Effects of Extracellular Calcium on Electrical Bursting and Intracellular and Luminal Calcium Oscillations in Insulin Secreting Pancreatic β-Cells

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ABSTRACT The extracellular calcium concentration has interesting effects on bursting of pancreatic β-cells. The mechanism underlying the extracellular Ca^{2+} effect is not well understood. By incorporating a low-threshold transient inward current to the store-operated bursting model of Chay, this paper elucidates the role of the extracellular Ca^{2+} concentration in influencing electrical activity, intracellular Ca^{2+} concentration, and the luminal Ca^{2+} concentration in the intracellular Ca^{2+} store. The possibility that this inward current is a carbachol-sensitive and TTX-insensitive Na^+ current discovered by others is discussed. In addition, this paper explains how these three variables respond when various pharmacological agents are applied to the store-operated model.

INTRODUCTION

In mouse β-cells perifused with a medium containing a moderate amount of glucose, an increase in the extracellular Ca^{2+} concentration, $[Ca^{2+}]_o$, lowers the repolarization potential, raises the plateau potential, and shortens the spike and plateau lengths (see Fig. 1 *A*, modified from Fig. 1 of Henquin, 1990). When extracellular Ca^{2+} is depleted in a perifusion medium, the bursting transforms to repetitive spiking, while $[Ca^{2+}]_i$ decreases to the lowest level (Fig. 1 *B*, modified from Fig. 1 *b* of Worley et al., 1994b). The opposite effect is found by raising extracellular Ca^{2+} (Fig. 1 *C*). That is, repetitive spiking induced by adding suprathreshold glucose to the external medium can be transformed to bursting (Fig. 1 *C*, modified from Fig. 3 of Henquin, 1990). The restoration of the bursting from repetitive spiking by extracellular Ca^{2+} can also be demonstrated using drugs that block the ATP-sensitive K^+ channel (Fig. 1 *D*, modified from Fig. 2 of Rosario et al., 1993). Note in Figs. 1 *B* and *D* that in conjunction with this electrical bursting, the intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, oscillates. The shape of the $[Ca^{2+}]_i$ oscillation is such that $[Ca^{2+}]_i$ rises quickly during the depolarization, the short spikes are generated during the plateau phase, and then $[Ca^{2+}]_i$ falls slowly until the next cycle is initiated. Electrical bursting seen in Figs. 1 *B* and *C* are consistent with the earlier finding of Ribalet and Beigelman (1980), who showed that the amplitude of the bursting (i.e., the plateau potential minus the repolarized potential) decreases as $[Ca^{2+}]_o$ decreases.

Henquin (1990) hypothesized that the phenomenon associated with $[Ca^{2+}]_o$ is due to a feedback control of the ATP-sensitive K^+ channel by intracellular Ca^{2+}. Rosario et al. (1993), on the other hand, hypothesized that this burst is mediated by a Ca^{2+}-dependent K^+ channel and/or by a Ca^{2+}-dependent slow process of inactivation of Ca^{2+} current. These hypotheses are not sufficient, however, since they do not explain the repetitive spiking observed in the absence of extracellular calcium (see Fig. 1 *B*). Depolarization that accompanies the depletion of extracellular calcium (see Fig. 1 *B*) is taken to indicate the existence of I_{NS}, a store-regulated nonselective cationic current (Worley et al., 1994b). Although the depolarization induced by a decrease of $[Ca^{2+}]_o$ may be explained by I_{NS}, the repetitive spiking in the absence of extracellular Ca^{2+} indicates that the spikes in β-cells may not be entirely due to a calcium current.

Earlier, we studied the extracellular Ca^{2+} effect using a mathematical model developed by us (Lee et al., 1983; Chay, 1985a). While we were able to mimic some features of electrical bursting in response to $[Ca^{2+}]_o$, we were unable to explain the spiking that arises as a result of extracellular Ca^{2+} depletion. Since the formulation of our initial mathematical model, many more interesting phenomena on β-cell physiology have been uncovered, which negates the underlying assumptions in our mathematical models (see Discussion). In this paper, the mechanism involved in the extracellular Ca^{2+} effect is elucidated using the recently formulated store-operated model of Chay (1995; 1996a, b) which incorporates luminal Ca^{2+} in the intracellular calcium stores and a store-regulated I_{NS}. To this store-operated model, a low-threshold inward current distinct from the Ca^{2+} current, I_{fast}, and a voltage-independent Ca^{2+}-sensitive K^+ current, $I_{K(Ca)}$, are included. Exclusion of I_{fast} makes it difficult to explain the external Ca^{2+} effects shown in Fig. 1. The possible existence of I_{fast} in pancreatic β-cell is discussed (see Discussion). See Results for how the store-operated model is capable of producing many interesting electrophysiological phenomena observed in pancreatic β-cells (in addition to the external Ca^{2+} effect). The underlying mechanisms involved in these phenomena are discussed in relation to the calcium stores, ion channels in the plasma membrane, and intracellular Ca^{2+} ions.

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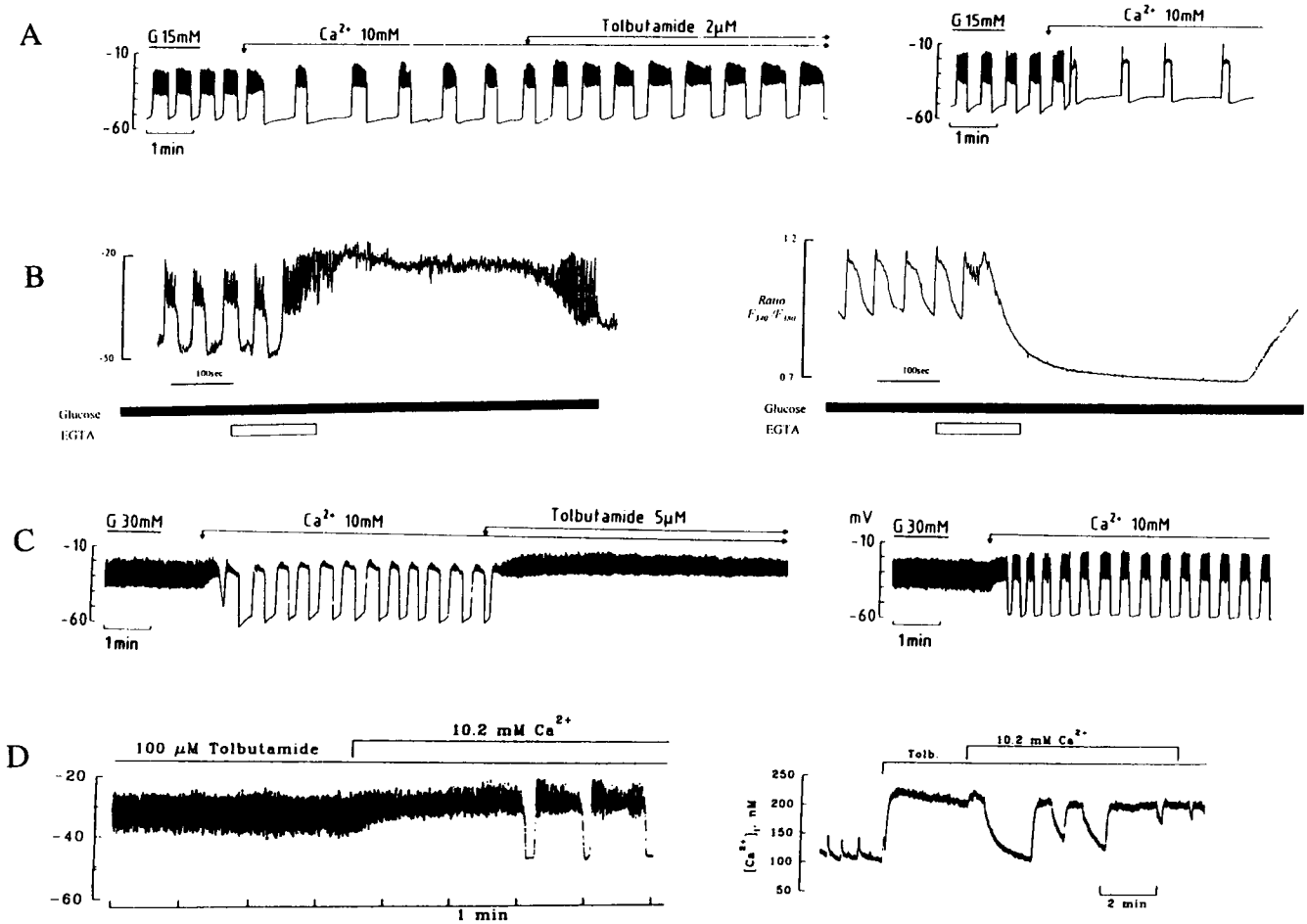

FIGURE 1 Extracellular calcium effects on burst of electrical activity and $[Ca^{2+}]_i$ oscillations. (*A*) Concentration of Ca^{2+} was increased from 2.5 to 10 mM, while the concentration of glucose (G) was held at 15 mM throughout. The left and right frames came from two different cells from different mice. Retouched from Henquin (1990). (*B*) Simultaneous recordings of membrane potential and $[Ca^{2+}]_i$, when 5 mM EGTA (and no added Ca^{2+}) was briefly introduced to remove external Ca^{2+}. The glucose concentration was 12 mM throughout. Retouched from Fig. 3 of Worley et al. (1994a). (*C*) Concentration of Ca^{2+} was increased from 2.5 to 10 mM, while glucose was held at 30 mM throughout. Retouched from Fig. 3 of Henquin (1990). (*D*) Extracellular Ca^{2+} concentration was raised from 2.56 to 10.2 mM in the presence of tolbutamide, while the glucose concentration was held at 11 mM throughout. Retouched from Rosario et al. (1993).

THE MODEL

The model used in this paper is schematically presented in Fig. 2. As shown in this figure, three signaling-pathways exist in β-cells: 1) the phosphatidylinositol (PI-) signaling pathway (*left*) that can activate protein kinase C (PKC), 2) the glucose-sensing pathway (*lower right* and *center*) that can activate calcium-calmodulin kinase (Ca-CAM K), and 3) the adenylate-cyclase (AC-) transduction pathway (*right*) that can activate cyclic AMP-dependent kinase (PKA). These kinases can release insulin from its granules via phosphorylation (Prentki and Matschinsky, 1987). An agonist (e.g., hormones such as glucagon or neurotransmitters such as acetylcholine) activates the GTP-bound G-protein (G) when it is bound to the receptor (REC). This agonist produces a secondary messenger such as cyclic adenosine monophosphate (*right*) and inositol (1,4,5) triphosphate (*left*). The ER contains a calcium releasing channel (CRC), which releases the luminal Ca^{2+} upon a rise of the secondary messengers. It also contains a Ca^{2+}-ATPase pump that pumps intracellular Ca^{2+} into the ER. The SGs sequester intracellular Ca^{2+} via its Ca^{2+}-ATPase pump and then release insulin and intragranular Ca^{2+} into the external medium during exocytosis.

As proposed in Chay (1993a), a "hot" spot exists underneath a cluster of L-type Ca^{2+} channels. This channel opens when the membrane is depolarized, permitting extracellular Ca^{2+} ions to come into the cell and inactivates when $[Ca^{2+}]_i$ is undesirably high (Chay, 1987; Plant, 1988a). The depolarization was initiated by a closure of the ATP-sensitive K^+ channels (the lower right of Fig. 2). A voltage-independent small-conductance Ca^{2+}-sensitive K^+ (K-Ca) channel (Ammala et al., 1991, 1993) coexists along with the L-type channels. In the vicinity of these two channels lies a voltage-independent cationic nonselective channel (NS), which is activated when $[Ca^{2+}]_{lum}$ becomes low (Worley et al., 1994b). In the hot spot lie two types of intracellular

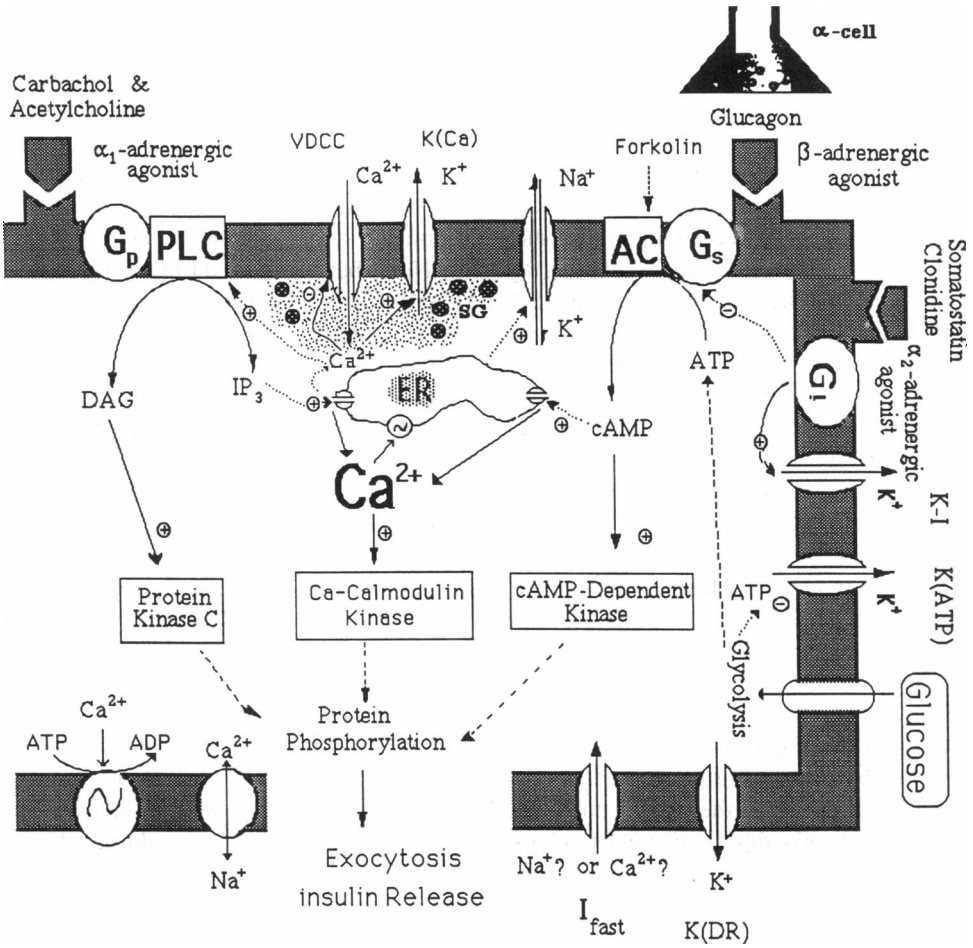

FIGURE 2 Three signaling pathways involved in secretion of insulin from pancreatic β-cells: the phosphatidylinositol-signaling pathway (*left*), the glucose-pathway (*lower right* and the *center*), and the adenylate-cyclase transduction pathway (*right*). Here, the symbols + and − indicate activation and inhibition, respectively. This figure is modified from Prentki and Matschinsky (1987) and Ashcroft and Rorsman (1989). For the notations see the text.

Ca^{2+} stores (ICSs): the endoplasmic reticulum (ER) and insulin containing secretory granules (Bokvist et al., 1995). The ER contains an IP_3-sensitive calcium releasing channel (CRC) and a ryanodine-sensitive CRC whose activity is modulated by cAMP (Karlsson and Ahren, 1996).

Outside this hot spot exist voltage-dependent delayed-rectifying K^+ [DR(K)] channels (Rorsman and Trube, 1986) and ATP-sensitive K^+ [K(ATP)] channels (Cook and Hales, 1984; Ashcroft, 1988; Ribalet et al., 1989). The new feature of this model is an inclusion of a low-threshold transient fast current that activates rapidly upon depolarization and inactivates in a voltage-dependent manner. This current is assigned as I_{fast} without a loss of generality.

In this model, a rise of $[Ca^{2+}]_i$ is due to two sources—influx of Ca^{2+} ions through the Ca^{2+} channels and a release of luminal Ca^{2+} from the ER (the first and third terms in Eq. 1). The fall of $[Ca^{2+}]_i$ is due to two effluxes—sequestration of intracellular Ca^{2+} into the SGs (second) and that into the ER (fourth). Accordingly, the $[Ca^{2+}]_i$ dynamic is expressed as

$$\frac{d[Ca^{2+}]_i}{dt} = -\phi I_{Ca} - k_{Ca}[Ca^{2+}]_i + k_{rel}([Ca^{2+}]_{lum} - [Ca^{2+}]_i) - k_{pump}[Ca^{2+}]_i \quad (1)$$

where ϕ measures the surface-volume ratio and k_{Ca} is the sequestration rate of intracellular calcium by the SGs. The last two terms in Eq. 1 are due to the events taking place in the ER, i.e., the dynamic change of the calcium concentration in the ER, $[Ca^{2+}]_{lum}$,

$$\frac{d[Ca^{2+}]_{lum}}{dt} = -k_{rel}([Ca^{2+}]_{lum} - [Ca^{2+}]_i) + k_{pump}[Ca^{2+}]_i \quad (2)$$

where k_{rel} measures the activity of the calcium releasing channel, and k_{pump} is the pump activity of Ca^{2+}-ATPase in the ER. The calcium-induced calcium release mechanism considered earlier for the CRC (Chay, 1991; 1993; 1995; 1996a–c) is taken out since it is not a necessary component in β-cell bursting.

The membrane potential necessary to compute I_{Ca} in Eq. 1 can be found from the charge neutrality condition (Hodgkin and Huxley, 1952):

$$-C_m \frac{dV}{dt} = \sum I_{ionic} \quad (3)$$

where C_m is the membrane capacitance, and I_{ionic} is an ionic current component. The expression of each ionic compo-

nent and the basic parametric values associated with this component are given in the Appendix.

RESULTS

Figure 3 is the result obtained in the limit cycle by solving the differential equations in the Model. Here, the time course of $[Ca^{2+}]_i$ is represented by dashes, membrane potential by a solid curve, and $[Ca^{2+}]_{lum}$ by dots. Note that $[Ca^{2+}]_{lum}$ oscillates slowly between 78.2 μM and 105.0 μM. In conjunction with this slow oscillation, the membrane potential (V) bursts between -55 mV (the repolarization potential) and -41 mV (the plateau potential). On the top of the plateau appear fast electrical spikes. The plateau terminates when $[Ca^{2+}]_{lum}$ reaches a maximum, followed by the repolarization period. After the termination of the plateau, $[Ca^{2+}]_{lum}$ decreases slowly until V reaches the threshold potential of ~ -47 mV. Then, a rapid upstroke of V follows. In this model as in other store-operated models (Chay, 1995; 1996a,b) the slow luminal Ca^{2+} dynamic drives electrical bursting as well as the $[Ca^{2+}]_i$ oscillation.

Typical bursts in Fig. 3 start with rapid membrane depolarization, which is accompanied by a rapid rise in $[Ca^{2+}]_i$. The rise of $[Ca^{2+}]_i$ is due to a high-threshold I_{Ca}, which was activated by a lower-threshold fast-activating I_{fast}. The I_{Ca} thus elicited generates the plateau potential. The upstroke of the electrical spike during the plateau phase is mainly due to I_{fast} and the downstroke is due to the combined effect of $I_{K(DR)}$ and the inactivating component of I_{fast}. Along with the electrical spike, the Ca^{2+} spike appears (see the *upper trace*). Note here that although the maximum peak of the Ca^{2+} spike decreases during the plateau, the minimum peak increases gradually. During the gradual increase, $[Ca^{2+}]_i$ increases slowly, which in turn leads to an increase in $I_{K(Ca)}$. In fact, from the beginning of the plateau to the end, a net increase of $I_{K(Ca)}$ is $\sim$10.5 pA/cm^2. On the other hand, $[Ca^{2+}]_{lum}$ also increases during the plateau, and this increase leads to a decrease in I_{NS} of $\sim$3.5 pA/cm^2. The other inward current, I_{fast}, decreases $\sim$7 pA/cm^2 due to voltage-dependent inactivation. The termination of the plateau results when $I_{K(Ca)}$ exceeds $I_{fast} + I_{NS}$.

That the decrease in $[Ca^{2+}]_i$ during the silent phase is gradual is due to luminal Ca^{2+}, which is released from the CRC during this phase. This release prevents $[Ca^{2+}]_i$ from decreasing rapidly even after the Ca^{2+} channel closes. The slow decrease in $[Ca^{2+}]_i$, in turn, inactivates $I_{K(Ca)}$. Likewise, a decrease of $[Ca^{2+}]_{lum}$ activates I_{NS}. When I_{NS} exceeds $I_{K(Ca)}$, the burst is initiated. I_{Ca} contributes little during the repolarization phase.

Figure 4 reveals how the six current components behave during bursting. The bottom trace is a combined current of the ATP-sensitive K^+ current $I_{K(ATP)}$ and the sodium leak current $I_{Na,L}$. The spike activity is generated by I_{fast} (*second from the bottom*) and $I_{K(DR)}$ (*third from the bottom*). The two currents, I_{Ca} (*third from the top*) and $I_{K(Ca)}$ (*second from the top*), are delicately balanced to control the plateau phase. The termination of the plateau is in part attributed to I_{NS} (*top*). The $I_{K(ATP)}$ (*bottom*) and I_{NS} (*top*) control the silent phase (the contribution of $I_{Na,L}$ is almost insignificant). Thus, the slow depolarization during the silent phase is attributed to the combined effect of I_{NS} and $I_{K(Ca)}$. Activation of I_{NS} during the silent phase prevents the membrane potential fall below the repolarization potential of ~ -55 mV.

Figure 5 reveals how extracellular calcium affects electrical bursting, $[Ca^{2+}]_i$, and $[Ca^{2+}]_{lum}$. Consistent with experiments shown in Fig. 1, when extracellular Ca^{2+} is depleted, electrical bursting transforms to repetitive spiking (*top trace*). The spikes seen here are not Ca^{2+} spikes since I_{Ca} is absent when external Ca^{2+} is depleted. During the spiking, $[Ca^{2+}]_i$ decreases to the lowest level (*top trace*).

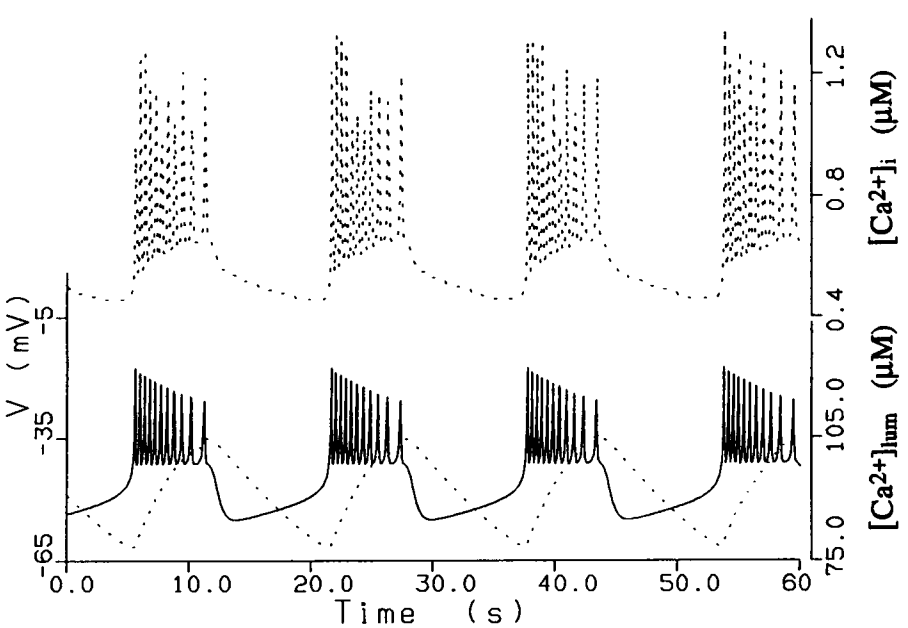

FIGURE 3 Electrical activity (the *solid line* in the *lower trace*), the intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ (the *upper trace*), oscillation of the luminal Ca^{2+} concentration, $[Ca^{2+}]_{lum}$ (*dashes* in the *lower trace*) based on the store-operated model. The parametric values used for the simulation are listed in the Appendix.

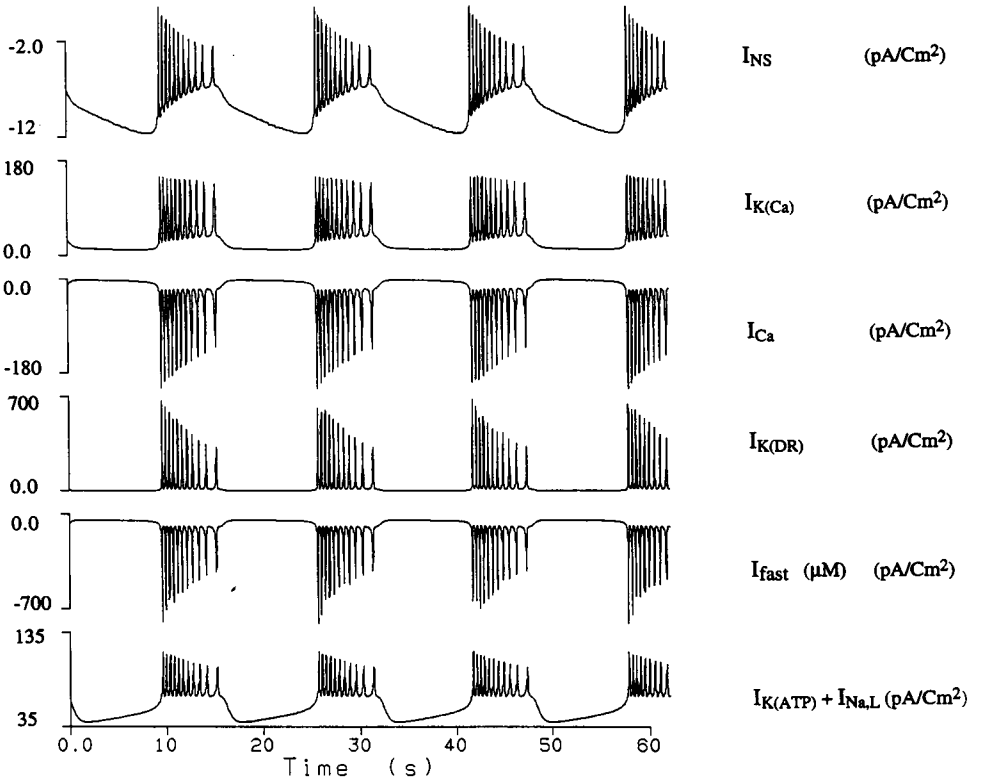

FIGURE 4 The six currents that participate during bursting in Fig. 3. The unit of the current is pA/cm^2.

Because of low $[Ca^{2+}]_i$ both I_{Ca} and $I_{K(Ca)}$ are completely inoperative, while I_{NS} is in its maximal strength (because of low $[Ca^{2+}]_{lum}$). This simulation thus reveals that the depolarization that accompanies depletion of external Ca^{2+} is due to maximal activation of I_{NS} (which oscillates between -30.1 pA/cm^2 and -6.5 pA/cm^2). In our earlier model (Lee et al., 1983; Chay, 1985a) the depolarization that arises by lowering $[Ca^{2+}]_o$ is solely due to weakened $I_{K(Ca)}$. In this model, depolarization is due to the two sources—maximal activation of I_{NS} and a near absence of $I_{K(Ca)}$.

When $[Ca^{2+}]_o$ is high (see the bottom trace), $[Ca^{2+}]_i$ oscillates with a high amplitude between 0.54 μM and 2.61 μM. $[Ca^{2+}]_{lum}$ also oscillates at a high level between 88.6 μM and 166.9 μM. One may ask, why does the burst amplitude lengthen when $[Ca^{2+}]_o$ is raised? This can be explained by the following sequences: 1) The repolarization potential is much lower than the usual one (i.e., -62.9 mV versus -55 mV) since I_{NS} is weak (due to high $[Ca^{2+}]_{lum}$) and $I_{K(Ca)}$ is strong (due to high $[Ca^{2+}]_i$). 2) Since $[Ca^{2+}]_i$ is high, the repolarization phase is long (it takes a long time to pump out intracellular Ca^{2+}). 3) At the end of this long period, the h-variable attains near its maximal value (i.e., unity). 4) During the fast depolarization upstroke, the m-variable also attains near unity while the h-variable remains still near unity. 5) As a consequence, I_{fast} gains a full strength, and this leads to the high plateau potential. These five events lead to the long burst amplitude. Then, why is the plateau period so short? The higher plateau potential induces fuller activation of I_{Ca}, and this in turn raises $[Ca^{2+}]_i$. Since $[Ca^{2+}]_i$ is high, $I_{K(Ca)}$ gains its maximal strength, and this in turn shortens the plateau length.

Figure 6 demonstrates the effect of varying activity of the ER Ca^{2+}-ATPase on electrical activity of β-cells. As this pump is blocked, bursting transforms to continuous spiking (see the bottom two traces of Fig. 6). This transformation is consistent with experiments of Worley et al. (1994a), who observed that when thapsigargin (a blocker of the ER Ca^{2+}-ATPase) is added to the medium bursting transforms to continuous spiking. The question is, how does the repetitive spiking arise when the pump is blocked? This can be seen by comparing the bottom trace ($k_{pump} = 10$ s^{-1}, i.e., a low pump rate) with the top trace ($k_{pump} = 40$ s^{-1}, a high pump rate). Note that $[Ca^{2+}]_{lum}$ oscillates at a much lower level when the pump rate is lower, i.e., $[Ca^{2+}]_{lum}$ oscillates between 40.8 μM and 41.7 μM when $k_{pump} = 10$ s^{-1}, while it oscillates between 93 μM and 150 μM when $k_{pump} = 40$ s^{-1}. Because of a low $[Ca^{2+}]_{lum}$, I_{NS} is stronger when the pump rate is lower, i.e., I_{NS} oscillates between -22.8 pA/cm^2 and -10.8 pA/cm^2 when $k_{pump} = 10$ s^{-1}, while it oscillates between -9.0 pA/cm^2 and 2.4 pA/cm^2 when $k_{pump} = 40$ s^{-1}. On the other hand, $[Ca^{2+}]_i$ oscillates at a higher level when the pump rate is lower, i.e., $[Ca^{2+}]_i$ oscillates between 0.61 μM and 1.40 μM when $k_{pump} = 10$ s^{-1}, while it oscillates between 0.43 μM and 1.20 μM when $k_{pump} = 40$ s^{-1}. Because of a high $[Ca^{2+}]_i$ $I_{K(Ca)}$ is stronger when k_{pump} is lower, i.e., $I_{K(Ca)}$ oscillates between 28.9 pA/cm^2 and 154.0 pA/cm^2 when $k_{pump} = 10$ s^{-1}, while it oscillates between 9.0 pA/cm^2 and 137 pA/cm^2 when $k_{pump} = 40$ s^{-1}.

The repetitive spiking seen in the absence of pump activity can be explained by the strength of these two currents: when the pump is blocked both $I_{K(Ca)}$ and I_{NS} become

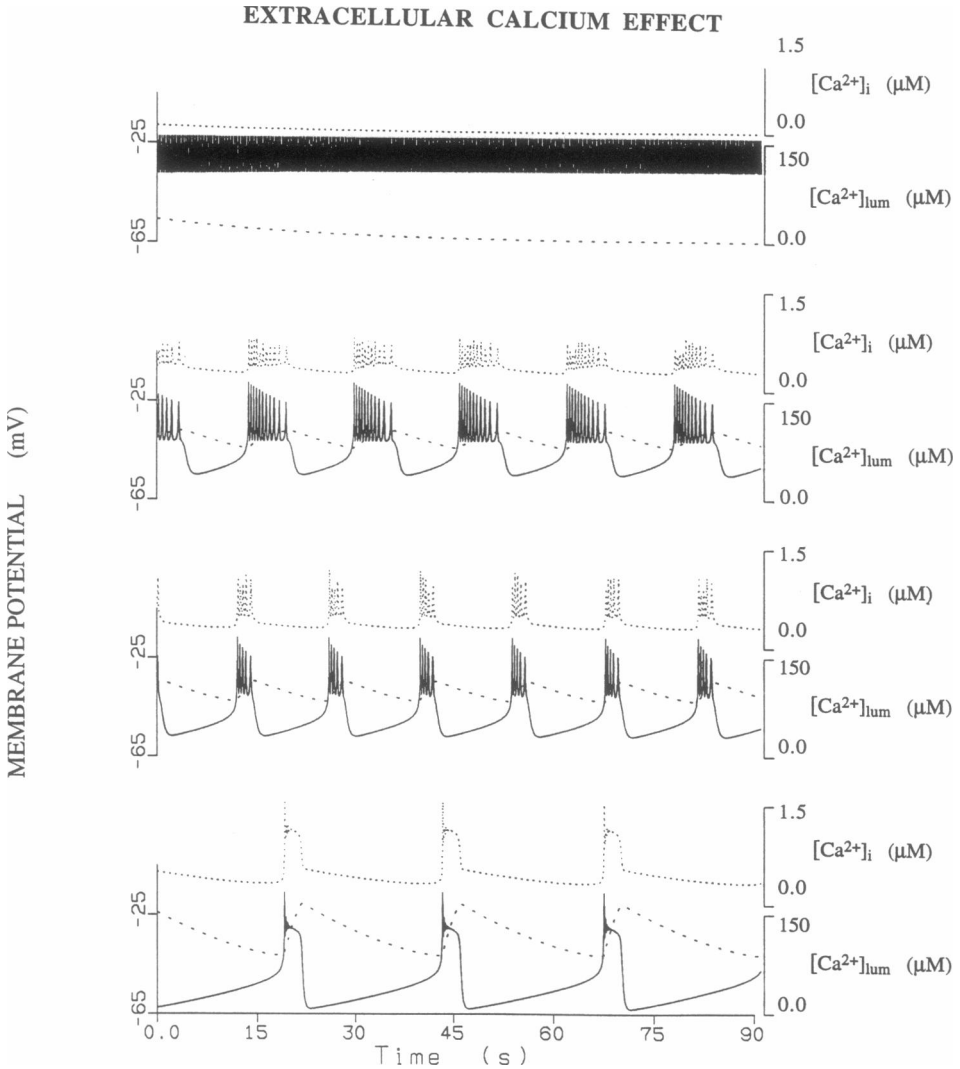

FIGURE 5 The role of the extracellular Ca^{2+} in electrical bursting and $[Ca^{2+}]_i$ oscillation. The small dots represent $[Ca^{2+}]_i$, large dots $[Ca^{2+}]_{lum}$, and solid lines the membrane potential. From the top trace to the bottom, $[Ca^{2+}]_o$ varied from 0 mM to 2.5 mM, to 5.0 mM, to 7.5 mM.

strong, but the latter current gains more strength than the former. Thus, depolarization that accompanies blocking of the Ca^{2+}-ATPase is due to enhanced I_{NS} that is caused by lowering of $[Ca^{2+}]_{lum}$. Without the presence of I_{NS} it is difficult to explain the thapsigargin effect.

In Fig. 7, increasing k_{Ca} increases the plateau fraction (i.e., the ratio between the plateau and repolarization lengths). When k_{Ca} becomes very large, the burst disappears completely and only repetitive spiking remains (see the bottom trace). This simulation mimics the glucose effect (Meissner and Schmelz, 1974) and thus suggests that a metabolite of glycolysis may enhance the sequestration rate of SGs in the limit cycle regime. Note that k_{Ca} has little effect on the repolarization potential or the plateau potential. Note also that k_{Ca} lowers the amplitude of the $[Ca^{2+}]_i$ oscillation slightly, e.g., $[Ca^{2+}]_i$ oscillates between 0.49 μM and 1.37 μM when $k_{Ca} = 3.0\ s^{-1}$ and 0.45 μM and 1.29 μM when $k_{Ca} = 9.0\ s^{-1}$. On the other hand, k_{Ca} lifts the level of $[Ca^{2+}]_{lum}$ and its amplitude significantly, i.e., $[Ca^{2+}]_{lum}$ oscillates between 75.6 μM. and 92.8 μM when $k_{Ca} = 3.0\ s^{-1}$ and between 79.7 μM and 112.8 μM when $k_{Ca} = 9.0\ s^{-1}$.

Why k_{Ca} affects little on the repolarization and plateau potentials can be explained by noting that the combined current (i.e., $I_{K(Ca)}$ plus I_{NS}) is almost the same in the bursting regime. To be more specific, take two cases, $k_{Ca} = 3.0\ s^{-1}$ (*top trace*) and $k_{Ca} = 9.0\ s^{-1}$ (*second from the bottom*). When k_{Ca} is low (i.e., $k_{Ca} = 3.0\ s^{-1}$), $I_{K(Ca)}$ oscillates between 12.9 pA/cm^2 and 151.0 pA/cm^2 and I_{NS} oscillates between -12.5 pA/cm^2 and -0.2 pA/cm^2. On the other hand, when k_{Ca} becomes high (i.e., $k_{Ca} = 9.0\ s^{-1}$), $I_{K(Ca)}$ oscillates between 10.5 pA/cm^2 and 150.0 pA/cm^2 and I_{NS} oscillates between -11.2 pA/cm^2 and 2.0 pA/cm^2. This is why there is little change in the repolarization potential or the plateau potential when k_{Ca} varies.

In my earlier model k_{Ca} was attributed to the mitochondrial sequestration (Chay, 1996b). There is no evidence that mitochondria exist in the hot spot. There, however, exists strong evidence that SGs coexist in the hot spot (Bokvist et al., 1995). Thus, it is more likely that k_{Ca} is due to the

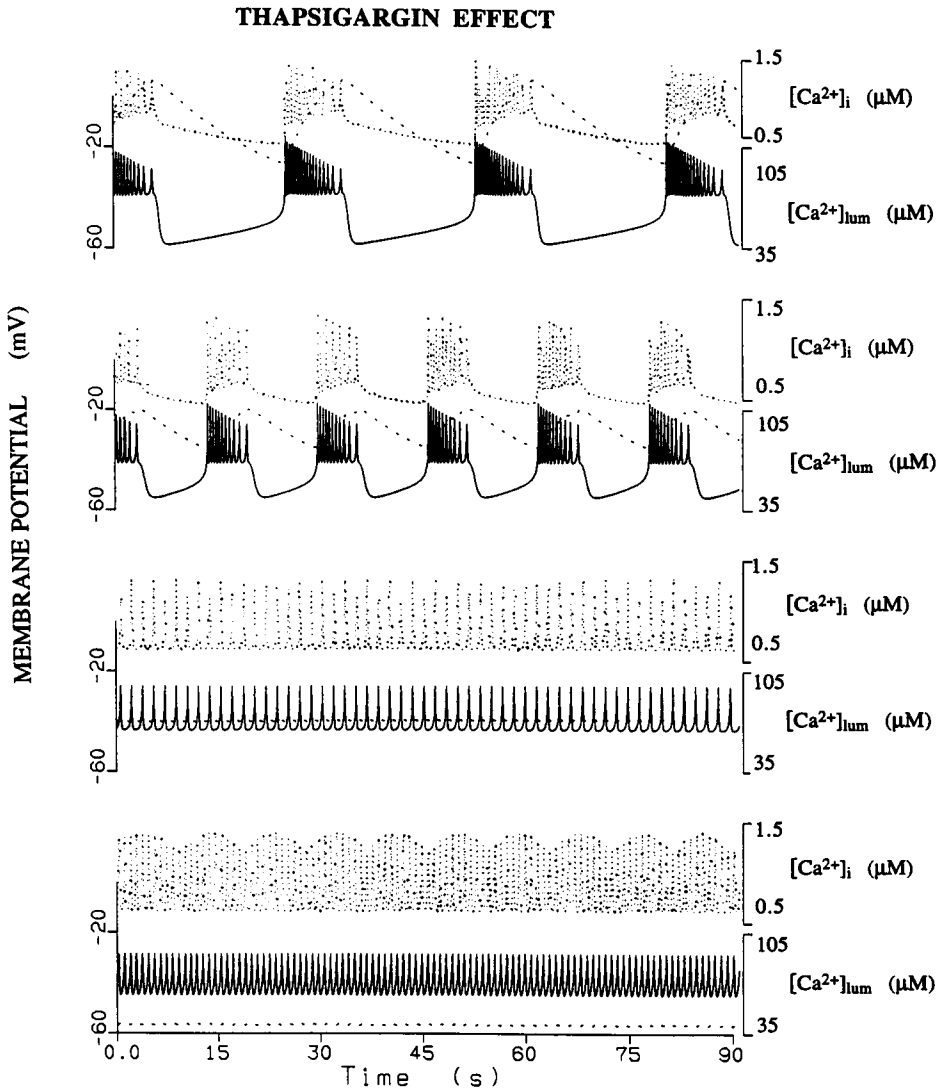

FIGURE 6 The effect of varying the Ca^{2+}-ATPase rate, k_{pump}, of the endoplasmic reticulum, where k_{pump} is decreased from 40 s^{-1}, to 30 s^{-1}, to 20 s^{-1}, and to 19 s^{-1} from the top trace to the bottom.

sequestration of intracellular Ca^{2+} by SGs than it is due to mitochondrial sequestration. An increase of $[Ca^{2+}]_{lum}$ (as k_{Ca} increases) may suggest that intragranular Ca^{2+} is a necessary component for exocytosis to take place.

As in our earlier models (Chay, 1985a; Himmel and Chay, 1997; Chay et al., 1990), electrical patterns similar to those observed in Fig. 7 can be also observed by blocking the ATP-sensitive K^+ sensitive current (see Fig. 8). Unlike the case in Fig. 7 (where $[Ca^{2+}]_i$ is almost invariant), blocking of the K(ATP) channel raises the level of $[Ca^{2+}]_i$, e.g., $[Ca^{2+}]_i$ oscillates between 0.25 μM and 1.18 μM when g_{ATP} = 3.0 $\mu S/cm^2$ and between 0.46 μM and 1.35 μM when g_{ATP} = 2 $\mu S/cm^2$. Along with the increase in $[Ca^{2+}]_i$, $[Ca^{2+}]_{lum}$ also increases as the K(ATP) channel is blocked, e.g., $[Ca^{2+}]_{lum}$ oscillates between 38.7 μM and 61.7 μM when g_{ATP} = 3.0 $\mu S/cm^2$ and between 78.2 μM and 105.0 μM when g_{ATP} = 2.0 $\mu S/cm^2$. An increase in $[Ca^{2+}]_i$ activates $I_{K(Ca)}$ while an increase in $[Ca^{2+}]_{lum}$ inactivates I_{NS}. The net effect is an increase in the outward current. This increase compensates a decrease in $I_{K(ATP)}$ due to the blocking of the K(ATP) channel. This is why the g_{ATP} affects little the repolarization potential or the plateau potential.

As demonstrated by the store-operated model of Chay (1995, 1996a,b), an increase in activity of the CRC (modeled by varying k_{rel}) leads to a increase in the frequency of bursting and a decrease in the amplitude of the $[Ca^{2+}]_{lum}$ oscillation. This is also seen using the present model, and the result is presented in Fig. 9. This simulation is done under the hypothesis that a secondary messengers such as cAMP can modulate the CRC. Note that increasing k_{rel} increases the frequency of electrical bursting. It also lifts the repolarization potential and shortens the plateau fraction. The bursting shown in the top trace reminds of that observed in the presence of somatostatin (Pace and Tarvin, 1981). The simulation in the bottom trace is reminiscent of the bursting observed in the presence of glucagon (Ikeuchi and Cook, 1984).

In addition to inducing bursting, there is an increase in $[Ca^{2+}]_i$ when k_{rel} increases. The increase is such that when

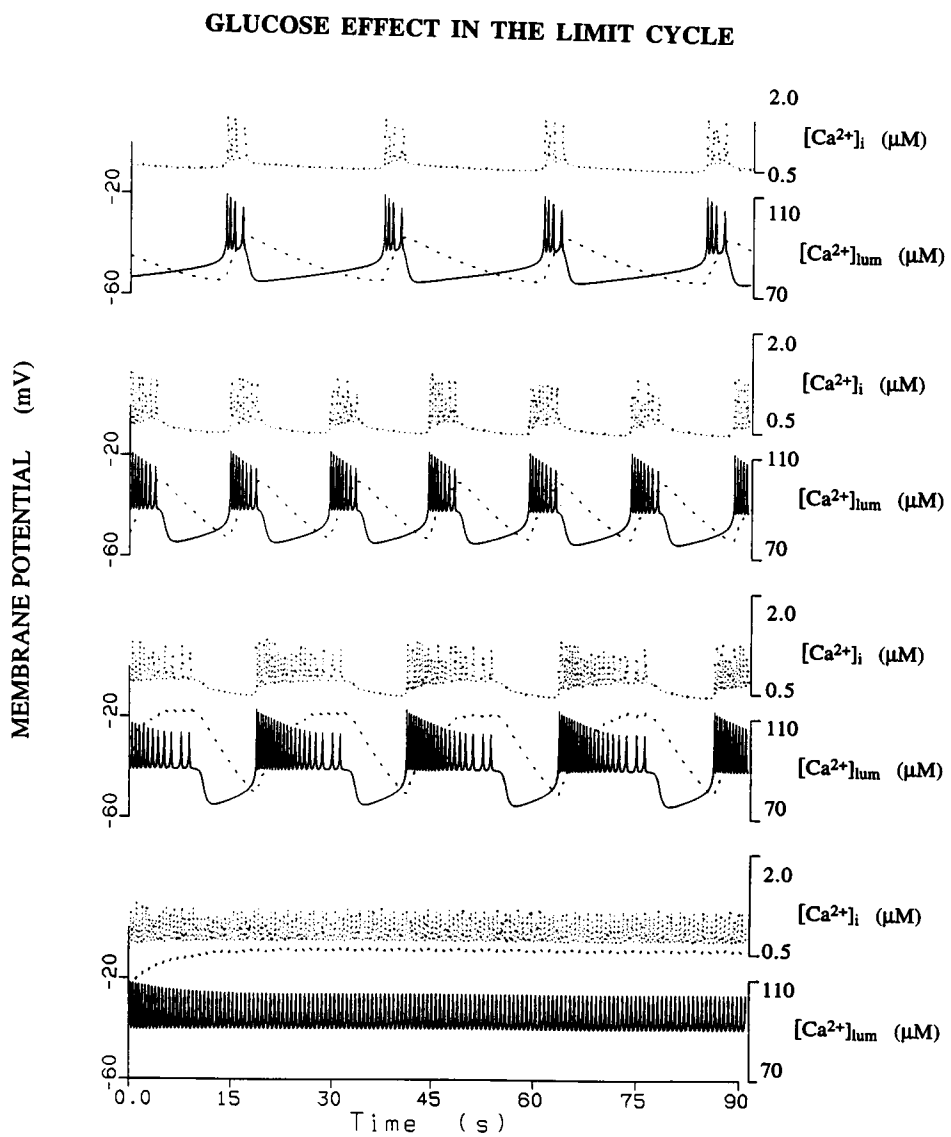

FIGURE 7 The effect of varying the sequestration rate k_{Ca} of the insulin-containing granule. The parameter k_{Ca} is increased from 3 s^{-1}, to 6 s^{-1}, to 9 s^{-1}, and to 12 s^{-1} from the top trace to the bottom.

k_{rel} = 0.03 s^{-1} $[Ca^{2+}]_i$ oscillates between 0.28 μM and 1.33 μM, but when k_{rel} is raised to 0.5 s^{-1} it oscillates at a higher level between 0.58 μM and 1.35 μM. Increasing k_{rel} significantly lowers the level and amplitude of the $[Ca^{2+}]_{lum}$ oscillation, i.e., $[Ca^{2+}]_{lum}$ oscillates between 308.8 μM and 603.4 μM when k_{rel} = 0.03 s^{-1}, while it oscillates between 39.9 μM and 50.8 μM when k_{rel} = 0.50 s^{-1}.

How k_{rel} modulates the frequency of electrical bursting can be readily understood by examining $[Ca^{2+}]_i$ and $[Ca^{2+}]_{lum}$. At a low k_{rel}, luminal Ca^{2+} is released from the store very slowly. Because of the slow release of luminal calcium, $[Ca^{2+}]_{lum}$ stays at a very high level. Since $[Ca^{2+}]_{lum}$ is high, I_{NS} carries little current, and the burst seen in the top trace (i.e., k_{rel} = 0.03 s^{-1}) owes its origin to $I_{K(Ca)}$. At this low k_{rel}, it requires a long time for $[Ca^{2+}]_i$ 1) to decrease sufficiently to inactivate $I_{K(Ca)}$ during the repolarization phase and 2) to increase sufficiently to activate the same current during the plateau phase. At high k_{rel}, on the other hand, I_{NS} is significantly strong; thus, the mechanism by which β-cell burst owes its origin largely to I_{NS}, i.e., the presence of I_{NS} facilitates the depolarization during the repolarization phase and shorten the plateau length during the plateau.

DISCUSSION

Through mathematical modeling, I have demonstrated how the luminal and intracellular calcium concentrations and the ion channels in the plasma membrane participate in controlling burst activity of pancreatic β-cells in response to the variation of extracellular Ca^{2+} and various pharmacological agents. To demonstrate the effect of $[Ca^{2+}]_o$ shown in Fig. 1, it was necessary to include I_{fast} in Chay's store-operated model (1995, 1996a–d). That is, without this current it was difficult to simulate repetitive spiking when extracellular calcium depletes. Also, without this current it was difficult to simulate the spike free high amplitude of electrical bursting when $[Ca^{2+}]_o$ becomes high.

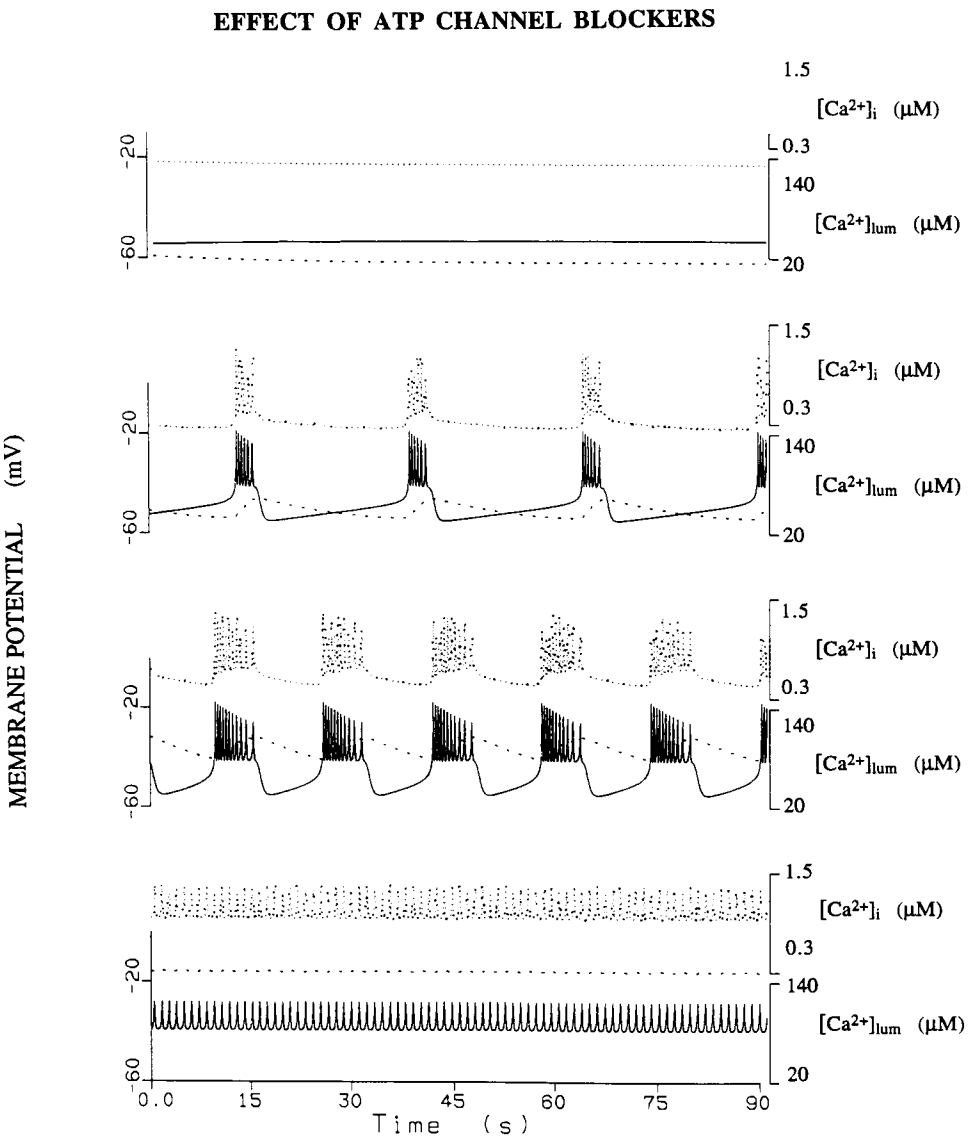

FIGURE 8 The effect of varying the conductance of the K(ATP) channel, g_{ATP}, where g_{ATP} used for the computation are 4 $\mu S/cm^2$, 3 $\mu S/cm^2$, 2 $\mu S/cm^2$, and 1 $\mu S/cm^2$ from the top trace to the bottom traces, respectively.

I have previously shown that three dynamic variables with feedback mechanisms are all that are necessary to generate electrical bursting (Chay, 1985b). Therefore, the question is not whether the proposed model can produce the bursting, but the question should be directed to: does a theoretical model generate crucial experiments (e.g., Fig. 1) observed in pancreatic β-cells? Figs. 3–9 demonstrate that the present model is capable of simulating most of the important experimental observations. However, there may arise some questions in regard to the present model, and I will answer those questions below.

Does the low-threshold transient inward current exist?

It is possible that I_{fast} in this paper could be the low-threshold transient T-type Ca^{2+} current, I_T, discovered in rat β-cells and HIT cells (but never in mouse β-cells). Note, however, in Fig. 1 *B* that the spikes appear even in the absence of external Ca^{2+}. These spikes cannot possibly be generated by I_T, unless the T-type channel allows Na^+ to pass through when external Ca^{2+} is depleted. If it is I_T, a uniform rise of $[Ca^{2+}]_i$ should be detected underneath the entire membrane area when the potential depolarizes. Spectrofluorometric measurements on β-cells reveal that a rise of $[Ca^{2+}]_i$ is confined to a few localized spots, not uniform along the membrane subspace (Bokvist et al., 1995).

A current that depends on extracellular Na^+ has been observed in ACh-stimulated β-cells (Ashcroft and Rorsman, 1989). It was suggested that this current may be coupled to the muscarinic receptors (Gilon et al., 1995) and is mediated by guanine nucleotide-binding proteins (Cohen-Armon and Sokolovsky, 1986). It is not the NS current since a rise of intracellular Na^+ is unaffected by thapsigargin (Gilon et al., 1995; Miura et al., 1996). Also, it is not the sodium channels discovered by Plant (1988b) in mouse and

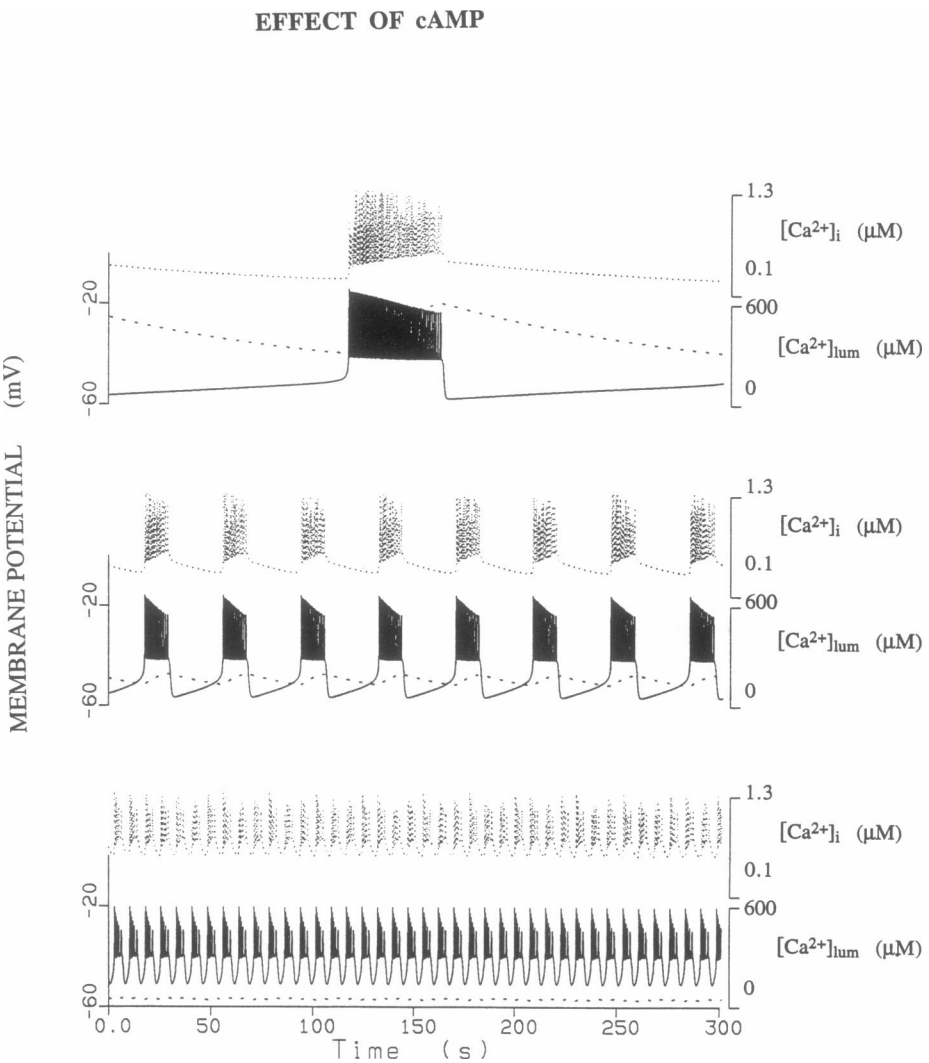

FIGURE 9 The effect of varying the release rate k_{rel} of the calcium-releasing channel. The parameter k_{rel} is increased from 0.03 s^{-1} (*top*) to 0.1 s^{-1} (*middle*) to 0.05 s^{-1} (*bottom*).

Hiriart and Matteson (1988) in rats, since the ACh-activated Na^+ channel is not sensitive to TTX (Gilon and Henquin, 1993).

If I_{fast} is the ACh-activated I_{Na}, then how could it be activated in the absence of carbachol? A reasonable hypothesis is that I_{fast} is modulated by an agonist that activates the phosphatidylinositol (PI-) signaling pathway. There are several agonists that may activate this pathway when glucose is added to the medium. For example, the ATP that is released during exocytosis could activate the PI-signaling pathway (Li et al., 1991) which in turn activates I_{fast}.

If the activation threshold for I_{Ca} is as high as that reported by Rorsman and Trube (1986), then a lower-threshold, fast-activating current is absolutely necessary to elicit the slower, higher threshold I_{Ca}. If indeed a glycolysis product has the ability to shift the activation curve of the L-type channel to a more negative membrane potential (as reported by Smith et al., 1989 and Velasco et al., 1988), there is every reason to believe that this product can also shift the activation threshold of I_{fast} to a negative direction. How the shifting of the half-maximal activation potential, V_m, affects the bursting is demonstrated in Fig. 10, where V_m is varied from −20 mV to −30 mV (from the *top* to the *bottom*). The V_m higher than −22 mV is too high for the membrane to depolarize (*top trace*). When V_m is lowered to −25 mV, one observes bursting (*middle*). When V_m is lowered further to −30 mV bursting disappears and repetitive spiking arises (*bottom*). Note that a decrease of V_m leads to a rise of both $[Ca^{2+}]_i$ and $[Ca^{2+}]_{lum}$. This rise of $[Ca^{2+}]_i$ is due to activation of a higher-threshold I_{Ca}, and a rise of $[Ca^{2+}]_i$ in turn gives rise to $[Ca^{2+}]_{lum}$.

Is luminal calcium necessary for exocytosis?

Recent experimental evidence (Blondel et al., 1994, 1995; Bokvist et al., 1995) indicates that the SGs may be involved in $[Ca^{2+}]_i$ in a nanodomain (Schwitzer et al., 1995), where a cluster of L-type Ca^{2+} channels exists. The $[Ca^{2+}]_i$ in the nanodomain is estimated to be as high as several micromolar, which seems to be required for exocytosis (Bokvist et al., 1995). In addition, the granular membrane contains an

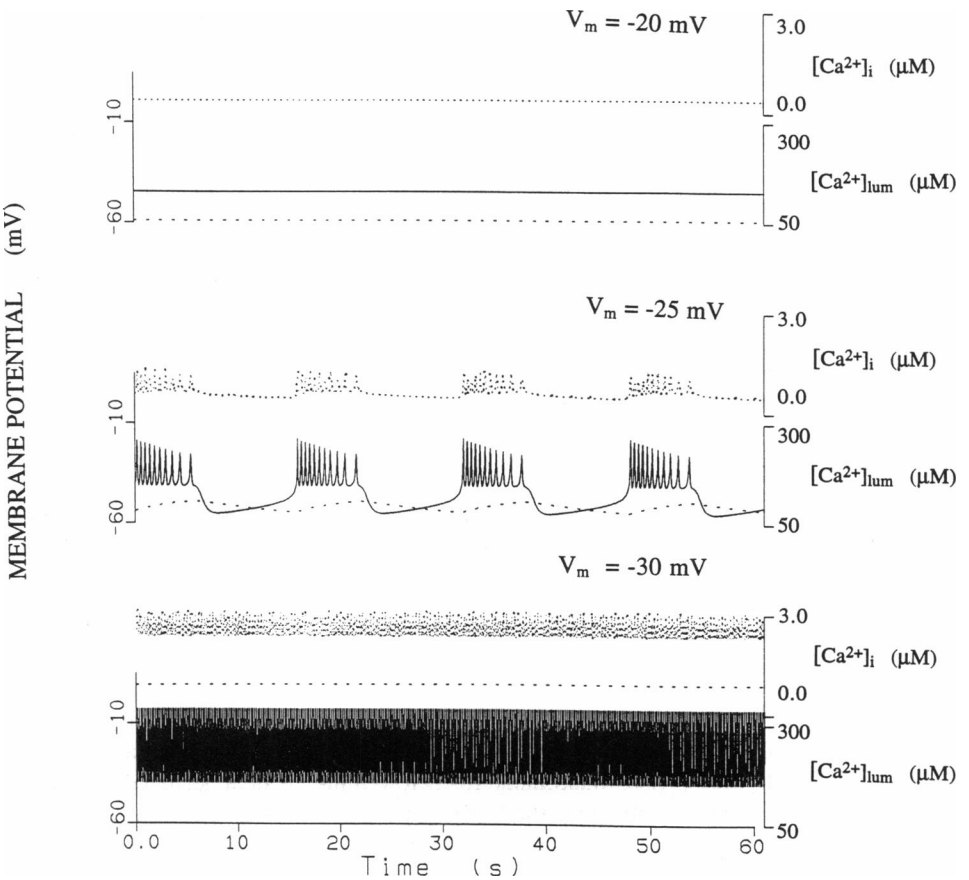

FIGURE 10 The effect of varying the half-maximal activation potential V_m of I_{fast}. The parameter V_m is increased from −20 mV (*top*) to −25 mV (*middle*) to −30 mV (*bottom*).

IP_3-receptor as well as a Ca^{2+}-ATPase (Blondel et al., 1994). It may even contain a cyclic ADP-ribose-releasable Ca^{2+} channel in addition to an IP_3-receptor controlled Ca^{2+} channel (Petersen, 1996). In this paper, it is shown (see Fig. 8) that the sequestration of SGs increases the plateau fraction. It is also shown that the increase in k_{Ca} leads to a slight decrease in $[Ca^{2+}]_i$ (although this decrease may not be detectable experimentally). This is somewhat puzzling since it is commonly believed that the higher $[Ca^{2+}]_i$ induces more insulin release and insulin is believed to be released during the plateau (Scott et al., 1981). The work in this paper suggests that filling the SG store with intragranular Ca^{2+} may be more important for exocytosis than a rise of $[Ca^{2+}]_i$.

Is exocytosis frequency-modulation or amplitude-modulation?

Insulin secretion is believed to be a frequency-modulated phenomenon (Schofl et al., 1995, 1996a, b), i.e., more insulin is released from β-cells as the frequency of bursting becomes faster. This can be seen by comparing bursting of single β-cells (whose burst period is several minutes, Smith et al., 1990; Hellman et al., 1990) with that of intact β-cells (whose burst period is tens of seconds, Dean and Mathews, 1970). In agreement with the frequency modulation hypothesis, the β-cells release 30-fold more insulin from islets than from single β-cells (Pipeleers, 1984). This higher release of insulin by intact β-cells is attributed to the concentration of cAMP released from the neighboring α-cells (Schuit and Pipeleers, 1985). This is further evidenced by the secretory response, which is markedly amplified when single β-cells are incubated in the presence of α-cells or glucagon (Gorus et al., 1984).

In my store-operated model, the calcium releasing channel in the ER is modulated by a secondary messenger, and I have demonstrated that an increase in activity of the CRC can bring about a drastic change in the frequency of bursting (see Fig. 9). However, note that the faster bursting causes a decrease in the amplitude of both electrical bursting and $[Ca^{2+}]_i$ oscillation. Note also that the $[Ca^{2+}]_i$ level becomes higher as the frequency becomes faster. This led to the conclusion that the ER may be an internal clock that regulates insulin secretion by adjusting the burst frequency as well as its amplitude (Chay, 1996d), i.e., both frequency and amplitude modulate insulin release.

How does coupling between β-cells affect synchronization?

It has been shown that a cluster of β-cells bursts faster (Gylfe et al., 1991) and secretes more insulin than do single cells (Pipeleers, 1984). Glucose has been shown to strengthen coupling between β-cells (Gylfe et al., 1991).

What are the mechanisms underlying these phenomena? We know that simple coupling does not cause a faster bursting (Chay and Kang, 1988). In fact, a simple coupling gives rise to a decrease in the frequency as more β-cells aggregate (Chay and Kang, 1988). Then, what cause the faster bursting? My hypothesis is that the faster bursting in a β-cell cluster is caused by the ATP (that is released along with insulin) trapped in a narrow interstitial space (Li et al., 1991). This trapped ATP can activate the P_{2y} receptor in β-cells, which in turn will release luminal Ca^{2+} from the ER. Why then are more β-cells synchronized when the glucose concentration is raised? My hypothesis is that synchronization is achieved by IP_3, which diffuses through gap junctions. Since more IP_3 can be produced by a higher glucose concentration (Kelly et al., 1994), wider synchronization can be achieved.

How would one explain triphasicity in islet of Langerhans?

Fig. 10 suggests the role that I_{fast} plays on the triphasicity after addition of glucose (Roe et al., 1993). Triphasicity consists of phase 0 where $[Ca^{2+}]_i$ decreases; phase 1 where V depolarizes and $[Ca^{2+}]_i$ rises maximally for a few minutes; and phase 2 where the cell enters the limit cycle. In the limit cycle, the $[Ca^{2+}]_i$ oscillates in a much higher level than that in the resting level. First, note that the triphasicity is a phenomenon associated with β-cells in the islet, i.e., isolated cells lacks phase 2. Second, the course of the triphasicity follows insulin release in the islet (Wollheim and Sharp, 1981). Third, a rise of $[Ca^{2+}]_i$ lags behind membrane depolarization (Worley et al., 1994). If the depolarization is a result of emptying the store, then depolarization should lag behind the $[Ca^{2+}]_i$ change. This implies that depolarization cannot possibly be due to activation of I_{NS} (in contrast to what was suggested earlier, Chay, 1996a, b). The initial depolarization is most likely triggered by a closure of the ATP-sensitive K^+ channel (Cook and Hales, 1984; Chay, 1985a; Himmel and Chay, 1987; Chay et al., 1990; Ghosh et al., 1991; Ronner et al., 1993).

Counting these facts, the triphasicity may be explained as follows: in the subthreshold glucose concentration of 2.8 mM, the two currents, I_{NS} and $I_{K,ATP}$, hold the resting potential of −70 mV. After raising the glucose concentration to 11.1 mM, the following sequence of events occurs. First, $[ATP]_i$ rises, and this activates the ICS ATPases. Phase 0 is due to sequestration of $[Ca^{2+}]_i$ by the Ca^{2+}-ATPases in ER and SGs. Sequestration of $[Ca^{2+}]_i$ by SGs is necessary for exocytosis to take place. Membrane depolarizes slowly due to a closure of the K(ATP) channels. Glucose may activate the PI-signaling pathway (Kelly et al., 1994), which in turn lowers the threshold potential of I_{fast}. When it is lowered sufficiently, the cell enters into phase 1. During phase 1 luminal Ca^{2+} is released from the ER, which further increases $[Ca^{2+}]_i$ and depolarizes membrane potential. The V_m will be lowered maximally first, then it will lift upward as the insulin release lessens. Phase 2 follows when insulin release enters the steady-state level. The simulations shown in Figs. 3–10 are those in the limit cycle regime.

Why then do single cells lack phase 2? In intact β-cells $[ATP]_i$ raised by a metabolite of glycolysis can activate the AC-transduction pathway (the pathway on the *right* in Fig. 2) due to neighboring α-cells, which secrete glucagon. The cAMP thus produced from this pathway enhances CRC activity. As shown in Fig. 9, enhanced CRC activity induces a higher frequency of bursting and a higher level of the $[Ca^{2+}]_i$ oscillation. This explains why intact β-cells exhibit triphasicity when glucose concentration is raised.

Why was reformulation of the mathematical model necessary?

Perhaps it would be informative to mention why and how our mathematical model of bursting cells has transformed over the past decade and half. The first bursting model is based on a slowly changing $[Ca^{2+}]_i$, which activates a voltage-independent small conductance Ca^{2+}-sensitive K^+ channel (Chay, 1983; Lee et al., 1983) or a voltage-dependent large-conductance K-Ca channel (Chay, 1986). The Ca^{2+} current in these models has the property of a T-type current (rather than that of an L-type). When evidence accumulates that the Ca^{2+} channel in β-cells is an L-type channel that is inactivated by intracellular Ca^{2+} ion, I showed that the L-type current alone can generate bursting, i.e., $I_{K\text{-}Ca}$ is not necessary for bursting (Chay, 1987). To obtain more robust bursting, Chay and Kang (1988) introduced two types of Ca^{2+} current—a low-threshold L-type current and a noninactivating high-threshold N-type current. In this class of models, $[Ca^{2+}]_i$ changes slowly, and this slow $[Ca^{2+}]_i$ change is what causes the slow underlying wave required for the bursting. These models are no longer acceptable since we know now that $[Ca^{2+}]_i$ is not a slow variable (i.e., the change of $[Ca^{2+}]_i$ takes place within tens of milliseconds when depolarization potential is applied).

To account for the fast $[Ca^{2+}]_i$ response that accompanies depolarization, several mathematical models have been proposed (Chay, 1990a–c; for review see 1993a,b). The essence of these models is that while the $[Ca^{2+}]_i$ is dynamically fast, the inactivation gating process of I_{Ca} is slow (i.e., takes place in the order of tens of seconds). In Chay (1990a,b) it was assumed that a Ca^{2+} channel contains a time- and voltage-dependent slow gating variable f, while in Chay (1990c) it was assumed that the binding-unbinding process of the Ca^{2+} ion at its receptor site takes place slowly in a voltage-dependent manner. These models give rise to $[Ca^{2+}]_i$ oscillation, which varies concomitantly with electrical bursting.

These models are based on evidence that I_{Ca} inactivates slowly in a voltage-dependent manner (Satin and Cook, 1989; Hopkins et al., 1990; Cook et al., 1991). However, there are two flaws in this mechanism. First, it lacks a slow

disappearance of $[Ca^{2+}]_i$ during the silent phase, evidenced by simultaneous measurements of membrane potential and $[Ca^{2+}]_i$ (Valdeolmillos et al., 1989; Worley et al., 1994a,b). This falling phase of $[Ca^{2+}]_i$ is slow enough to generate bursting via the $Ca^{2+}{}_i$-dependent ion channels in the plasma membrane. Second, the channel gating process is in general a fast dynamic process which takes place in the order of several hundred milliseconds at most—not several seconds or more as required to generate the bursting.

Then the question arises: why did Cook's group (Satin and Cook, 1989; Hopkins et al., 1990; Cook et al., 1991) observe slow inactivation of I_{Ca}? My hypothesis is that their patches are contaminated with other channels, which are patched along with the Ca^{2+} channels. The $I_{K(Ca)}$ and I_{NS} are resistant to ATP and TEA, and the slow inactivation could be due to these channels. The fact that their patches became outward at a step potential of ~40 mV and above supports my contamination hypothesis. Furthermore, voltage-dependent inactivation is due to a conformational transformation of the proteins induced by a charge movement. The conformation transformation is dynamically a very fast process. In view of these issues, it is not likely that the underlying wave of bursting owes its origin to the Hodgkin–Huxley-type voltage-dependent inactivation of the calcium channel.

When evidence accumulates that the ER is involved in bursting of R-15 in *Aplysia* neurons (Scholz et al., 1989), I began to examine the influence of the ICS on electrical bursting and $[Ca^{2+}]_i$ oscillation in pancreatic β-cells (Chay, 1991, 1993). Under the hypothesis that $[Ca^{2+}]_{lum}$ is the primary oscillatory, I have proposed several mathematical models that utilize the ICS. My first model (Chay, 1995) is based on the assumption that I_{NS} is responsible for the bursting. However, enough evidence exists that the bursting in β-cell depends on intracellular Ca^{2+} (Rosario et al., 1993). My second model (Chay, 1996c), utilizes the calcium-release activated current, I_{crac}, that is activated by depletion of the ICS (Randriamampita and Tsien, 1993). The existence of I_{crac} in β-cells, however, is not supported by experiment, which shows that calcium does not enter when I_{Ca} is blocked (Worley, 1994a,b). My third model (Chay, 1996a,b) incorporates a Ca^{2+}-blockable Ca^{2+} channel (L-type) in addition to I_{NS}. In this model, the slow disappearance of $[Ca^{2+}]_i$ is sufficient to generate bursting via Ca^{2+}-dependent inactivation of the L-type channel. In this paper, I have included $I_{K(Ca)}$ and I_{fast} in order to explain the extracellular Ca^{2+} effect. In the present model, the lower-threshold fast-activating I_{fast} activates the slower higher-threshold I_{Ca}, and $I_{K(Ca)}$ prevents an indefinite rise of membrane potential. Whether $I_{K(Ca)}$ coexists with I_{Ca} (where the inactivation mechanism of I_{Ca} is ineffective), or I_{Ca} with an effective Ca^{2+}-dependent inactivation mechanism exists alone, awaits experimental testing.

The central assumption of the store-operated models is that the bursting process is an intracellularly driven event, not a Hodgkin–Huxley-type mechanism. These models predict that $[Ca^{2+}]_{lum}$ oscillates slowly and that this slow oscillation is what gives rise to a slow component in $[Ca^{2+}]_i$. The slow component of $[Ca^{2+}]_i$, in turn, gives rise to electrical bursting via the $Ca^{2+}{}_i$-dependent ion channels in the plasma membrane. The dynamic change of $[Ca^{2+}]_{lum}$ may also control the bursting via I_{NS}. The CRC in the store-operated models is controlled by the neurotransmitters and hormones that influence the PI- and AC-signaling pathways.

Could oscillating G-protein generate the large-amplitude oscillation?

It should be pointed out that other possibilities may exist in regard to the mechanism involved in the large amplitude $[Ca^{2+}]_i$ oscillation (Grapengiesser et al., 1991). Evidence was presented in β-cells that $[IP_3]$ oscillates, and this oscillation drives the bursting via a small-conductance TEA-insensitive, apamin-insensitive Ca^{2+}-sensitive K^+ current (Ammala et al., 1991). This ties in very well with the receptor-operated model (Cuthbertson and Chay, 1991; Chay et al., 1995). This model assumes that the G-protein in the PI-signaling pathway is a slow dynamic variable and this slow variable drives the oscillations in IP_3 and protein kinase C (PKC). According to the Cuthbertson–Chay model, the agonist concentration controls the rate of the formation of the active form of the G-protein. The rate, in turn, controls the interval of the $[Ca^{2+}]_i$ oscillation—the interval ranging from several minutes to several seconds. The applicability of this model to β-cell bursting should certainly be pursued. Another possibility exists where the slowly oscillating G_S-protein may influence the K(ATP) channel. Indeed, evidence was presented by Ribalet et al. (1989) that the K(ATP) channel depends on cAMP-dependent protein. Further evidence was presented by Liu et al. (1994, 1995) that thapsigargin-sensitive store may not be involved in the large-amplitude Ca^{2+} oscillation, and it was suggested that the K(ATP) channel is involved in this type of oscillation.

Conclusion

The benefit of our modeling approach is that it helps clarify the main events that take place in the various interacting processes as well as predicting the areas requiring further experimental work. Our model predicts that 1) $[Ca^{2+}]_{lum}$ in the ER oscillates slowly in pancreatic β-cells, and 2) intragranular Ca^{2+} in SGs is involved in exocytosis. Experiments using fluorescent dyes such as mag-fura-2-AM (Chatton et al., 1995) or aequorin could provide relevant information. The existence of the transient low-threshold inward current (which may be different from a transient Ca^{2+} current) should be elucidated experimentally, perhaps by pursuing the line that Henquin's group has taken (Henquin et al., 1988; Gilon and Henquin, 1993; Gilon et al., 1995; Miura et al., 1996). This current is essential to explain the extracellular Ca^{2+} effect. To demonstrate its existence,

one suggestion is to demonstrate a rise of the repetitive firing in the absence of Ca^{2+} when TTX is present. The work presented in this paper is a clear demonstration of the power and usefulness of mathematical modeling.

APPENDIX

A) Fast current

$$I_{fast} = \bar{g}_{fast} m_\infty^3 h (V - V_{fast})$$

where

$$h_\infty = \frac{1}{1 + \exp[(V_h - V)/S_h]}$$

and

$$\tau_h^{-1} = \lambda_h \left(\exp\left[\frac{V_h - V}{2\,S_h}\right] + \exp\left[\frac{V - V_h}{2\,S_h}\right] \right)$$

B) Ca^{2+} current

$$I_{Ca} = p_{Ca} d f_\infty \frac{2FV}{RT} \left\{ \frac{[Ca^{2+}]_o - [Ca^{2+}]_i \exp(2FV/RT)}{1 - \exp(2FV/RT)} \right\}$$

where

$$d_\infty = \frac{1}{1 + \exp[(V_d - V)/S_d]},$$

$$\tau_d^{-1} = \lambda_d \left(\exp\left[\frac{V_d - V}{2\,S_d}\right] + \exp\left[\frac{V - V_d}{2\,S_d}\right] \right),$$

and

$$f_\infty = \frac{K_{Ca}}{K_{Ca} + [Ca^{2+}]_i}$$

C) Cationic nonselective inward current

$$I_{NS} = \bar{g}_{NS} \frac{K_{NS}^2}{K_{NS}^2 + [Ca^{2+}]_{lum}^2} \left(\frac{V - V_{NS}}{1 - \exp(0.1(V_{NS} - V))} - 10 \right)$$

D) Delayed-rectifying K^+ current

$$I_{K(DR)} = \bar{g}_{K(DR)} n (V - V_K)$$

where

$$n_\infty = \frac{1}{1 + \exp[(V_n - V)/S_n)]}$$

and

$$\tau_n^{-1} = \lambda_n \left(\exp\left[\frac{V_n - V}{2S_n}\right] + \exp\left[\frac{V - V_n}{2S_n}\right] \right)$$

E) Small-conductance calcium-sensitive K^+ current

$$I_{K(Ca)} = \bar{g}_{K(Ca)} \frac{[Ca^{2+}]_i^3}{K_{Ca}^3 + [Ca^{2+}]_i^3} (V - V_K)$$

F) ATP-sensitive K^+ current

$$I_{K(ATP)} = g_{K(ATP)} (V - V_K)$$

G) Na^+ leak current

$$I_{Na,L} = g_{Na,L} (V - V_{Na})$$

The basic parametric values in the model are as follows: $C_m = 1\ \mu F\ cm^{-2}$, $g_{fast} = 600\ \mu S\ cm^{-2}$, $p_{Ca} = 2.0\ nA\ cm^{-2}$, $g_{K(DR)} = 600\ \mu S\ cm^{-2}$, $g_{K(Ca)} = 5.0\ \mu S\ cm^{-2}$, $g_{NS} = 5.0\ \mu S\ cm^{-2}$, $g_{K(ATP)} = 2.0\ \mu S\ cm^{-2}$, $g_{Na,L} = 0.5\ \mu S\ cm^{-2}$, $V_{fast} = 80$ mV, $V_K = -75$ mV, $V_{NS} = -20$ mV, $V_{Na,L} = 80$ mV, $V_m = -25$ mV, $S_m = 9$ mV, $V_h = -48$ mV, $S_h = -7$ mV, $V_d = -10$ mV, $S_d = 5$ mV, $V_n = -18$ mV, $S_n = 14$ mV, $\lambda_d^{-1} = 0.08\ s^{-1}$, $\lambda_h^{-1} = 0.4$ s, $\lambda_n^{-1} = 0.08$ s, $K_{Ca} = 1.0\ \mu M$, $K_{NS} = 50\ \mu M$, $k_{Ca} = 7.0\ s^{-1}$, $k_{pump} = 30\ s^{-1}$, $k_{rel} = 0.2\ s^{-1}$, $\phi = 0.2$, $[Ca^{2+}]_o = 2500\ \mu M$, and T = 37°C.

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