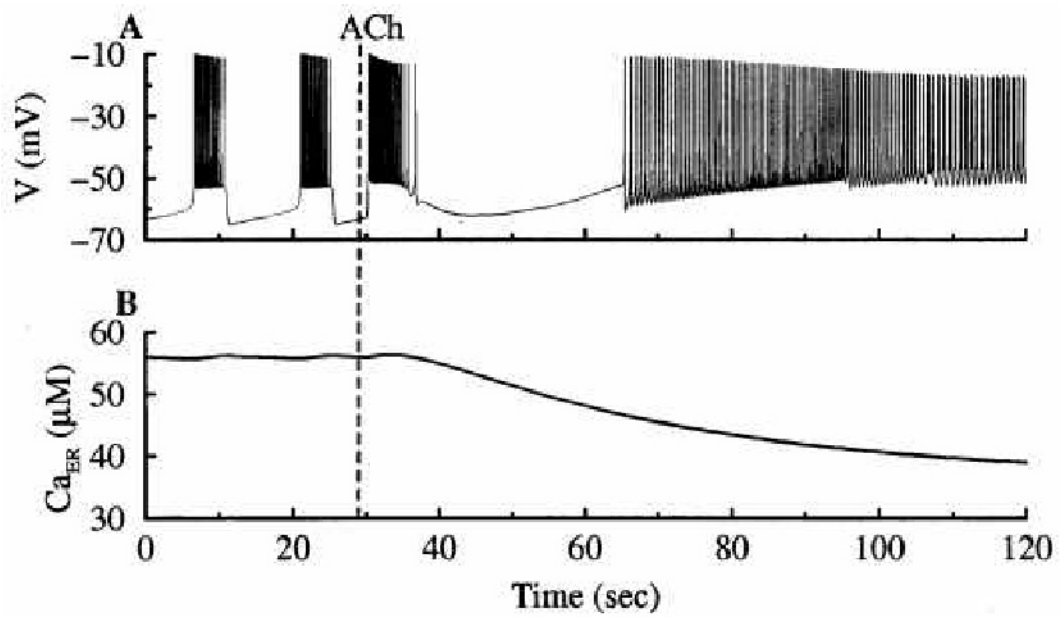

Figure 6. (**A**) The affects of acetycholine (ACh) on glucose-induced bursting simulated with a β-cell model. Rapidly-activated I_{Ach} and a more slowly-activated I_{CRAC} were added to the model from Mears *et al* (1997). (**B**) The ER Ca^{2+} concentration is reduced as the result of production of IP_3. Following ACh application, the IP_3 concentration increases exponentially to an asymptotic value of 0· 25 μM with a time constant of 5 s.

-ing the silent phase and increasing the current during the active phase. However, Rosario *et al* (1993) showed that medium bursting persists in the presence of a K(ATP) channel blocker, arguing against a role for oscillations in $I_{K(ATP)}$ in medium bursting. Recent K(ATP) channel data (Dryselius *et al* 1994; Larsson *et al* 1996) and imaging data of glycolytic intermediaries and oxygen consumption (Longo *et al* 1991; Nilsson *et al* 1996) suggest that the ATP/ADP ratio may indeed oscillate, but on a time scale more appropriate for the generation of slow bursts than for medium bursts.

Another potential slow variable for medium bursting is the voltage-dependent inactivation of Ca^{2+} current, which has a time constant of several seconds (Satin and Cook 1988; Satin *et al* 1994). A slow activity-dependent oscillation in this inactivation variable is the basis of bursting in several mathematical models (Chay and Cook 1988; Keizer and Smolen 1991; Smolen and Keizer 1992; Bertram *et al* 1995b). In these models, the Ca^{2+} current inactivates during the active phase and deinactivates during the silent phase, restarting the burst. With an inactivation time constant of just a few seconds, however, this mechanism seems to be a more likely candidate for fast bursting than for medium bursting.

Finally, it has been suggested that slow activity-dependent oscillations in the Ca^{2+} concentration in the ER ($[Ca^{2+}]_{er}$) drive fast, medium, and slow bursting (Chay 1996, 1997). According to this model, $[Ca^{2+}]_{er}$ declines during the silent phase and rises during the active phase, due to Ca^{2+} influx into the cell through Ca^{2+} channels. The ER Ca^{2+} concentration affects the membrane indirectly through the cytoplasmic Ca^{2+} concentration, which in turn inactivates the Ca^{2+} current. The CRAC current, I_{CRAC}, modifies burst period and membrane potential levels in this model, but is not necessary for bursting. The IP_3-induced Ca^{2+} efflux rate from the ER is the primary controller of burst period; fast, medium, and slow bursting can be obtained by varying the efflux rate from high to low. In terms of the fast-slow analysis described earlier (figure 5B), the slow manifold is stretched when the Ca^{2+} efflux rate from the ER is reduced, so that the distance between the left saddle node and the homoclinic bifurcation is increased, and conversely when the efflux rate is increased. Similar features are found in a recent, simpler model (Gall and Susa 1999; see their model III). Unfortunately, this explanation for slow bursting seems to be at odds with data demonstrating that slow bursting persists in the presence of thapsigargin (Liu *et al* 1995), an agent that blocks ER Ca^{2+} pumps and empties the stores.

Given the many biophysical processes in β cells that are "slow" in some sense, and given the wide range of burst frequencies observed in β cells, we favour a scenario in which two or more slow variables interact to produce bursting. This scenario is the basis of a recent model (Bertram *et al* 2000), in which fast, medium, and slow bursting are produced by the interaction of two slow variables, s_1 and s_2. In this model, s_1 is only moderately slow, with a time constant appropriate for changes in the cytosolic Ca^{2+} or the inactivation of Ca^{2+} current, and s_2 is very slow, with a time constant appropriate for changes in the ATP/ADP ratio or $[Ca^{2+}]_{er}$. The burst period is determined by the conductance of the s_1 current, g_{s1}. When g_{s1} is large the bursting is driven entirely by s_1. Since this variable has a time constant of a few seconds, fast bursting is produced. When g_{s1} is small the bursting is driven entirely by s_2, which has a time constant of a couple of minutes. Thus, slow bursting is produced. Finally, with intermediate values of g_{s1}, oscillations in both s_1 and s_2 are essential for driving bursting, which has a period greater than that for fast bursting, and less than that for slow bursting. This medium bursting is novel in that no single biophysical process with a time constant appropriate for medium bursting is required, which could explain why no such process has been observed after several decades of experimentation.

9. Conclusion

Pancreatic β cells have very rich dynamics that both intrigue and frustrate β cell researchers. The islet structure in which β cells are found make it difficult to analyse the dynamics of the individual cell, while removal of the cell from the islet significantly alters the cell's behaviour. Sometimes the cells burst with a high frequency, sometimes with a low frequency, and sometimes with a medium frequency (but only when in an islet). The resetting behaviour of isolated β cells and β cells in islets defy all of the physiologically-based β cell models. Adding to the complexity, there appears to be a great deal of heterogeneity among β cells, and mathematical studies have shown that this adds robustness to endogenous bursting patterns when cells are electrically coupled. Perhaps most frustrating is that despite several decades of investigation, the identity of the slow process or processes driving medium bursting in β cells is still not known. The slow and fast modes of bursting observed over the past few years introduce additional unidentified slow processes that must now be identified. Fortunately, all this complexity ensures a role for mathematical modelling for many years to come.

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