Bursting and calcium oscillations in pancreatic β-cells: specific pacemakers for specific mechanisms

L. E. Fridlyand, N. Tamarina, and L. H. Philipson

Department of Medicine, University of Chicago, Chicago, Illinois

Submitted 18 March 2010; accepted in final form 9 July 2010

Fridlyand LE, Tamarina N, Philipson LH. Bursting and calcium oscillations in pancreatic β-cells: specific pacemakers for specific mechanisms. Am J Physiol Endocrinol Metab 299: E517–E532, 2010. First published July 13, 2010; doi:10.1152/ajpendo.00177.2010.—Oscillatory phenomenon in electrical activity and cytoplasmic calcium concentration in response to glucose are intimately connected to multiple key aspects of pancreatic β-cell physiology. However, there is no single model for oscillatory mechanisms in these cells. We set out to identify possible pacemaker candidates for burst activity and cytoplasmic calcium oscillations in these cells by analyzing published hypotheses, their corresponding mathematical models, and relevant experimental data. We found that although no single pacemaker can account for the variety of oscillatory phenomena in β-cells, at least several separate mechanisms can underlie specific kinds of oscillations. According to our analysis, slowly activating Ca2+-sensitive K+ channels can be responsible for very fast Ca2+ oscillations; changes in the ATP/ADP ratio and in the endoplasmic reticulum calcium concentration can be pacemakers for both fast bursts and cytoplasmic calcium oscillations, and cyclical cytoplasmic Na+ changes may underlie patterning of slow calcium oscillations. However, these mechanisms still lack direct confirmation, and their potential interactions raises new issues. Further studies supported by improved mathematical models are necessary to understand oscillatory phenomena in β-cell physiology.

IN PANCREATIC β-CELLS, glucose-stimulated insulin secretion is mediated by an elevated cytosolic free calcium concentration ([Ca2+]c). This results from calcium influx through voltage-dependent Ca2+ channels (VDCCs) located in the plasma membrane (PM), which open in response to secretagogues, primarily glucose. Stimulation-secretion coupling in β-cells is different from most other cell types, because instead of being mediated by receptor binding, glucose must be transported into the cytoplasm and metabolized.

Glucose initiates changes in the PM potential via an increase in the cytoplasmic ATP/ADP ratio derived from glycolysis and oxidative phosphorylation. This results in closure of ATP-sensitive K+ (KATP) channels. Closure of these channels leads to PM depolarization up to a threshold potential, causing the cell to move from quiescence to initiate electrical activity and VDCC opening. Ca2+ influx through VDCCs leads to increased [Ca2+]c, which is a key signal in the initiation of insulin secretion from the pancreatic β-cells. Consensus mechanisms of glucose-induced PM, cytosolic, and mitochondrial processes are summarized in Fig. 1 (for recent review, see Refs. 71, 74, 76, and 140).

The β-cell membrane is hyperpolarized to a resting potential of about −60 mV at low glucose levels (~3–6 mM). Dean and Matthews (32, 33) showed that when glucose rises the PM depolarizes and then generates an electrical activity organized into slow depolarizing waves, called bursting, with a plateau from which action potentials (APs) rapidly fire. Bursts (or active phases) are separated by quiescent (resting) periods at potentials below the AP threshold (Fig. 2). This bursting process results from the metabolic processes and electrical activity of ion channels localized on the β-cell PM and generates as long as the glucose concentration is elevated. Rapid depolarization at the beginning of a burst activates VDCCs, whereas the fast repolarization at the end of a burst results in inactivation of VDCCs. This leads to synchronized cyclical spike-burst activity and corresponding cytoplasmic Ca2+ oscillations in response to a rise in extracellular glucose that ultimately determine the oscillatory nature of insulin secretion (71, 76, 140).

Increased glucose concentration induces several types of cyclical spike-burst activity and [Ca2+]c oscillations in β-cells with period ranges from several seconds to several minutes (11, 19, 71). We have divided these oscillations into several classes: 1) bursts and [Ca2+]c oscillations with a period of several seconds we term “ultrafast”; 2) oscillations with a period from 10 to 60 s, denoted as fast (Fig. 2A); 3) Ca2+ oscillations with a period ranging from 1 to several minutes and long bursts (Fig. 2B), designated as slow; and 4) mixed (or compound) [Ca2+]c oscillations, characterized by ultrafast or fast [Ca2+]c oscillations superposed on slow oscillations; this is due to periodic bursts, called compound bursting (Fig. 2, C and D) (17).
Ultrafast oscillations can be observed in isolated single β-cells (91, 150). Fast electrical bursting and cytoplasmic Ca^{2+} oscillations are usually observed in isolated mouse islets (11, 57). Slow and mixed cytoplasmic Ca^{2+} oscillations were found in single cells, β-cells clusters, and isolated islets at stimulating glucose concentrations (10, 11, 57, 70, 99). Slow and mixed oscillations most likely constitute a physiological oscillatory pattern in β-cells (57, 140).

Gap junctions are responsible for tight electrical coupling at least in mouse islets. This leads to the synchronization of bursting and calcium oscillations in an islet (12, 94, 144). For this reason, as a first approximation we can consider the islet as a community of coupled β-cells with similar electrical behavior.

Experimental data and theoretical concepts support the idea that the AP bursts and the corresponding [Ca^{2+}]_c oscillations reflect a periodic depolarization of the PM (7, 11, 37, 48). Whereas a depolarizing component predominates at the beginning of a burst, a progressive increase in outward and/or decrease in inward cation currents take place during the burst, leading to a repolarization and burst termination. Slow depolarization in a resting (silent) phase leads again to a burst initiation (7, 48). Experimental data and theoretical analysis agree that, after the large depolarizing effect following K_{ATP} channel closure, the subsequent generation and termination of the bursts can be maintained by small cyclical changes of any PM current (9, 48, 132, 133).

Consequently, initiation and termination of bursts can be connected directly with activation and deactivation of different electrogenic channels, pumps, or exchangers, which can be modulated in turn by numerous intracellular components. Therefore, it is not surprising that a variety of different proposals and corresponding mathematical models have been advanced, underlining the nature of these components in the β-cells. A list of the compounds to be considered here is shown in Table 1. Several of these modulators can act simultaneously, generating AP bursts and corresponding [Ca^{2+}]_c oscillations. However, the exact physiological variables and their underlying mechanisms that drive bursting or Ca^{2+} oscillations in β-cells remain uncertain. Here, we attempt to identify the possible pacemaker candidates for burst activity and cytoplasmic Ca^{2+} oscillations in the pancreatic β-cells.

We define a pacemaker as a component of some pacemaker mechanism that serves to establish a rhythmic physiological activity inside a cell. To distinguish likely pacemaker proposals from less likely hypotheses, we will reject a hypothesis that some component is a part of a pacemaker mechanism if Ca^{2+} oscillations (or the AP bursting pattern) persist even though the proposed pacemaker mechanism was blocked in an experiment. We will term this condition the main criterion for the rejection of a proposed pacemaker mechanism. Disappearance or a change of bursting or Ca^{2+} oscillation behavior under the influence of an agent or process will we term a “modulation,” and an agent that creates it we will term a “modulator.”

A pacemaker component can also act as a modulator with a certain concentration dependence, depending on its activity or experimental conditions. However, a modulator in this article is a component that is not a part of a proposed pacemaker mechanism.
mechanism. Several examples of such pacemakers and modulators will be considered.

We performed an analysis of the existing hypothesis, experimental data, and the corresponding mathematical models and compared the simulations with the experimental results. In this way we tested both the validity of the initial hypotheses and the models. Furthermore, predictions for unmeasured quantities could be made, and the model could then be tested. However, many of the mechanisms that could control electrical activity and Ca\(^{2+}\) handling have not been adequately experimentally characterized in pancreatic \(\beta\)-cells, so we can consider them only briefly. Although most of the analyzed experimental work employed mouse islets with some studies in other species, it is difficult to generalize given the different existing animal and insulinoma models of pancreatic \(\beta\)-cells. We have emphasized primary tissues where possible.

There are several reasons why a clear understanding of internal oscillatory mechanisms in \(\beta\)-cells is worth pursuing. First, bursting and \([Ca^{2+}]_c\) oscillations are readily observable phenomena in cell culture and in isolated mouse islets. Numerous hormones and small molecule agonists as well as toxins can modulate oscillatory behavior. The study of the changes in bursting or \([Ca^{2+}]_c\) oscillations can provide a flexible approach to understand the action of specific modulators on the pancreatic \(\beta\)-cell. However, this approach requires a comprehensive knowledge of the pacemaker mechanisms of these oscillations.

We consider here primarily the internal pacemaker mechanisms for individual \(\beta\)-cells that represent behavior of isolated cells or the electrically synchronized islet. A further consideration of the oscillations in insulin secretion is beyond the scope of the present article. However, islet Ca\(^{2+}\) oscillations appear to be the framework behind the phenomenon of pulsatile insulin release observed in vivo (71, 112, 117, 140). Interestingly, disruptions in rhythmic function may be an important early biomarker of islet dysfunction leading to diabetes in animal models (72, 79, 140) and man (116). Although pulsatility appears to be a natural function of islets both in vivo and in vitro, this emphasizes the need for careful study of the oscillatory phenomena on the cellular level.

**EXPERIMENTAL PROCEDURES AND MODELING**

Ca\(^{2+}\) imaging measurements of relative cytoplasmic Ca\(^{2+}\) concentrations are presented as the 340/380 nm fluorescence excitation ratio obtained with the indicator fura-2, as described previously (137). Fura-2 was from Invitrogen (Carlsbad, CA). All other chemicals were from Sigma (St. Louis, MO).

The “complex model” of processes (51) was used for the following simulations, excluding the model for slowly activated Ca\(^{2+}\)-sensitive K\(^{+}\) channels, that are described in the APPENDIX. This model is available for direct simulation on the web site “Virtual Cell” (www.nrcam.uchc.edu) in the “MathModel Database” on the “math workspace” in the library “Fridlyand” with name “Chicago 1.”

**RESULTS AND DISCUSSION**

We can distinguish two main types of mechanisms underlying bursting and \([Ca^{2+}]_c\) oscillations.

**Mechanisms With Ca\(^{2+}\) Feedback Effect**

In this case, a fast increase of \([Ca^{2+}]_c\) during an active phase should activate (or deactivate) some processes, leading to slow changes in the intermediate pacemaker components. Slow changes of these components should gradually lead to a membrane repolarization down to the threshold potential for spike activity, shutting down the spikes and inactivating the VDCCs. Then Ca\(^{2+}\) influx decreases sharply, leading to decreased PM potential and a resting phase. Processes occur in opposite directions during a resting (repolarized) phase. The \([Ca^{2+}]_c\) decreases because cytoplasmic Ca\(^{2+}\) is pumped into the extra-cellular space and into the endoplasmic reticulum (ER) by specific ATP-consuming Ca\(^{2+}\) pumps. Decreases in \([Ca^{2+}]_c\), deactivate (or activate) specific processes of the slow changes in the intermediate pacemaker components, leading to PM depolarization up to the threshold potential, reentry of spike activity, Ca\(^{2+}\) influx, and an active phase.

**Table 1. Candidates for pacemakers regulating \(\beta\)-cell bursting and \([Ca^{2+}]_c\) oscillations**

<table>
<thead>
<tr>
<th>Compound or Process</th>
<th>Reference No(s.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca(^{2+})-dependent K(^{+}) current</td>
<td>8, 26</td>
</tr>
<tr>
<td>Slowly activated Ca(^{2+})-sensitive K(^{+}) current</td>
<td>58, 59</td>
</tr>
<tr>
<td>Voltage-gated Ca(^{2+}) current</td>
<td>28, 88</td>
</tr>
<tr>
<td>[ATP]/[ADP] ratio</td>
<td>3, 126, 133</td>
</tr>
<tr>
<td>Glycolytic oscillations</td>
<td>17, 19, 142, 145</td>
</tr>
<tr>
<td>([Ca^{2+}]_c) in endoplasmic reticulum</td>
<td>20, 25, 51, 125, 148</td>
</tr>
<tr>
<td>Intracellular Na(^{+})</td>
<td>51, 93</td>
</tr>
<tr>
<td>Inositol (1,4,5)-trisphosphate</td>
<td>This article</td>
</tr>
<tr>
<td>cAMP</td>
<td>47</td>
</tr>
<tr>
<td>Mitochondrial metabolic oscillations</td>
<td>14, 101</td>
</tr>
</tbody>
</table>

\([Ca^{2+}]_c\), cytosolic free calcium concentration.
Ca\textsuperscript{2+}-Independent Mechanisms

This type of mechanism was suggested for oscillations such as glycolytic oscillations that can lead to oscillations in the [ATP]/[ADP] ratio changing the conductance of K\textsubscript{ATP} channels (19, 142). In this case, Ca\textsuperscript{2+}-independent mechanisms create the oscillations of compounds that can lead to cyclic changes in PM potential, generating the bursts and corresponding Ca\textsuperscript{2+} oscillations (101, 142).

[Ca\textsuperscript{2+}]c as the Pacemaker Component and Ca\textsuperscript{2+}-Dependent K\textsuperscript{+} Channels

Fast-activated Ca\textsuperscript{2+}-dependent K\textsuperscript{+} (SK1–3 and BK) channels. Initially, a mechanism with [Ca\textsuperscript{2+}]c as the pacemaker component and a Ca\textsuperscript{2+} feedback effect was proposed for burst generation and [Ca\textsuperscript{2+}]c oscillations via cyclic activation of a Ca\textsuperscript{2+}-dependent K\textsuperscript{+} current. According to this hypothesis, Ca\textsuperscript{2+} influx during the active phase would cause a slow rise in [Ca\textsuperscript{2+}]c, which activates fast Ca\textsuperscript{2+}-dependent K\textsuperscript{+} (K\textsubscript{Ca}) channels repolarizing the PM potential down to a threshold potential. This would shut down AP spiking and inactivate VDCCs, leading to a resting phase. A decrease in the [Ca\textsuperscript{2+}]c level during the resting period would lead to decreased current through K\textsubscript{Ca} channels, PM depolarization, and burst initiation. According to this proposal, a cyclical change in [Ca\textsuperscript{2+}]c is a candidate islet pacemaker for burst behavior and [Ca\textsuperscript{2+}]c oscillations per se (8). Corresponding mathematical models have incorporated the cyclical activation and deactivation of K\textsubscript{Ca} channels by bursting-induced changes in [Ca\textsuperscript{2+}]c (26).

The principle difficulty with this hypothesis is that [Ca\textsuperscript{2+}]c should slowly increase during a period of depolarization, leading to a slowly increasing current through K\textsubscript{Ca} channels and then to a repolarization only at the end of a burst period. However, subsequent Ca\textsuperscript{2+} imaging data indicate that the time for the [Ca\textsuperscript{2+}]c increase is short relative to the oscillation period (129). Another problem with this hypothesis was that apamin, a K\textsubscript{Ca} (SK1–3) channel blocker, does not change mouse islet bursting or alter β-cell K\textsuperscript{+} currents in normal mouse islets (4, 58, 59, 96). Slow [Ca\textsuperscript{2+}]c oscillations were also observed in the presence of 10–20 mM tetraethylammonium (TEA), i.e., in conditions when TEA-sensitive K\textsubscript{Ca} channels should be nearly completely blocked (124, 125). For these reasons, this mechanism for generation of [Ca\textsuperscript{2+}]c oscillation was ruled out (see Refs. 125 and 132). Despite these issues, mathematical models for β-cells continue to appear with slow [Ca\textsuperscript{2+}]c oscillations on the basis of this hypothesis (36, 108). However, although here we focus on pacemaker mechanisms, some changes in channel conductance may change PM polarization such that oscillation behavior can be modified. For this reason, although K\textsubscript{Ca} (SK1–3 and BK) channels do not directly take part in the creation of the pacemaker mechanism, their inhibition can contribute to PM depolarization and lead to changes in the oscillatory behavior (139).

Slow-activated Ca\textsuperscript{2+}-sensitive K\textsuperscript{+} channels. A specific, slowly activated TEA-insensitive Ca\textsuperscript{2+}-sensitive K\textsuperscript{+} current (SK-like current) may underlie the mechanisms of oscillations (58, 59). This current activates gradually with a time constant of 2.3 s and inactivates with a time constant of 6.5 s (59). It was assumed that this current regulates the duration of intervals between successive AP bursts observed when β-cells are exposed to moderately elevated glucose (59, 151).

We developed a special mathematical model to simulate this proposal (see APPENDIX). Here, fast K\textsubscript{Ca} channels (SK1–3 and BK; current I\textsubscript{KCa} in Fig. 1) as well as slowly activated K\textsubscript{Ca} channels (SK-like current; I\textsubscript{KCa} in Fig. 1), which according to Düfer et al. (38) can be represented partly by SK4 channels, were modeled, and the time constants for activation gating variables reported by Gopal et al. (59) were used for modeling of I\textsubscript{KCa}. The behavior of [Ca\textsuperscript{2+}]c, V\textsubscript{m}, and the I\textsubscript{KCa} is shown in Fig. 3. The reversible slow cyclical changes in I\textsubscript{KCa} were obtained, leading to bursting behavior and [Ca\textsuperscript{2+}]c oscillations. The simulated [Ca\textsuperscript{2+}]c increased rapidly in the beginning of burst period, stabilized by activation of the fast K\textsubscript{Ca} (SK1–3) channels and kept at a high level during this period. I\textsubscript{KCa} increased during a burst period, leading to repolarization, termination of the spikes, and transition to a silent phase. Inhibition of I\textsubscript{KCa} during a resting period led to repolarization and reentry to an active phase.

However, the simulated bursts and [Ca\textsuperscript{2+}]c oscillations had a period of several seconds and corresponded well only to ultrafast bursting and [Ca\textsuperscript{2+}]c oscillations. Therefore, the experimental data on the dynamics of this slow SK-like current and our simulation support the idea that ultrafast bursts and Ca\textsuperscript{2+} oscillations can be driven by an interaction of these slow-activated K\textsubscript{Ca} channel parameters with [Ca\textsuperscript{2+}]c changes.

Cyclic Changes in K\textsubscript{ATP} Channel Conductance: The Pacemaker Role of [ATP]/[ADP] Ratios

K\textsubscript{ATP} channels contribute the predominant β-cell K\textsuperscript{+} conductance in resting low-glucose conditions. Following glucose entry and metabolism, their conductance is greatly diminished. However, the small remaining K\textsubscript{ATP} conductance in elevated glucose is still comparable with the conductances of other channels, exchangers, and pumps (133). For this reason, cyclical changes in the K\textsubscript{ATP} channel conductance have been
proposed as a possible mechanism underlying oscillatory behavior of β-cells (3, 68, 110, 126, 133). In this conceptualization, the [ATP]/[ADP] ratio decreases slowly when [Ca\(^{2+}\)]\(_{c}\) increases during a burst, leading to a slow opening of K\(_{ATP}\) channels and subsequent PM repolarization as the K\(^+\) efflux increases. Actually, the opposite should occur since the [ATP]/[ADP] ratio gradually increases during a silent phase in the presence of stimulatory glucose, when [Ca\(^{2+}\)]\(_{c}\) decreases, leading to the closing of K\(_{ATP}\) channels, PM depolarization, and burst initiation (34, 86, 87, 103–105, 126). According to our classification, this is a mechanism with a Ca\(^{2+}\) feedback effect, whereas the [ATP]/[ADP] ratio is a pacemaker “compound.”

Several mechanisms and corresponding mathematical models for the [ATP]/[ADP] ratio changes and [Ca\(^{2+}\)]\(_{c}\) oscillations have been proposed. An empirical equation for [ATP]/[ADP] ratio changes was introduced in an early mathematical model (133) such that [ATP]/[ADP] ratio slowly decreased as [Ca\(^{2+}\)]\(_{c}\) increased and with decreased [Ca\(^{2+}\)]\(_{c}\). Oscillatory changes in the [ATP]/[ADP] ratio, K\(_{ATP}\) channel conductance, bursting, and [Ca\(^{2+}\)]\(_{c}\) oscillations were simulated using this model (17, 18, 133).

Mechanistic hypotheses underlying the possible influence of [Ca\(^{2+}\)]\(_{c}\), changes on the [ATP]/[ADP] ratio have also been proposed. Decreased [ATP]/[ADP] ratio during [Ca\(^{2+}\)]\(_{c}\) rise and an increase of this ratio with [Ca\(^{2+}\)]\(_{c}\) decline could result from either stimulated ATP hydrolysis or inhibition of ATP production.

[Ca\(^{2+}\)]\(_{c}\)-activated ATP consumption. Ca\(^{2+}\) activates Ca\(^{2+}\) pumps on the PM and in the ER as well and certain other intracellular reactions that use ATP. In this case, increased [Ca\(^{2+}\)]\(_{c}\), can increase ATP consumption and decrease the [ATP]/[ADP] ratio. An opposite process that increases the [ATP/ADP] ratio due to a decline in ATP consumption can also occur with decreased [Ca\(^{2+}\)]\(_{c}\) (34, 51).

We illustrated this mechanism using our mathematical model (51) that includes a simulation of ATP consumption due to the work of Ca\(^{2+}\) pumps (PM and ER) as well as the Ca\(^{2+}\)-activated ATP consumption in some cytoplasmic processes. The simulated phase relations for the conditions when the free ADP (and corresponding ATP) concentration in the cytoplasm is the only slow pacemaker parameter in this complex model are shown in Fig. 4. Other slow parameters such as cytoplasmic concentrations of Na\(^+\), inositol 1,4,5-trisphosphate (IP\(_3\)), and Ca\(^{2+}\) in ER ([Ca\(^{2+}\)]\(_{ER}\)) were frozen at constant levels, blocking other possible mechanisms underlying Ca\(^{2+}\) oscillations in this model. A Ca\(^{2+}\) feedback effect model was responsible for bursting and Ca\(^{2+}\) oscillations; a rise in [Ca\(^{2+}\)]\(_{c}\) during the active phase leads to increased Ca\(^{2+}\)-activated ATP consumption in the cytoplasm and by Ca\(^{2+}\) pumps. As this occurs, free [ADP] increases (and the [ATP]/[ADP] ratio decreases), slowly opening K\(_{ATP}\) channels. This in turn leads to a slowly increasing \(I_{\text{KATP}}\) and PM depolarization, damping potential spikes and leading to decreased [Ca\(^{2+}\)]\(_{c}\). These processes have the opposite direction after decreased [Ca\(^{2+}\)]\(_{c}\) in the resting period. K\(_{Ca}\) channels serve to damp depolarization and terminate the [Ca\(^{2+}\)]\(_{c}\) increase after initial Ca\(^{2+}\) increase during the active phase.

Negative [Ca\(^{2+}\)]\(_{c}\) effects on ATP production. In several mathematical models the uptake of Ca\(^{2+}\) by β-cell mitochondria has large energy-dissipative effects, leading to decreased oxidative phosphorylation suppressing the production rate of ATP (15, 103–105). This proposal also leads to a decreased [ATP]/[ADP] cytoplasmic ratio with increased [Ca\(^{2+}\)]\(_{c}\), as in first case, and the models can simulate [Ca\(^{2+}\)]\(_{c}\) oscillations through the cyclical changes in [ATP]/[ADP] ratio (103–105). However, recent experimental data convincingly shows that “the primary role of mitochondrial Ca\(^{2+}\) is the stimulation of oxidative phosphorylation” (24), suggesting that [Ca\(^{2+}\)]\(_{c}\) effects on ATP production are stimulatory rather than inhibitory.

Glycolytic oscillations as a mechanism for cyclical changes in the [ATP]/[ADP] ratio. A number of hypotheses have been proposed to explain the mechanism of [ATP]/[ADP] ratio oscillations through glycolytic oscillations. The fundamental proposal of this model is that Ca\(^{2+}\)-independent glycolytic oscillations that produce [ATP]/[ADP] ratio oscillations are mediated by the positive feedback of fructose 1,6-bisphosphate. This is the product of the glycolytic enzyme phosphofructokinase (PFK), stimulating PFK activity and subsequently depleting substrate (83, 142). Mathematical models for β-cell oscillations have been constructed on the basis of this mechanism (17–19, 81, 145).

However, the direct evidence for glycolytic oscillations in β-cells such as measurements of glycolytic metabolite concentrations is lacking. Periodic variations in mitochondrial membrane potential, NADH, and respiration have been offered as evidence of glycolytic oscillations (18). However, these phe-
nomina can also be a consequence of independent Ca\(^{2+}\) cytoplasmic oscillations (see below). The available evidence supports the idea that glycolytic processes are not a requirement at least for slow cytoplasmic Ca\(^{2+}\) oscillations. Slow [Ca\(^{2+}\)]\(_e\) oscillations can be induced at low glucose levels using K\(_\text{ATP}\) and K\(_\text{ATP}\) channel blockers (such as TEA and sulfonyleureas) in islets and β-cell lines (see Fig. 5 and Refs. 51 and 125). Slow [Ca\(^{2+}\)]\(_e\) oscillations can be generated even in the absence of glucose using such mitochondrial substrates as leucine (64), α-ketoisocaproic acid (69, 106), and methyl pyruvate (69). Fast [Ca\(^{2+}\)]\(_e\) oscillations were found in islets incubated solely in the presence of glyceraldehyde or α-ketoisocaproic acid (31). PFK itself is not required for these oscillations, since [Ca\(^{2+}\)]\(_e\) oscillations remain normal after 95–98% suppression of PFK-M activity in mouse islets (121). These data demonstrate that an oscillatory capacity is preserved when glycolytic flux is small or absent and that the slow components of [Ca\(^{2+}\)]\(_e\) oscillations cannot be fully explained by mechanisms incorporating glycolytic oscillations. However, we cannot entirely rule out that this mechanism could contribute to ultrafast (and possibly fast) [Ca\(^{2+}\)]\(_e\) oscillations. If K\(_\text{ATP}\) channel conductivity are unlikely to function as a pacemaker component for coupled slow oscillatory mechanisms.

However, there are several valid arguments against a K\(_\text{ATP}\) channel-dependent mechanism for slow bursting and [Ca\(^{2+}\)]\(_e\) oscillations. If K\(_\text{ATP}\) channels function as a pacemaker, the conductance of the K\(_\text{ATP}\) channels should oscillate during bursting electrical activity following changes in the [ATP]/[ADP] ratio. However, effective K\(_\text{ATP}\) channel blockers such as sulfonyleureas do not stop slow bursting and Ca\(^{2+}\) oscillations in pancreatic islets and can even induce them in single β-cells or β-cell clusters isolated from mouse islets (see Fig. 5 and Refs. 43, 65, and 109) and in βTC3-neo cells (125). Slow [Ca\(^{2+}\)]\(_e\) oscillations were also found in a mouse lacking functional K\(_\text{ATP}\) channels (40, 67, 120, 135). These results suggest that changes in the [ATP]/[ADP] ratio and, consequently, in K\(_\text{ATP}\) channel conductivity are unlikely to function as a pacemaker component for coupled slow oscillatory mechanisms.

However, studies are needed to carefully examine whether fast Ca\(^{2+}\) oscillations persist upon blocking K\(_\text{ATP}\) channels at low glucose levels or in K\(_\text{ATP}\) channel-null mice. Until such studies are reported, it is possible that ultrafast and fast bursting and the corresponding [Ca\(^{2+}\)]\(_e\) oscillations are in part due to [ATP]/[ADP] ratio and K\(_\text{ATP}\) channel conductance changes acting as a pacemaker.

Intracellular [Na\(^{+}\)] As a Slow Component in Pacemaker Mechanisms

Several components regulate Na\(^{+}\) dynamics in β-cells. The most important is the electrogenic Na\(^{+}\)/K\(^{+}\)-ATPase that extrudes three Na\(^{+}\) ions in exchange for two K\(^{+}\) ions for each molecule of ATP hydrolyzed, generating a net outward flow of cations through the PM. This pump is highly active, since it determines the high cytoplasmic K\(^{+}\) concentration (113). Like most other cells, the β-cells are also equipped with a PM Na\(^{+}\)/Ca\(^{2+}\) exchanger, an electrogenic transporter that couples the exchange of 3 Na\(^{+}\) for 1 Ca\(^{2+}\). Consequently, changes in the cytoplasmic Na\(^{+}\) concentration ([Na\(^{+}\)]\(_c\)) lead to changes of inward and outward currents and the PM potential. Changes in [Na\(^{+}\)]\(_c\) can have a significant effect on generation of slow cytoplasmic Ca\(^{2+}\) oscillations in β-cells (56, 93).

For example, Kitasato et al. (93) proposed a specific oscillatory mechanism, including Na\(^{+}\) pumps and transporters. They suggested that increased [Ca\(^{2+}\)]\(_e\) during an active phase results in an activation of the Na\(^{+}\)/Ca\(^{2+}\) exchanger and a corresponding increase in [Na\(^{+}\)]\(_c\). An increase in cytosolic Na\(^{+}\) would lead to an enhanced ATP consumption at Na\(^{+}\)/K\(^{+}\) pump sites and to a decreased ATP/ADP ratio. This in turn could lead to activation of K\(_\text{ATP}\) channels followed by membrane repolarization. A decrease in [Ca\(^{2+}\)]\(_c\) results in the opposite order of events during a silent phase. This hypothesis was modeled by Miwa and Imai (110) as the underlying mechanism of [Ca\(^{2+}\)]\(_e\) oscillations. This mechanism is a variant of K\(_\text{ATP}\) channel-dependent mechanism for a generation of bursts and [Ca\(^{2+}\)]\(_e\) oscillations. It does not explain the experimental data, suggesting that the [ATP]/[ADP] ratio and K\(_\text{ATP}\)
channel conductance changes are not the main driving force for slow [Ca$^{2+}$]$_c$ oscillations (see above).

We proposed and modeled a mechanism with a Ca$^{2+}$ feedback effect where the intracellular [Na$^+$]$_c$ is a dynamic pacemaker variable that can govern bursts and slow [Ca$^{2+}$]$_c$ oscillations, although K$_{ATP}$ channels do not change their activity (51). Figure 6 illustrates the roles of the Na$^+$,K$^+$-ATPase, the Na$^+$/Ca$^{2+}$ exchanger, and [Na$^+$]$_c$ dynamics in this model. Other slow parameters such as cytoplasmic concentrations of [ADP], [Ca$^{2+}$]$_{ER}$, and [IP$_3$] were frozen at constant levels, blocking other possible mechanisms of bursting and [Ca$^{2+}$]$_c$ oscillations. The following mechanism was responsible for bursting and Ca$^{2+}$ oscillations; increased [Ca$^{2+}$]$_c$ during an active period activates Na$^+$ influx through Na$^+$/Ca$^{2+}$ exchangers. The resultant increase in intracellular [Na$^+$]$_c$ leads to a slow increase in outward current through electrogenic Na$^+$/K$^+$ pumps ($I_{NaK}$ in Fig. 1), with corresponding plasma membrane repolarization, so that the cell reenters a silent phase. Decreased [Ca$^{2+}$]$_c$ during a silent phase leads to a slow decrease in [Na$^+$]$_c$, because Na$^+$ influx through the Na$^+$/Ca$^{2+}$ exchanger decreases. This leads to a decreased outward current through Na$^+$/K$^+$ pumps and then in turn to PM depolarization and a generation of bursts and [Ca$^{2+}$]$_c$ oscillations.

The effect of modulators leads to changes in the shape of simulated [Ca$^{2+}$]$_c$ oscillations, bringing them closer to experimental results. For example, Fig. 7 (which reproduces the third figure from our earlier article (51)) shows slow-simulated [Ca$^{2+}$]$_c$ oscillations that are similar to the experimental data represented in Fig. 2B. In this simulation, as opposed to that in Fig. 6, [ADP], [Ca$^{2+}$]$_{ER}$, and [IP$_3$] were not frozen at constant levels and thus played a role as modulators. In this case, cytoplasmic [Na$^+$] as a slowly variable component functioned as the pacemaker mechanism. We will discuss the possible mechanisms of this kind of influence of modulators on the shape of Ca$^{2+}$ oscillators below.

Some indirect experimental evidence supports the hypothesis that cyclic [Na$^+$]$_c$ changes can act as a pacemaker to induce bursting and slow [Ca$^{2+}$]$_c$ oscillations. For example, in isolated individual β-cells or cell clusters, the slow glucose-induced Na$^+$ oscillations were found under conditions known to induce oscillations of [Ca$^{2+}$]$_c$. Partial suppression of the Na$^+$/K$^+$ pump by ouabain resulted in an increased amplitude of slow Na$^+$ and Ca$^{2+}$ oscillations with decreased frequency; slow Na$^+$ and Ca$^{2+}$ oscillations were converted to a tonically elevated Na$^+$ and Ca$^{2+}$ level following ouabain inhibition of the Na,K-ATPase (62, 63). Interestingly, slow Ca$^{2+}$ oscillations were converted to a sustained elevated Ca$^{2+}$ level following ouabain even in sulfonylurea type 1 receptor (SUR1)-null mouse islets, where K$_{ATP}$ channels were blocked (39).

Our computational simulation resulted in a similar picture (see the 7th figure in Ref. 51) showing that these experiments can be modeled exactly by a partial inhibition of the Na$^+$/K$^+$ pumps by ouabain. According to this simulation, decreasing the Na$^+$/K$^+$ pump maximal activity reduced the rate of Na$^+$ efflux and corresponding net outward current, leading to increased [Na$^+$]$_c$ and PM depolarization. This resulted in increased
amplitude of slow Na\(^+\) and Ca\(^{2+}\) oscillations with decreased frequency. Greater inhibition of the Na\(^+/K\(^{+}\) pump maximal activity led to a collapse of this Na\(^+/\)dependent mechanism, the disappearance of bursting and slow Ca\(^{2+}\) oscillations, and tonically elevated [Ca\(^{2+}\)]\(_{c}\).

Veratridine causes voltage-dependent Na\(^+\) channels to stay open during a sustained membrane depolarization by decreasing inactivation (143). Partial activation of Na\(^+\) channels by veratridine resulted in a decreased amplitude and increased frequency of slow Ca\(^{2+}\) oscillations, and slow Ca\(^{2+}\) oscillations were converted to a tonically elevated Ca\(^{2+}\) level in isolated individual β-cells or cell clusters (44). We were able obtain similar results in isolated pancreatic islets (Fig. 8).

We simulated the effects of veratridine by shifting the half-activation potential for Na\(^+\) channels toward a more positive voltage using our general model (51). According to this simulation (Fig. 9), increased Na\(^+\) influx through voltage-dependent Na\(^+\) channels leads to an additional PM depolarization as a result of increased Na\(^+\) inward current and an accelerated Na\(^+\) accumulation during an active phase. This resulted in increased amplitude and frequency of slow Na\(^+\) and Ca\(^{2+}\) oscillations. The simulations are in reasonably good agreement with experimental data (compare Figs. 8 and 9).

Correlating the model simulations with experimental data shows that cyclic [Na\(^+\)]\(_{c}\) changes can act as a pacemaker to induce bursting and slow [Ca\(^{2+}\)]\(_{c}\) oscillations as opposed to the proposals on the role of K\(_{ATP}\) channels. However, this mechanism underlying oscillations remains incompletely investigated. Simultaneous measurements of [Ca\(^{2+}\)]\(_{c}\) and [Na\(^+\)]\(_{c}\), although difficult, can directly test this hypothesis.

**Role of the ER and [Ca\(^{2+}\)]\(_{ER}\) as a Slow Pacemaker**

The ER is a high-affinity and high-capacity organelle for calcium storage. The β-cell ER sequesters Ca\(^{2+}\) when the cytoplasmic calcium level is high and releases it when [Ca\(^{2+}\)]\(_{c}\) is low or under the influence of intracellular messengers (55, 122, 123, 141). Ca\(^{2+}\) from the cytosol is transported into the ER by P-type ATPases using ATP and exits through two types of ER Ca\(^{2+}\) channels, the IP\(_3\) receptor (IP\(_3\),R) channels and the ryanodine receptor channels (Fig. 1) (56, 82, 123, 138). However, Ca\(^{2+}\) influx from the extracellular space through VDCCs is the principal regulator of cytoplasmic Ca\(^{2+}\) homeostasis in β-cells under physiological conditions (6, 51, 127, 146).

[Ca\(^{2+}\)]\(_{ER}\) and store operating currents. Changes in [Ca\(^{2+}\)]\(_{ER}\) may regulate PM potential. Depletion of intracellular Ca\(^{2+}\) stores in β-cells activates a special Ca\(^{2+}\) release-activated current [or store operating current (SOC)] through nonselective PM cation channels and can represent an inward cation current, leading to PM depolarization that potentiates glucose-induced Ca\(^{2+}\) influx through VDCCs (20, 30, 97, 125, 147). This current may be Na\(^+\) influx through nonselective PM cation channels, which have been described in insulin-secreting β-cells (30, 97, 125). Transient receptor potential (TRP) ion channels were proposed for this role (74, 78, 125). Although the detailed mechanism for coupling of ER Ca\(^{2+}\) store depletion with the nonselective PM channels activation is uncertain, it likely involves translocation of STIM1 and STIM2 proteins from the ER calcium stores to the PM, where they interact with ORAI1 and related proteins associated with cation influx channels (119, 137).

Several mechanisms of oscillations with [Ca\(^{2+}\)]\(_{ER}\) as a pacemaker employing a Ca\(^{2+}\) feedback effect have been proposed (20, 25, 51, 53, 107, 125, 148, 150). These proposals were primarily based on the characteristic properties of SOC that lead to cell depolarization with decreased [Ca\(^{2+}\)]\(_{ER}\). Several mathematical models have also been developed for these proposals using empirical equations for activation of SOC with decreased [Ca\(^{2+}\)]\(_{ER}\) (20, 25, 53, 150). SOC increased with decreased [Ca\(^{2+}\)]\(_{ER}\) and vice versa in simulations using the empirical equations from these models, yielding periodical changes of [Ca\(^{2+}\)]\(_{ER}\), bursting, and [Ca\(^{2+}\)]\(_{c}\) oscillations.

We developed a model where “SOC” includes Na\(^+\) influx through nonselective cation channels that open following de-
creases in $[\text{Ca}^{2+}]_{\text{ER}}$ (SOC was termed “CRAN current” in our model) (51), following the earlier work of Roe et al. (125).

The proposed mechanism is illustrated in Fig. 10. In this case, other possible pacemaker mechanisms of $[\text{Ca}^{2+}]_{i}$ oscillations in the model (51) were blocked by freezing the cytoplasmic concentrations of [ADP], [Na$^+$], and [IP$_3$] at constant levels. In this simulation, a rapid rise in $[\text{Ca}^{2+}]_{i}$ at the beginning of the active phase led to increased Ca$^{2+}$ pumping into the ER and slow $[\text{Ca}^{2+}]_{\text{ER}}$ accumulation with corresponding closure of PM nonselective cation channels. This decreased inward Na$^+$ influx current ($I_{\text{SOC}}$; Fig. 1) through these channels (i.e., SOC) resulted in PM repolarization, termination of the spiking, and the transition to a silent phase. Slowly decreasing $[\text{Ca}^{2+}]_{\text{ER}}$ during a silent phase as a result of Ca$^{2+}$ leak through ryanodine or IP$_3$R then led to increased nonselective cation channel conductance, increased inward Na$^+$ current, and PM depolarization. This resulted in subsequent activation of VDCCs and a new burst. In this case, $[\text{Ca}^{2+}]_{\text{ER}}$ is a slow pacemaker component for $[\text{Ca}^{2+}]_{i}$ oscillations with a Ca$^{2+}$ feedback effect.

However, an explanation of slow oscillations on the basis of $[\text{Ca}^{2+}]_{\text{ER}}$-dependent mechanisms involves several difficulties, since it seems to be at odds with data demonstrating that the slow oscillations can persist in the presence of thapsigargin, a SERCA inhibitor (Fig. 5 and Refs. 6, 51, 55, 98, 109, and 136). Slow $[\text{Ca}^{2+}]_{i}$ oscillations also persist in islets from a SERCA3 knockout mouse, where fast oscillations disappear (11). These results argue against a role of $[\text{Ca}^{2+}]_{\text{ER}}$ changes as a pacemaker for slow $[\text{Ca}^{2+}]_{i}$ oscillations.

Slow $[\text{Ca}^{2+}]_{i}$ oscillations can also persist even though $K_{\text{ATP}}$ channels and SERCA activity are blocked simultaneously in mouse islets by tolbutamide and thapsigargin (Fig. 5) or in isolated islets from the SUR1-null mouse lacking $K_{\text{ATP}}$ channels when SERCA is inhibited (39). These complex experimental data show that a mechanism of slow $[\text{Ca}^{2+}]_{i}$ oscillations cannot be explained solely by an interaction of $K_{\text{ATP}}$ channels and ER-dependent mechanisms.

SERCA blockade has led to conflicting results for fast bursts and $[\text{Ca}^{2+}]_{i}$ oscillations in β-cells converted to a sustained elevated $[\text{Ca}^{2+}]_{i}$ level by thapsigargin (55, 86, 109, 136). However, Tamarina et al. (138) found that an addition of thapsigargin was accompanied by a gradual increase in the amplitude and frequency of fast $[\text{Ca}^{2+}]_{i}$ oscillations in some islets. Bertram et al. (20) had previously found that thapsigargin induced a gradual increase in the amplitude plateau fraction and in electrical burst frequency in the β-cell membrane potential. It is possible that in some experiments only partial SERCA blockade was obtained, leading to a partial decrease in $[\text{Ca}^{2+}]_{\text{ER}}$, thereby preserving the ability of the β-cells to create an oscillatory mechanism. More profound SERCA block in other experiments could decrease $[\text{Ca}^{2+}]_{\text{ER}}$ to a threshold level where oscillatory mechanisms with $[\text{Ca}^{2+}]_{\text{ER}}$ as a pacemaker are suppressed. This suggests that the mechanism discussed above in which $[\text{Ca}^{2+}]_{\text{ER}}$ is the pacemaker component could be responsible for fast bursting and $[\text{Ca}^{2+}]_{i}$ oscillations.

Recently, null mice for one of the TRP ion channels (Trpm5) were found to have islets that had lost the ability to create fast $[\text{Ca}^{2+}]_{i}$ oscillations, whereas slow $[\text{Ca}^{2+}]_{i}$ oscillations were intact (27). The disappearance of fast $[\text{Ca}^{2+}]_{i}$ oscillations in this case is consistent with the hypothesis that Trpm5 channels can be a part of the SOC (or CRAN) mechanism with Na$^+$ influx (75, 118) that is in part responsible for the fast, not slow, $[\text{Ca}^{2+}]_{i}$ oscillation (see above).

$[\text{Ca}^{2+}]_{\text{ER}}$ changes can regulate the shape of slow glucose-induced $[\text{Ca}^{2+}]_{i}$ oscillation in pancreatic β-cells (11, 99). Our simulations also support the hypothesis that slow $[\text{Ca}^{2+}]_{i}$ oscillation frequency and the generation of the waveform can be modulated by changes in the rates of Ca$^{2+}$ leak from the ER, although $[\text{Ca}^{2+}]_{\text{ER}}$ does not account for the pacemaker mechanism for slow oscillations per se (51, 138). For example, Fig. 7 (reproduced from Ref. 51) shows the changes in the shape of $[\text{Ca}^{2+}]_{i}$ oscillations during simulation of SERCA block that lead to a decrease in $[\text{Ca}^{2+}]_{\text{ER}}$. This is at conditions where the changes in $[\text{Na}^+]_i$, drive the pacemaker mechanism for slow $[\text{Ca}^{2+}]_{i}$ oscillations. In this case, Ca$^{2+}$ is gradually released into the cytosol, and a decrease in $[\text{Ca}^{2+}]_{i}$ can be slow at the beginning of the silent period of slow oscillations (Fig. 7). When $[\text{Ca}^{2+}]_{\text{ER}}$ is small after SERCA inhibition, no additional Ca$^{2+}$ release occurs during the silent period of slow oscillations, and the $[\text{Ca}^{2+}]_{i}$ decrease is sharp. A similar result was obtained experimentally (51, 55).

Pacemaker due to $[\text{Ca}^{2+}]_{\text{ER}}$ regulated by IP$_3$. In pancreatic β-cells, as well as in other cell types, external agonists can act on G protein-coupled receptors to stimulate phospholipase C (PLC) activity. Ca$^{2+}$-activated PLC catalyzes the formation of IP$_3$ from phosphatidylinositol-4,5-bisphosphate (Fig. 1) (23). The resulting rise in IP$_3$ initiates Ca$^{2+}$ discharge from the ER through IP$_3$R channels (13, 56, 138). IP$_3$ can also activate K$^+$ or Na$^+$ conductances that can lead to PM repolarization (5, 134).

Changes in IP$_3$ can play a crucial role in creating Ca$^{2+}$ oscillations through activation of the ER IP$_3$ receptor in non-electrically excitable types of cells (13, 130). Several mathe-
matical models employed this mechanism for nonelectrically excitable cells. The first step is that a \([\text{Ca}^{2+}]\), increase is caused by \(\text{Ca}^{2+}\) release from the ER. One intracellular signal for this release is IP3, which triggers \(\text{Ca}^{2+}\) release by binding to the IP3Rs on the surface of the ER. This opens ER \(\text{Ca}^{2+}\) channels, allowing \(\text{Ca}^{2+}\) to flow out into the cytoplasm. Subsequent inactivation of the IP3Rs and removal of \(\text{Ca}^{2+}\) by ER membrane pumps then reduces the \(\text{Ca}^{2+}\) concentration to lower levels. It can initiate a repeating cycle of release and uptake of \(\text{Ca}^{2+}\) from the ER, resulting in \(\text{Ca}^{2+}\) oscillations if the IP3 concentration is in the correct range (13, 130). However, \(\beta\)-cells are electrically excitable cells, and as discussed above the changes in PM potential and corresponding \(\text{Ca}^{2+}\) influx from the extracellular space through VDCCs may be the principal regulator of cytoplasmic \(\text{Ca}^{2+}\) homeostasis in \(\beta\)-cells. For this reason, IP3\(_s\) and IP3R interactions are not likely to function as a pacemaker for \([\text{Ca}^{2+}]\) oscillations in \(\beta\)-cells.

We recently investigated the dynamics of IP3 in mouse primary \(\beta\)-cells and in MIN6 mouse insulinoma cells upon stimulation with glucose and a muscarinic agonist (carbamoylcholine) (138). We found that IP3 concentrations in the cytoplasm oscillated in parallel with \([\text{Ca}^{2+}]\) oscillations upon stimulation with glucose. Although this could be interpreted as evidence supporting IP3 oscillations as a pacemaker for \(\text{Ca}^{2+}\) oscillations, this case IP3 oscillations can be readily explained by \(\text{Ca}^{2+}\)-induced activation of PLC leading to IP3\(_s\) changes, as simulated in our model (Fig. 7E). This suggests that slow cyclical changes in IP3 concentration may follow from the activation of PLC by independent cyclical \([\text{Ca}^{2+}]\) changes; i.e., IP3 may not be a pacemaker for \([\text{Ca}^{2+}]\) oscillations (51, 138). However, this problem needs further investigation, perhaps by PLCK inhibition, to determine the existence of \([\text{Ca}^{2+}]\) oscillations in the absence of IP3 oscillations.

However, the rate of IP3 production can have a dramatic effect on oscillations even though IP3 is not a pacemaker compound; i.e., IP3 can be a strong modulator of oscillatory processes. For example, activation of IP3 synthesis can cause a transformation of slow oscillations into fast ones (20, 138). Although the mechanism is unclear, it may be due to additional PM depolarization following from the opening of SOC or the recently found NALCN channels (134) with decreased \([\text{Ca}^{2+}]\)\(_{\text{ER}}\), or this could follow from activation of PM K\(^+\) channels by IP3.

**cAMP as a Pacemaker Component**

The cAMP pathway has \([\text{Ca}^{2+}]\)-dependent components such as some isoforms of adenyl cyclase and phosphodiesterases (PDEs) (Fig. 1) (29, 60). cAMP may also increase \(\text{Ca}^{2+}\)-induced \(\text{Ca}^{2+}\) release from the ER through ryanodine and IP3Rs in mouse pancreatic \(\beta\)-cells and may activate some PM channels via phosphorylation or protein binding (66, 84, 85, 131). The suggestion was made for several types of cells that \([\text{Ca}^{2+}]\) oscillations could result from interactions between cAMP and \([\text{Ca}^{2+}]\)\(_{\text{EC}}\) (61, 102). Therefore, an oscillatory mechanism with \(\text{Ca}^{2+}\) feedback effect and \([\text{cAMP}]\) as a slow pacemaker component could also occur in \(\beta\)-cells.

However, recent experimental data does not support the hypothesis that cAMP is a pacemaker component for an oscillatory mechanism, at least for slow \([\text{Ca}^{2+}]\) oscillations in \(\beta\)-cells. For example, in insulinoma \(\beta\)-cell lines a PDE inhibitor (IBMX) abolished cAMP oscillations without inhibiting \([\text{Ca}^{2+}]\) oscillations (see the 5th figure in Ref. 95). Dynchok et al. (42) showed that IBMX can stimulate \([\text{Ca}^{2+}]\) oscillations even during elevated, nonoscillating cAMP levels, measured using real-time imaging. For this reason, according to our rules for hypothesis rejection (see introduction), we believe that cAMP oscillations are not part of a pacemaker mechanism, at least for slow \([\text{Ca}^{2+}]\) oscillations. We recently analyzed oscillatory behavior of cAMP using our model of cAMP dynamics and found that slow \([\text{Ca}^{2+}]\) oscillations are a pacemaker for cAMP oscillations in pancreatic \(\beta\)-cells and not the other way around (47).

However, cAMP (as well as IP3) may modulate the dynamics of the intracellular processes and PM potential, leading to the changes in the oscillation pattern. For example, both IBMX and forskolin, an activator of adenyl cyclase, increase the level of cAMP, transforming \([\text{Ca}^{2+}]\) oscillations from slow into fast and further increasing their frequency (10). Other agents that increase the concentration of cAMP, such as glucagon, also promote the appearance of fast \([\text{Ca}^{2+}]\) oscillations (99). The underlying mechanisms of these transformations remain an open question but can be partially explained by altering PM depolarization.

**The Pacemaker Role of Metabolic Oscillations**

Metabolic cycles can control glucose-stimulated insulin secretion (80), and a number of hypotheses have implicated metabolic oscillations in determining \(\text{Ca}^{2+}\) oscillations (101, 142). Oscillations of a metabolic signal in \(\beta\)-cells may result from intrinsic properties of various pathways, although glycolytic oscillations are not likely to account for the mechanism of other metabolic oscillations (see above). We cannot rule out that some \(\text{Ca}^{2+}\)-independent mechanism can create oscillations of compounds that can change the PM potential, leading to \([\text{Ca}^{2+}]\) oscillations. For example, based on the existence of citrate oscillations in isolated islet mitochondria, it was proposed that there is an independent mitochondrial oscillator and that the exported citrate may feed back and coordinate mitochondrial and cytosolic oscillations (101). Several mathematical models of possible mitochondrial oscillatory mechanisms were discussed recently (14), and these should be testable in \(\beta\)-cells.

Oscillations of the metabolic signals in \(\beta\)-cells may be secondary to oscillations in \([\text{Ca}^{2+}]\). As was pointed out by Kennedy et al. (89), an appearance of metabolic oscillations does not prove that metabolic variations are the real pacemakers of oscillations, because these metabolic changes can indeed reflect the \([\text{Ca}^{2+}]\) oscillations. For example, variations in \([\text{Ca}^{2+}]\) can change ATP consumption because the rate of work of ATP-consuming \(\text{Ca}^{2+}\) pumps depends on \(\text{Ca}^{2+}\) concentrations. Using our mathematical model for \(\beta\)-cell processes (49, 50), we found that cyclic [ATP] and [ADP] oscillations in the cytoplasm due to the oscillations in \([\text{Ca}^{2+}]\) can stimulate the oscillations in metabolic processes such as oxygen consumption, the mitochondrial inner membrane potential, the rate of ATP production in mitochondria, and NAD(P)H levels in \(\beta\)-cells, i.e., in the oscillating processes that were found in experiments using \(\beta\)-cells (89, 92, 100, 111). We can also expect oscillations in metabolism if specific metabolic enzymes can be modulated by cyclic \(\text{Ca}^{2+}\) concentration changes in cytoplasm or mitochondria. For this reason, the oscillations...
in metabolic variables mentioned above are most likely secondary and likely follow rather than precede bursting and \([\text{Ca}^{2+}]_c\) oscillations.

Activation or Deactivation of the Voltage-Dependent Channels As a Pacemaker Component

As we pointed out in the Introduction, the cyclic activation and deactivation of several channels can underlie mechanisms that drive bursting and \([\text{Ca}^{2+}]_c\) oscillations in β-cells. Here, we consider other possible suggestions related to regulation of ion channels.

For example, it was suggested that PM repolarization and termination of spikes could occur during a burst if some parameters of the voltage-gated \([\text{Ca}^{2+}]_c\) current could be slowly changed, leading to a decreased current (28). On the other hand, a regenerative recovery from inactivation and reactivation of \([\text{Ca}^{2+}]_c\) currents in the rest period could lead to a depolarization and a beginning of a new burst. This mechanism for burst generation requires mathematical modeling of time-dependent changes in maximal conductance or other coefficients in the equations for VDCC (88). However, this mechanism for the changes in \([\text{Ca}^{2+}]_c\) current also lacks sufficient experimental support.

Similarly, we can suggest that slow activation of any \(K^+\) currents (other than current through \(K_{\text{ATP}}\) and slow \(K_{\text{Ca}}\) channels, which we have considered above) in the active period can lead to repolarization and burst termination. A regenerative deactivation of these currents in the silent interval can lead to depolarization and to a beginning of a burst. However, the available experimental evidence argues against this hypothesis, at least for slow \([\text{Ca}^{2+}]_c\), oscillations. For example, stimulation of βTC3-neo or MIN6 insulinoma cells with glucose results only in a modest nonscillatory increase in \([\text{Ca}^{2+}]_c\). However, subsequent addition of TEA (15–20 mM, sufficient to substantially suppress most non-\(K_{\text{ATP}}\) \(K^+\) channels) can induce fast and slow \([\text{Ca}^{2+}]_c\) oscillations (41, 136, 139) so that inhibiting most \(K^+\) channels activates rather than suppresses \([\text{Ca}^{2+}]_c\) oscillations. Slow bursting and \([\text{Ca}^{2+}]_c\) oscillations are not lost in mouse islets lacking \(K_v 2.1\) channels (77). According to our rule for rejection, this indicates that \(K_v 2.1\) channels are not a part of a pacemaker mechanism, but they do have important modulating effects.

Interactions of Mechanisms and Compound Patterns of Bursting and \([\text{Ca}^{2+}]_c\) Oscillations

Fast and slow modes of oscillation can occur together, as in the mixed pattern, or separately, as in the fast and slow patterns (Fig. 2; see also Refs. 19 and 31). This strongly argues that they stem from distinct mechanisms. These mechanisms can be linked reciprocally and often occur together and can also proceed largely independently of each other. Mixed oscillations also occur in isolated cells, suggesting that this peculiar pattern does not result from the sum of signals produced in distinct β-cells in islets (6).

Modeling approaches show that compound bursting and corresponding mixed \([\text{Ca}^{2+}]_c\) oscillations can be simulated. The results of the modeling favor a scenario where two or more slow variables interact to produce complex oscillations. For example, Bertram and colleagues (17, 18) simulated the ultrafast and fast oscillations by small variations in \(K_{\text{ATP}}\) conductance using the empirical equation connecting the [ATP]/[ADP] ratio with \([\text{Ca}^{2+}]_c\) changes. The slow \([\text{Ca}^{2+}]_c\) oscillations were simulated as metabolic glycolytic oscillations such that the two interact to produce compound oscillations consisting of episodes of bursts separated by long periods of silence, or “accordion” oscillations, that consist of fast bursts with a slowly modulated duty cycle. Similar behavior could be achieved by defining the different slow and fast pacemaker variables (16). For example, slow \([\text{Ca}^{2+}]_c\) oscillations in a compound picture may be a result of a \([\text{Ca}^{2+}]_\text{ER}\) dependent mechanism, and a \(K_{\text{ATP}}\)-dependent mechanism may be responsible for fast \([\text{Ca}^{2+}]_c\) oscillations on the top of the slow (35). According to our analysis, it is most likely that \([\text{Na}^+]_c\) can represent a slow-changing compound for slow oscillations and changes in the [ATP/ADP] ratio and/or \([\text{Ca}^{2+}]_\text{ER}\) for fast oscillations.

Modulators of Oscillation Behavior and External Pacemaker Mechanisms

Changes in channel conductance or \([\text{Ca}^{2+}]_c\) handling can change PM polarization or \([\text{Ca}^{2+}]_c\) redistribution. This, in turn, may lead to changes in bursting and \([\text{Ca}^{2+}]_c\) oscillations even though the cellular components do not participate directly in pacemaker oscillatory mechanisms. For example, activation of anion channels may cause depolarization of the β-cell PM (21, 22). Glucose stimulation of β-cells is coupled to an increase in \(Cl^-\) permeability, and oscillatory \([\text{Ca}^{2+}]_c\) signaling may be critically dependent on transmembrane \(Cl^-\) fluxes (45). This indicates that agents that regulate anion channels may modulate oscillations. Cyclic nucleotide-gated (HCN) channels may also have a modulating effect because they can influence β-cell PM potential (152). External insulin can also be considered a modulator because it may be able to activate \(K_{\text{ATP}}\) channels (90).

Cyclical changes in external modulators can create or synchronize the alterations in PM polarization as well as the corresponding intracellular \([\text{Ca}^{2+}]_c\) concentration. This property of β-cells can be important for synchronization of oscillations of insulin release in vivo in the pancreas. This synchronization is likely achieved through intrapancreatic innervation. This is probably due to cholinergic effects synchronizing islets (1, 149). Interestingly, a single cholinergic pulse per several internal \([\text{Ca}^{2+}]_c\) oscillations can synchronize these oscillations (46, 149).

Other external pacemaker mechanisms can also contribute to islet synchronization. For example, insulin secreted by islets acts on the liver, resulting in a reduction in plasma glucose concentration that can lead to glucose oscillations in blood, synchronizing an insulin secretion in islets (57, 114, 117). Additional synchronizing factors can be external ATP acting on purinergic receptors (115, 128).

Conclusion

Our analysis of several different hypotheses for pacemakers of oscillatory processes in β-cells shows that the following proposals may be most consistent with the existing experimental data. 1) Changes in the conductivity of slow \(K_{\text{Ca}}\) channels and \(K_{\text{ATP}}\) channels can be the pacemakers for ultrafast \([\text{Ca}^{2+}]_c\) oscillations; 2) changes in [ATP]/[ADP] ratio and/or \([\text{Ca}^{2+}]_\text{ER}\) can be the pacemakers for fast bursts and \([\text{Ca}^{2+}]_c\) oscillations; and 3) cyclical \([\text{Na}^+]_c\) changes may be important to explain the pattern of slow bursting and calcium oscillations. However, the exact
physiological variables and their underlying mechanisms that drive bursting or Ca$$^{2+}$$ oscillations in β-cells remain uncertain. Additional dynamic experiments and mathematical models are necessary to better understand this emerging aspect of β-cell physiology.

GRANTS

This work has been partially supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DRTC P60-DK-020595, DK-48494, and DK-063493 and a Research Grant from the Keck Foundation.

DISCLOSURES

The authors have nothing to disclose.

APPENDIX

Modeling of Slow-Activated Ca$$^{2+}$$-Sensitive K$$^{+}$$ Channel-Dependent Bursting and [Ca$$^{2+}$$]c Oscillations

Equations and coefficients for channels and pumps are taken from the previous model (51) or represented here. Slow-activated Ca$$^{2+}$$-sensitive K$$^{+}$$ channels as well as the fast-activated Ca$$^{2+}$$-sensitive K$$^{+}$$ channels were modeled, with considerably increased time-dependent parameters for activation reported in Ref. 59.

The units are time in milliseconds (ms), voltage in millivolts (mV), concentration in micromoles/liter (µM), current in femtoamperes (fA), conductance in picosiemens (pS), and capacitance in femtofarads (fF). Numerical integration of the model was carried out using standard numerical methods (51). This model is available for direct simulation on the web site “Virtual Cell” (www.nrcam.uchc.edu) in the “MathModel Database” on the “math workspace” in the library “Fridlyand” with the name “SlowCa+ acted Kchan.”

\[
\begin{align*}
\text{Ca}^{2+} \text{ Current (I}_{\text{VCa}}) & \quad I_{\text{VCa}} = g_{\text{mVCa}} d_{\text{Ca}} f_{\text{VCa}} (V_p - E_{\text{Ca}}), \\
\frac{d d_{\text{Ca}}}{d t} & = \frac{d_{\text{Cab}} - d_{\text{Ca}}}{\tau_{d_{\text{Ca}}}}, \\
\tau_{d_{\text{Ca}}} & = 2.2 - 1.79 \times \exp[-(V_p - 9.7)/70.2]^2, \\
f_{\text{VCa}} & = f_{\text{VCab}} = \frac{1}{1 + \exp[-(15 + V_p)/6]},
\end{align*}
\]

where \(g_{\text{mVCa}} = 800 \text{ pS}, E_{\text{Ca}} = 100 \text{ mV}.\)

Delayed Rectifier K$$^{+}$$ Current (\(I_{Kr}\))

\[
\begin{align*}
\text{I}_{kr} & = g_{\text{mKr}} d_{kr} f_{kr} (V_p - E_{kr}), \\
\frac{d d_{kr}}{d t} & = \frac{d_{krb} - d_{kr}}{\tau_{d_{kr}}}, \\
\tau_{d_{kr}} & = 1 + \exp[(-9 - V_p)/5], \\
d_{krb} & = \frac{1}{1 + \exp[(-9 - V_p)/5]},
\end{align*}
\]

where \(g_{\text{mKr}} = 30,000 \text{ pS}, \tau_{d_{kr}} = 23 \text{ ms}, E_{kr} = -75 \text{ mV}.\)

Fast Ca$$^{2+}$$-Activated K$$^{+}$$ Current (\(I_{KCa}\))

\[
\begin{align*}
\text{I}_{KCa} & = g_{\text{mKCa}} d_{Ca} [Ca^{2+}]_c (V_p - E_{kr}), \\
d_{KCa} & = \frac{[Ca^{2+}]_c^4 + (0.25)^4}{[Ca^{2+}]_c^4 + (0.25)^4},
\end{align*}
\]

where \(g_{\text{mKCa}} = 10 \text{ pS}.\)

Slow-Activated Ca$$^{2+}$$-Sensitive K$$^{+}$$ Channels

\[
\begin{align*}
\text{I}_{KCa} & = g_{\text{mKCa}} d_{KCa} (V_p - E_{K}), \\
\frac{d d_{KCa}}{d t} & = \frac{d_{KCab} - d_{KCa}}{\tau_{d_{KCa}}}, \\
\tau_{d_{KCa}} & = \frac{[Ca^{2+}]_c^4}{[Ca^{2+}]_c^4 + (0.25)^4},
\end{align*}
\]

where \(G_{mKCas} = 90 \text{ pS} \text{ and } \tau_{d_{KCas}} = 2,300 \text{ ms}.\)

\[
\begin{align*}
ATP-Sensitive K$$^{+}$$ Channel Current (\(I_{KATP}\)) & \quad I_{KATP} = g_{mKATP} O_{KATP} (V_p - E_k),
\end{align*}
\]

where

\[
O_{KATP} = 0.08(1 + 2[MgADP]_i/17 + 0.89[MgADP]_i/17)^2/[(1 + [MgADP]_i/17)^2(1 + 0.45[MgADP]_i/26 + ATP_{free})/50]
\]

where \([\text{MgADP}]_i = 0.55[\text{ADP}]_i\),

where \(O_{KATP}\) is the fraction of open K$$^{+}$$ATP channels, \([\text{MgADP}]_i\) is the concentration of free Mg-bound ADP, \(G_{mKATP} = 30,000 \text{ pS}, \text{ and } [\text{ADP}]_i = 20 \text{ µM}.\)

\[
\begin{align*}
\text{Plasma Membrane Ca}^{2+} \text{ Pump Current (I}_{\text{Cap}}) & \quad I_{\text{Cap}} = P_{mCap} \frac{[Ca^{2+}]_i^4}{[Ca^{2+}]_i^4 + (0.2)^4},
\end{align*}
\]

where \(P_{mCap} = 4,300 \text{ fA}.\)

Na$$^{+}$$ Background Current (\(I_{Na}\))

\[
\begin{align*}
\text{I}_{Na} & = g_{mNa} (V_p - E_{Na}),
\end{align*}
\]

where \(g_{mNa} = 12 \text{ pS} \text{ and } E_{Na} = -70 \text{ mV}.\)

The differential equation describing time-dependent changes in the PM potential (\(V_p\)) is the current balance equation:

\[
\begin{align*}
-C_m \frac{d V_p}{d t} & = I_{\text{VCa}} + I_{Kr} + I_{KATP} + I_{KCa} + I_{KCab} + I_{Cap} + I_{Na},
\end{align*}
\]

where \(C_m \) is the whole cell membrane capacitance. The PM currents are listed in Fig. 1.

Based on the consideration in our general model (51), and including only \(I_{VCa}\) and \(I_{Cap}\), the equations for \([Ca^{2+}]_c\) dynamics can be written as

\[
\begin{align*}
\frac{d [Ca^{2+}]_c}{d t} & = f_i (-I_{VCa} - 2I_{Cap}) - k_{eq}[Ca^{2+}]_c,
\end{align*}
\]

where \(f_i\) is the fraction of free Ca$$^{2+}$$ in cytoplasm, \(F\) is Faraday’s constant, \(V_i\) is the effective volumes of the cytosolic compartment, and \(k_{eq}\) is a coefficient of the sequestration rate of [Ca$$^{2+}$$]c.

REFERENCES


Kuznetsov A, Bindokas V, Marks JD, Philipson LH. Modeling of Ca\(^{2+}\) oscillations in pancreatic beta-cells by 10.220.33.3 on September 28, 2016 http://ajpendo.physiology.org/ Downloaded from


