RESEARCH ARTICLE

Ca²⁺ release or Ca²⁺ entry, that is the question: what governs Ca²⁺ oscillations in pancreatic β cells?

 Patrick A. Fletcher,[1] Ben Thompson,[2] Chanté Liu,[2] Richard Bertram,[3] Leslie S. Satin,[2] and Arthur S. Sherman[1]

[1]*Laboratory of Biological Modeling, National Institutes of Health, Bethesda, Maryland, United States;* [2]*Department of Pharmacology and Brehm Center for Diabetes Research, University of Michigan Medical School, Ann Arbor, Michigan, United States; and* [3]*Department of Mathematics and Programs in Neuroscience and Molecular Biophysics, Florida State University, Tallahassee, Florida, United States*

Abstract

The standard model for Ca²⁺ oscillations in insulin-secreting pancreatic β cells centers on Ca²⁺ entry through voltage-activated Ca²⁺ channels. These work in combination with ATP-dependent K⁺ channels, which are the bridge between the metabolic state of the cells and plasma membrane potential. This partnership underlies the ability of the β cells to secrete insulin appropriately on a minute-to-minute time scale to control whole body plasma glucose. Though this model, developed over more than 40 years through many cycles of experimentation and mathematical modeling, has been very successful, it has been challenged by a hypothesis that calcium-induced calcium release from the endoplasmic reticulum through ryanodine or inositol trisphosphate (IP3) receptors is instead the key driver of islet oscillations. We show here that the alternative model is in fact incompatible with a large body of established experimental data and that the new observations offered in support of it can be better explained by the standard model.

calcium-induced calcium release; calcium oscillations; endoplasmic reticulum; IP3 receptors; ryanodine receptors

INTRODUCTION

Science advances on two legs: an openness to new ideas and a healthy degree of skepticism. This Commentary will provide readers have the opportunity to exercise both of those legs. In a recent paper, Postić et al. (1) present a large body of new data on Ca²⁺ fluctuations and oscillations in islets of Langerhans observed in pancreatic slices from mice and argue that this study "demands an updated model of β cell activation and bursting activity."

The essence of their critique of the standard model and their proposed alternative is that standard methods consider only islets isolated from the pancreas, possibly damaging them, and then oscillatory responses are studied using unphysiological glucose concentrations. The baseline glucose that is typically used is 2–3 mM, which is below the in vivo baseline of ∼5 mM. In their view, the use of such low levels of glucose depletes the endoplasmic reticulum (ER) of Ca²⁺ and takes ER Ca²⁺ channels, ryanodine receptors (RYR), and inositol trisphosphate receptors (IP3R), "out of the game." Glucose is then often raised to 10–12 mM, well above typical postprandial levels seen under physiological conditions (e.g., in the absence of diabetes). Furthermore, cytosolic Ca²⁺ is most often monitored using Fura-2, a high affinity Ca²⁺ dye which they

consider to be too slow to pick up activity on the millisecond timescale and subject to saturation given its high affinity. Postić et al. use Calbryte 520 AM in their study, a lower affinity Ca²⁺ sensing dye that detects faster events and does not saturate in response to high Ca²⁺ transients. It is proposed that fast Ca²⁺ events due to ER Ca²⁺ release gated by ryanodine receptors combine, in some unspecified way, to generate the slow oscillations in activity observed on the time scale of tens of seconds to minutes.

In this Commentary we consider three questions: *1*) are the objections raised by Postić et al. to the standard methods of studying isolated islets valid? *2*) is the alternative model sketched out by the authors (see for example their Fig. 7) plausible? and *3*) can their observations be accounted for within the framework of the standard model? We announce in advance that the answers are "no," "no," and "yes." Our conclusions are consonant with another recent review of the mechanisms of islet Ca²⁺ oscillations, which found little evidence to support a major role for calcium-induced calcium release (CICR) (2). We will limit our discussion to mouse islets, the most well-studied preparation and the one also used by Postić et al., albeit in their case islets within intact pancreatic slices. We note, however, that there is considerable evidence for oscillations in human islets that are quite similar to those in mouse islets (3–5).

MATERIALS AND METHODS

Islet Isolation and Treatments

Pancreatic islets were isolated from 3-mo-old male Swiss-Webster mice (25–35 g) in accordance with the regulations of the University of Michigan Committee on the Use and Care of Animals (UCUCA). A previously described (6) and the approved animal protocol was followed. Isolated islets were cultured in standard RPMI 1640 medium containing 11 mM glucose, 10% fetal bovine serum (FBS), 10 mM HEPES, and 1% penicillin/streptomycin. To measure cytosolic Ca^{2+}, islets were either loaded with 2.5 μM of Fura-2AM for 45 min or with 10 μM Calbryte 520 AM for 1 h in a medium containing 11 mM glucose before imaging.

Modeling

Mathematical models were simulated using the `ode15s` function in MATLAB 2022b (Natick, MA: The MathWorks Inc.). Program code defining the models in both MATLAB and xppaut (https://sites.pitt.edu/~phase/bard/bardware/xpp/xpp.html) compatible files are freely available at https://doi.org/10.6084/m9.figshare.22529515.

Statistics

Plateau fractions and periods were extracted from raw Fura-2AM or Calbryte 520 AM data using a MATLAB program that we developed called OscAr (https://zenodo.org/record/7796008). Linear mixed effect modeling for Fig. 1 was carried out using the `fitlme` function in MATLAB 2021b. To assess the significance of glucose concentration (G) and Ca^{2+} sensor (S) for period and plateau fraction, we constructed the following model G and S as fixed variables with mouse and islet as random grouping variables:

$$X \sim 1 + G + S + (1 \mid \text{mouse}) + (1 \mid \text{islet}),$$

where X is either period or plateau fraction, and used the MATLAB compare function to compare that full model to one containing either only G or only S as fixed variables:

$$X \sim 1 + G + (1 \mid \text{mouse}) + (1 \mid \text{islet})$$

or

$$X \sim 1 + S + (1 \mid \text{mouse}) + (1 \mid \text{islet})$$

Five hundred bootstrap iterations were performed. The codes and sample data are posted at https://doi.org/10.6084/m9.figshare.22529515.

ARE THE OBJECTIONS TO THE STANDARD METHODS VALID?

The physiologically most relevant Ca^{2+}-related phenomena that occur in islets are oscillations having periods on two main time scales, fast bursting, with a period of 10–60 s (7) and slow bursting, with a period of 3–10 min (8–11). Fura-2 is
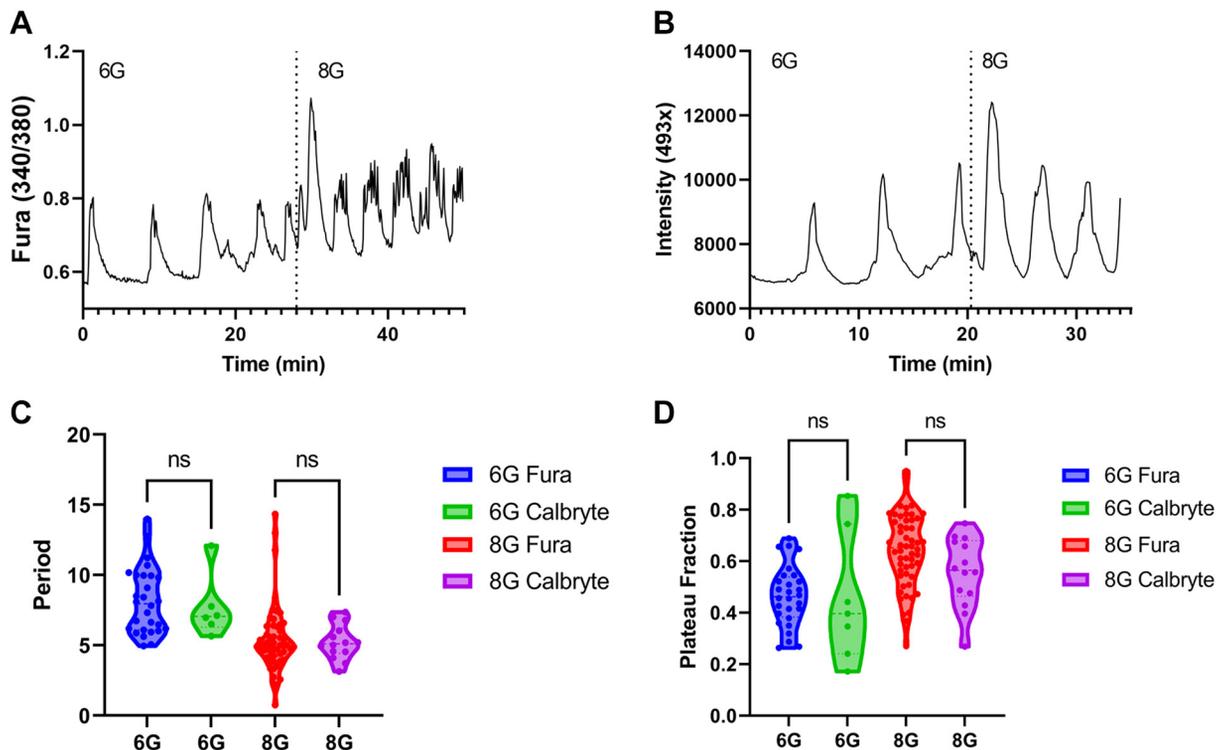
Figure 1. Comparison of cytosolic Ca^{2+} oscillations from isolated islets recorded using either Fura-2AM or Calbryte 520 AM. Representative whole islet recording of Fura-2AM (*A*) and Calbryte 520 AM (*B*) oscillations in 6 mM and 8 mM glucose solution. Comparisons of period by glucose and sensor are shown in *C* and of plateau fraction (PF) in *D*. Periods (means ± SD): 6 mM G: 8.1±2.4 (Fura), 7.7±2.3 (Calbryte); 8 mM G: 5.4±2.4 (Fura), 5.2±1.2 (Calbryte). Plateau fractions: 6 mM G: 0.47 ± 0.12 (Fura), 0.46 ± 0.13 (Calbryte); 8 mM G: 0.64 ± 0.14 (Fura), 0.56 ± 0.13 (Calbryte). Linear mixed effect analysis shows G highly significant for period (*P* = 2e-3) and PF (*P* = 2e-3), and sensor NS for both (*P* = 0.52 for period, 0.40 for PF). *n* = 58 islets from three mice for Fura-2AM and 14 islets from three mice for Calbryte 520 AM. 30/58 islets in Fura-2 AM and 7/14 islets in Calbryte 520 AM did not have oscillations in 6 mM glucose and were not included in the analysis. G, glucose concentration.

well able to capture both types of oscillations. These two time scales, together with the time scale of action potentials during each burst, correspond to the short, long, and ultrashort time scales shown in the Graphical Abstract of Postíc et al. These have all been studied in detail for over 30 years using Fura-2 and electrical recordings. The slower oscillations occur on the same time scale as pulsatile insulin secretion measured in vivo, which has a period of ~5 min [9]. Fura-2 has also been used to study changes in Ca²⁺ over a more diverse range of glucose concentrations than acknowledged by Postíc et al., where ER depletion is unlikely to play a role. See for example recordings made in 5 versus 8 mM glucose in the study by Glynn et al. [12] and 6–9 mM versus 11 mM in the study by Nunemaker et al. [13].

Fast Ca²⁺ transients to near 10 µM have been proposed to reflect nanodomains very near the mouth of voltage-activated Ca²⁺ channels (VACCs) of the β cell plasma membrane. These localized Ca²⁺ transients are likely important for insulin secretion, as a subset of insulin vesicles (the readily or immediately releasable pools) are tightly colocalized to the Ca²⁺ channels [14–18]. They also may play a role in inactivating the Ca²⁺ channels [19, 20] and activating large-conductance, voltage-dependent Ca²⁺-activated (BK) potassium channels [21, 22]. Although these processes (which occur on a milliseconds-seconds time scale) shape β-cell action potentials, they are unlikely to play a critical role in the slow Ca²⁺ oscillations governed by bursts of action potentials (time scale tens of seconds to minutes) described in detail later.

Published measurements of Fura-2 fluorescence values agree with independent measurements of membrane potential V_m with electrodes [6, 8, 23] and voltage-sensitive dyes [23], Perceval (to monitor ATP/ADP) [11, 24, 25], NAD(P)H auto-fluorescence [24–26], rhodamine 123 (to measure mitochondrial membrane potential) [27], oxygen consumption [28], and Zimir (to measure extracellular zinc coreleased with insulin) [28, 29], for instance. Electrophysiological measurements of V_m in particular can faithfully capture very fast events, but when measured simultaneously with Fura-2, the two align perfectly with respect to the slow events [6, 8], contrary to the implication of Postić et al. that oscillations in V_m are an order of magnitude faster than the oscillations recorded using Fura-2. The Fura-2 oscillations also align well with insulin secretion oscillations when the two are measured simultaneously [6, 8, 30].

If Fura-2 measurements were truly deficient, they would not agree with all these other types of measurements. Finally, while Postić et al. fail to mention metabolic oscillations, we have recently provided new data and modeling [31] that support metabolic oscillations as the key driver of slow oscillations, and not just a readout of free Ca²⁺ levels. Another model in current use differs with our model on this question but is solidly in the camp of the standard model, as oscillations in it too are governed primarily by Ca²⁺ influx, not Ca²⁺ release [32–34].

Figure 1 summarizes results obtained in our own experiments using either Fura-2AM or Calbryte-520 AM to monitor islet Ca²⁺ oscillations. Representative whole islet recording of Fura-2AM (Fig. 1A) and Calbryte 520 AM (Fig. 1B) oscillations recorded in 6 mM and 8 mM glucose are shown. Analyses of the periods and plateau fractions of the oscillations are shown

by the violin plots in Fig. 1, C and D. Recordings made using Calbryte 520 AM were less noisy than those of Fura-2, as expected from their differing Kds. Thus, the key parameters characterizing Ca²⁺ oscillations, their frequency and plateau fraction, do not differ significantly from those obtained from the analysis of Fura-2 oscillations, and therefore we do not believe that mechanistically important features of islet bursting have been overlooked by the use of Fura-2 in experiments.

Published electrical recordings made from mouse islets in vivo, an even more intact preparation than the pancreatic slices used by Postić et al., look strikingly similar to those measured in vitro [35, 36]. This confirms the essential validity of studies of isolated islets and conclusions drawn therein.

Oscillations measured in solutions containing 6 or 8 mM glucose look very similar in fact to those seen in 10–15 mM glucose, differing only quantitively, with lower frequency and half-width (Fig. 1). We therefore do not consider it plausible that different mechanisms control islet oscillations in 6 versus 10 mM glucose.

Although we agree that the ER can be depleted of free Ca²⁺ after islets are exposed to low glucose for several minutes, by the same token, the ER can also refill quickly once glucose is raised, as confirmed by Ravier et al. [37]. Since experimental studies of islet Ca²⁺ signaling last tens of minutes or longer, the initial filling state of the ER is unlikely to influence the measurements of steady-state free Ca²⁺ oscillations. We address later the nature of the initial transient rise of Ca²⁺ seen in response to a stepwise increase in glucose, another important question.

Taken together, the six main points raised earlier clearly do not support a need to overthrow the current model of islet oscillations, as suggested by Postić et al.

IS THE ALTERNATIVE MODEL PLAUSIBLE?

There are important theoretical considerations as well as established experimental observations that argue against an alternative model where the release of intracellular Ca²⁺ is the driver of islet oscillations, as detailed later.

In any model in which the rise in cytosolic-free Ca²⁺ occurs mainly because of ER Ca²⁺ release, changes in ER Ca²⁺ must be out of phase with those of cytosolic Ca²⁺. This was shown early on [38, 39] and illustrated in Fig. 2 using the model of Li and Rinzel [40]. The oscillations in this model are due to spike-like releases of Ca²⁺, a form of CICR mediated by IP3 receptors. The same phase relationship between cytosolic and ER Ca²⁺ would hold in a model based on CICR involving RyR. In islets, however, ER Ca²⁺ has been shown clearly to be in phase with cytosolic Ca²⁺, indicating that Ca²⁺ release cannot be the driver of rises in cytosolic Ca²⁺ [37].

This does not mean, however, that CICR or ER Ca²⁺ release more generally does not occur in β cells. Indeed, Ravier et al. [37] provide evidence (see Fig. 5 in Ref. 37) that Ca²⁺ release events can occur in response to strong depolarizing stimulation with KCl or tolbutamide. During those events, ER Ca²⁺ fell as cytosolic Ca²⁺ rose. Most importantly, repetitive Ca²⁺ releases did not occur in parallel with cytosolic Ca²⁺ oscillations in their studies. We are also not saying that ryanodine or IP3 receptors cannot play a role in

Figure 2. Simulations with a version of the Li–Rinzel model for endoplasmic reticulum (ER)-driven Ca²⁺ oscillations (40) show that cytosolic and ER Ca²⁺ are out of phase and that SERCA blockade, modeled as a 50% reduction in SERCA pump activity, acutely terminates oscillations (*A*). The two-parameter diagram of SERCA activity vs. inositol trisphosphate (IP3) concentration in (*B*) shows the border between oscillations (with period color-coded) and no oscillations (gray region).

type 2 diabetes or potentially be useful drug targets for treating disease. However, these are entirely different questions from how oscillations are generated by β cells. We do remind readers that the role of KATP channels in disease, including congenital hyperinsulinemia, neonatal diabetes mellitus, and type 2 diabetes, is well established (41–43).

As also shown in Fig. 2, oscillations in the Li-Rinzel model stop very soon after SERCA is blocked and long before the ER is depleted. This is because the oscillations depend on cycles of Ca²⁺ reuptake and release, not just Ca²⁺ release. The two-parameter plane shows that the threshold for oscillations depends on the balance between IP3 and the SERCA rate. It demonstrates that SERCA blockade robustly terminates oscillations, but the degree of block required depends on the concentration of IP3, and hence on the strength of the stimulus. The behavior shown by the model is characteristic of pituitary gonadotrophs, as confirmed experimentally in Fig. 4 of Li et al. (44).

In islets, however, slow oscillations persist with only quantitative changes in their frequency and shape after the ER is depleted by the SERCA blocker thapsigargin, indicating at best a minor role for ER Ca²⁺ dynamics in oscillation genesis (45, 46). This work is cited in passing by Postić et al., but without comment on its implications for their model. However, the persistence of oscillations when the ER is depleted is incompatible with any model in which ER Ca²⁺ release is the main driver of Ca²⁺ oscillations.

In contrast, the findings by Zhang et al. (6), Gilon et al. (30), and Arredouani et al. (45, 46) are fully compatible with the standard model in which rises in cytosolic Ca²⁺ are mainly due to Ca²⁺ entry through VACCs. Fast oscillations are more affected by ER Ca²⁺ depletion (47, 48), which Postić et al. confirm, but this can be explained by the activation of small inward plasma membrane ion currents due to store-operated channels (SOC) (47, 49). SOC turns on when the ER Ca²⁺ concentration is reduced (50, 51) (see CAN THE OBSERVATIONS OF POSTIĆ ET AL. BE EXPLAINED BY THE STANDARD MODEL? for more about modeling of SOC in β cells). SOC can link ER Ca²⁺ status to plasma membrane electrical activity and concomitant Ca²⁺ influx, so it was surprising that despite their emphasis on the importance of ER Ca²⁺ for oscillations, SOC does not appear to play a major role in their verbal model.

The standard model does not, however, require that SOC channels be activated to trigger islet electrical activation under normal circumstances. What is required, as long known, is that glucose be at a suprathreshold level (i.e., >6 or 7 mM). We believe that K(ATP) closure by glucose metabolism is necessary and sufficient (together with VACCs) to trigger electrical bursting and concomitant islet Ca²⁺ oscillations. However, given the high electrical impedance of islet cells, which is on the order of gigaohms, small currents mediated by other channels can push islets that are near threshold over the edge and trigger bursting. This is likely to be the case for the glucose concentrations studied in the study by Postić et al.

Postić et al. propose that the role of VACCs in β cells is primarily to refill the ER. There is a cell type in which oscillations work that way, the pituitary gonadotroph. Bursting in these cells is due to the activation of Ca²⁺-activated K⁺ (K(Ca)) channels, which interrupt otherwise continuous spiking with periodic transient silent phases. But in this case, V_m is out of phase with cytosolic Ca²⁺ (52). This was demonstrated experimentally by using K(Ca) channels as a reporter for cytosolic Ca²⁺, as shown in Fig. 3, *A* and *B*, reprinted from that paper. We also show this in a model simulation in Fig. 3C using a version of the Li-Rinzel model adapted from the same paper. Note that in gonadotrophs the action potentials, largely driven by Na⁺ current, bring in very little Ca²⁺, and almost all of the Ca²⁺ rise results from ER release (52).

In contrast, published recordings in which V_m and Ca²⁺ are measured simultaneously show that this is not the case in β cells (6, 8, 53). A model of β cells similar to the Li-Rinzel model was proposed many years ago for β cells (54), but objections were immediately raised based on the voltage-dependent nature of islet Ca²⁺ oscillations, evidenced notably by the ability of applied current pulses to switch between active and silent phases (55).

When glucose is first stepped from a subthreshold concentration to >7 mM, a first phase of intense action potential firing from a maintained plateau is observed that lasts a few minutes before the beginning of steady-state bursting. Postić et al. assert that this first phase is due to ER Ca²⁺ release, a hypothesis previously proposed by Worley et al. (56). If this were true, one would expect then that lowering ER below its
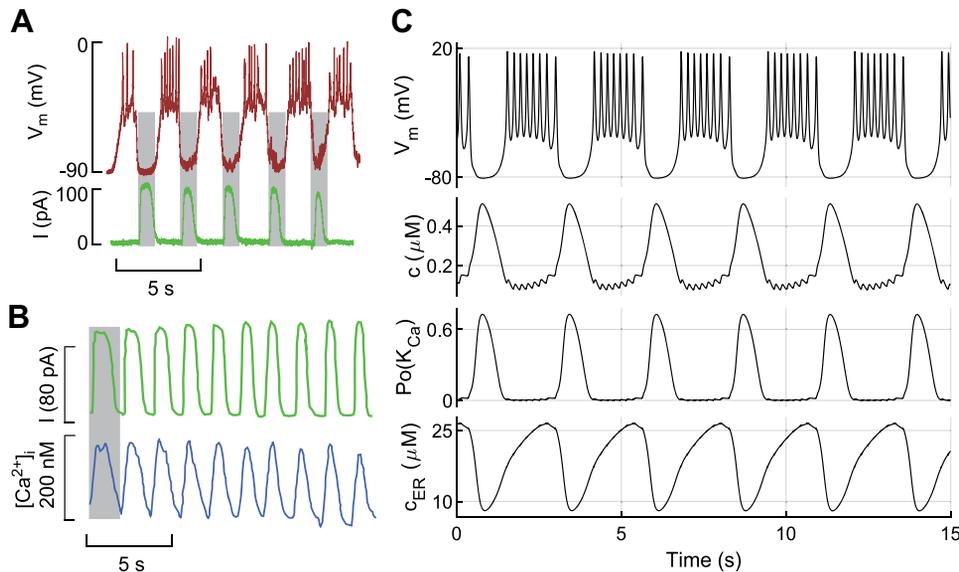
Figure 3. During endoplasmic reticulum (ER)-driven Ca^{2+} oscillations in pituitary gonadotrophs, membrane potential and cytosolic Ca^{2+} are out of phase. Experimental records reprinted with permission from Ref. 52 show that membrane potential is out of phase with K$^+$ current from K(Ca) channels (*A*) and that K(Ca) current is in phase with cytosolic Ca^{2+} (*B*). Together, these imply that V_m is out of phase with Ca^{2+}. *C*: simulations with the Li–Rinzel model (52) coupled to Hodgkin–Huxley type equations for membrane potential, again showing that membrane potential and cytosolic Ca^{2+} are out of phase. The ER subsystem is modeled as in Fig. 2.

level in low glucose would eliminate this first phase activity. In reality, the opposite happens: the first phase is prolonged when the ER is depleted by removing external Ca^{2+}. Conversely, increasing ER Ca^{2+} by applying KCl before raising glucose shortened the first phase (47). This result was predicted by simulations with mathematical models based on the hypothesis that the intense spiking during the first phase was a consequence of SOC activated when the ER is depleted (47). These observations are therefore incompatible with the hypothesis that the first phase is predominantly a Ca^{2+} release event but are consistent with the standard model.

Postić et al. cite a previous publication (57) as support for their hypothesis that the first-phase Ca^{2+} transient occurs due to Ca^{2+} release. However, the reference cited concerns the spike of cytosolic Ca^{2+} observed in response to acetylcholine in a slice preparation. Although that spike genuinely results from a Ca^{2+} release event that would be prevented by ER depletion, it is an entirely different phenomenon from the first phase Ca^{2+} rise that occurs when glucose is first raised above threshold. The addition of acetylcholine to 6 mM glucose initiated Ca^{2+} oscillations in the cited paper. We believe this is another example of SOC pushing the islet over threshold. If the glucose concentration had been lower, and hence K(ATP) conductance higher, acetylcholine addition would have been unable to initiate Ca^{2+} oscillations. Furthermore, if glucose had been higher, acetylcholine would have been unnecessary to trigger oscillations. Glucose is the primary physiological trigger of islet oscillations.

In summary, not only does the Ca^{2+}-release mechanism proposed by Postić et al., and others previously, make predictions that are contrary to established data for β cells, we believe that any alternative model based on intracellular Ca^{2+} release will fail to account for β-cell Ca^{2+} data.

CAN THE OBSERVATIONS OF POSTIĆ ET AL. BE EXPLAINED BY THE STANDARD MODEL?

The standard model we have been referring to has evolved over more than 40 years through an iterative process of model predictions and experimental testing. Importantly, the model has accumulated a strong record of accounting for a diverse array of observations from many individual laboratories. Furthermore, the continuous development of the model has occurred with its scope increasing as theoretical and experimental knowledge have advanced in parallel. The early development of the standard model is summarized by Sherman (58) and later developments are covered by Bertram et al. (59, 60) and Felix-Martinez and Godinez-Fernandez (61). Judging by the narrative in Postić et al., one might get the impression that the standard model is unable to explain the new observations from pancreatic slices. Nonetheless, it can.

Pioneering experiments by Atwater et al. (62), Dean and Matthews (63, 64), and Meissner and Schmelz (65) in the 1960s and 1970s first established that membrane potential oscillations in the form of electrical bursting (alternation of silent and active/spiking periods) are glucose-dependent, and later work showed that they are the cause of the parallel Ca^{2+} oscillations (6, 8, 53). The plateau fraction (duty cycle) of the bursting oscillations correlates well with the rate of insulin secretion (62, 65), and increases as the glucose concentration is increased, until a saturating point at which the spiking is continuous (i.e., the active phase never terminates).

In 1983, a mathematical model developed by Chay and Keizer (66) showed for the first time that these features could be explained by an ion channel-based model of the Hodgkin–Huxley family in which the spiking during the burst active phase brought in extracellular Ca^{2+}. This would progressively activate K(Ca) channels until the total level of K$^+$ conductance became too high to support spiking. In the model, pumping Ca^{2+} out of the cell during the silent phase reduced K(Ca) channel conductance, allowing a new active phase to begin. This "minimal model" also predicted that cytosolic Ca^{2+} would oscillate, which was confirmed experimentally in 1989 (67), albeit with some differences in shape from the initial predictions. These differences led to an appreciation that the ER has an important role in shaping the cytosolic Ca^{2+} transients, and an ER Ca^{2+} compartment was incorporated into revised mathematical models (49, 68,

69). These models predicted that ER Ca^{2+} would oscillate in-phase with cytosolic Ca^{2+}, which was confirmed by experiments using D4ER, a genetically encoded Ca^{2+} sensor targeted to the ER (37). As already indicated, a replete, oscillating ER was nonetheless not required for oscillations, especially slow oscillations, to occur. Instead, the ER acted like a low-pass filter and in doing so improved the model's ability to simulate the experimentally observed dynamics of islet cytosolic Ca^{2+} signaling.

In 1983, the ATP-dependent K$^+$ channel (K(ATP)), first discovered in cardiomyocytes (70), was found in β cells (71, 72) and shown by experiment to have the right properties to transduce changes in glucose concentration into the appropriate level of electrical activity without a need for other external factors. The K(ATP) channel essentially senses the cytosolic ATP/ADP ratio of the cell, an indicator of the rate of glucose metabolism, and regulates Ca^{2+} entry through VACCs. The Chay–Keizer model, equipped shortly thereafter with K(ATP) channels, was able to show that progressive block of the channel by increasing ATP/ADP would lead to increased plateau fraction and increased Ca^{2+} (73).

Postić et al. cite the well-known observation that the ATP/ADP ratio rises before K(ATP) channels close as evidence that other channels are involved in the bursting mechanism (74, 75). This is true: VACCs must also open, and that cannot happen until the K(ATP) conductance falls sufficiently. It is likely that a conductance active at low membrane potential, such as a leak conductance, is also needed to bridge the gap between K(ATP) channel closure and VACC opening, but the identity of such a conductance remains unknown (76). As K(ATP) channels are present in great excess, there is a delay between the ATP/ADP rise and Ca^{2+} entry. Indeed, a dip in cytosolic Ca^{2+} (called phase 0) due to SERCA activation generally precedes the initiation of action potential spiking and the frank rise in cytosolic Ca^{2+} (29, 56, 77). If anything, these observations support the primacy of Ca^{2+} entry over Ca^{2+} release.

Limitations of the earlier models based on K(Ca) channels led to a further extension of the original Chay–Keizer framework in which KATP channels were indirectly regulated by Ca^{2+} through its effects on the ATP/ADP ratio, by shunting the mitochondrial membrane potential that drives ATP production (78), activating dehydrogenases (79, 80), and by activating Ca^{2+} ATPases (81). Models combining K(Ca) and K(ATP) mechanisms were able to account in fine detail for the shapes of Ca^{2+} transients, the long first-phase of spiking that is observed when glucose is first elevated and precedes steady-state oscillations, and the depolarization and increased burst frequency seen when islets in elevated glucose are exposed to acetylcholine (6, 49). Finally, the models can account for fast oscillations, slow oscillations, and compound oscillations, in which the fast occur superimposed on the slow, within a quantitative and unified framework. In contrast to the alternative explanations put forth in Postić et al., which do not provide a mechanism by which fast elementary release events summate to produce slow oscillations, the standard model is based on well-established processes of Ca^{2+} accumulation and ATP production and consumption that have intrinsically slow timescales.

We now turn to the key specific observations contained in Postić et al. In their Fig. 3, they showed that stimulating RyR using low-dose ryanodine turned on oscillations in 6 mM glucose, where they usually did not see them, and in Fig. 4 they showed that blocking RyR using high-dose ryanodine inhibited oscillations in 8 mM glucose, where they usually did see them. They concluded from these observations that RyRs are both "necessary and sufficient" to mediate islet Ca^{2+} oscillations.

Our model offers a different interpretation. When glucose is near threshold (as was the case here, where glucose was 6 mM), a small variation in SOC conductance can turn bursting on and off because the effects of small differences in total membrane conductance on overall islet electrical behavior are amplified in this case. In contrast, at higher glucose levels, RyRs are generally unable to turn bursting on or off, and at best can only modulate the properties of islet bursting (illustrated in the model simulation using a version of the integrated oscillator model (IOM) (59, 82) in Fig. 4A).

The two-parameter plane in Fig. 4B shows that this is a robust feature of the model and that the boundary of the region in which oscillations occur (i.e., the threshold) depends on the balance between the rate of efflux of Ca^{2+} from the ER (p_{leak}) and glucose concentration. The rate of efflux determines the concentration of ER Ca^{2+}, and hence the SOC conductance, whereas the glucose concentration determines the K(ATP) conductance. As glucose increases, K(ATP) conductance decreases and less contribution from SOC is needed to support oscillations. Note that ER Ca^{2+} efflux is modeled here as a passive leak; in contrast to the Li–Rinzel simulations in Figs. 2 and 3, no spikes of release due to CICR are required to explain this phenomenon.

We have reported previously that a shift like this occurs spontaneously in β cells undergoing ER stress induced by tunicamycin and confirmed that the shifted threshold is mediated by SOC (83). At high glucose (11 mM), SOC and tunicamycin had only modest effects, as expected from the model parameter diagrams in Fig. 4B and Fig. 5B. The effects on electrical activity were paralleled by effects on insulin secretion. These results suggest that cells use SOC to adjust the threshold determined by K(ATP) in a physiological manner.

We have not been able to experimentally verify that a low dose of ryanodine consistently activates bursting in low G conditions, but we note that Postić et al said they saw this "occasionally." Also, it is not clear that the lack of need for ryanodine in higher glucose can be tested as ryanodine may not be reversible (84).

However, in similar fashion the model predicts that the SERCA inhibitor cyclopiazonic acid (CPA), which is readily reversible, can trigger oscillations in low G but is not needed in higher G (Fig. 5). The two-parameter diagram in Fig. 5 shows strikingly that the dependence of oscillations on SERCA activity in the IOM is opposite to that for the Li–Rinzel model (or any model of that class): in the IOM decreasing SERCA activity triggers oscillations, whereas in Li–Rinzel, it abolishes oscillations (compare with Fig. 2). Figure 6 shows experimental confirmation of this model prediction using isolated islets where cytosolic Ca^{2+} was imaged with Fura-2. The acute application of 25 μM CPA to a Fura-2 loaded islet triggered cytosolic Ca^{2+} oscillations in 5 mM glucose. Increasing the glucose concentration to 8 mM in the continued presence of CPA increased oscillation
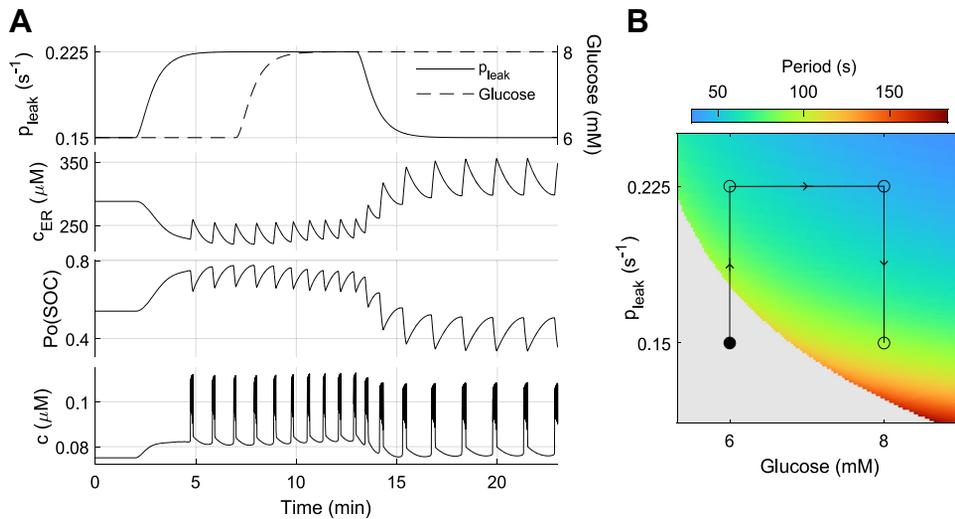
Figure 4. Simulations with the integrated oscillator model (IOM) (59) for coupled oscillations in membrane potential, cytosolic and endoplasmic reticulum (ER) Ca²⁺, and ATP/ADP ratio, as extended in Ref. 82. The model has been supplemented with a store-operated Ca²⁺ (SOC) current to represent the effects of ER Ca²⁺ on membrane potential. *A*: starting in 6 mM glucose, no oscillations are seen, but increasing ER Ca²⁺ leak rate (p_{leak}) by 50% to represent application of low-dose ryanodine triggers oscillations. After raising glucose to 8 mM, oscillations, increased leak rate is no longer required for oscillations to continue. *B*: two-parameter diagram shows regions of oscillation as ER leak rate and glucose concentration are varied (color indicates oscillation period; gray area denotes space where no oscillations occur).

frequency and plateau fraction. Notably, the oscillations persisted after removing CPA.

Consistent with our model, but in contradistinction to that of Postić et al., emptying the ER of Ca²⁺ using CPA triggered oscillations in 5 mM *G* rather than abolishing them, and CPA can be removed without abolishing bursting if glucose is raised to a sufficiently high level.

Regarding their reported effect that high dose (100 μM) ryanodine blocked Ca²⁺ oscillations in 8 mM glucose (their Fig. 4), we did not find this in our experiments. Figure 7 shows a representative recording of steady-state islet Ca²⁺ oscillations in 8 mM glucose before and after the acute application of 100 μM ryanodine (Ryan). These data showing that high-dose ryanodine failed to block islet Ca²⁺ oscillations support the hypothesis that Ca²⁺ influx, and not ryanodine-receptor mediated Ca²⁺ release, mediates islet Ca²⁺ oscillations. Nonetheless, the diagram in Fig. 4B predicts that blockage of RyR can push the cells below threshold in 8 *G* by reducing SOC if at rest RyRs contribute to Ca²⁺ efflux from the ER. This can also fail to occur depending on the boundary of the region of excitability and the size of the leak due to RyR, which may explain why we did not see block in our

own experiments. Thus, the work of Postić et al. is significant for pointing to the possible role of RyR in shaping the conditions under which Ca²⁺ influx operates.

Another objection to the standard model raised by Postić et al. involves the drug isradipine, an L-type VACC blocker. Their Fig. 5 showed that the effects of isradipine were slow to take effect, although the recorded Ca²⁺ transients they observed were eventually largely abolished. They attributed this slow action to the time required for the ER Ca²⁺ to drain, since there was no longer a way to restore ER Ca²⁺ when VACC-mediated Ca²⁺ influx to the cell was blocked by isradipine. Once the ER was sufficiently drained of Ca²⁺ their interpretation was that the ER Ca²⁺ release events would stop.

The literature is somewhat inconclusive regarding the effects of L-type channel blockers on islet Ca²⁺ activity, with some published experiments showing that Ca²⁺ oscillations can continue when L-type calcium channels are blocked (85), whereas the data on insulin secretion is more clearcut, with insulin secretion shown to be completely or almost completely abolished by Ca²⁺ channel blockers (85, 86). Figure 8 shows that in our hands steady-state islet Ca²⁺ oscillations in 11 mM glucose were rapidly and reversibly
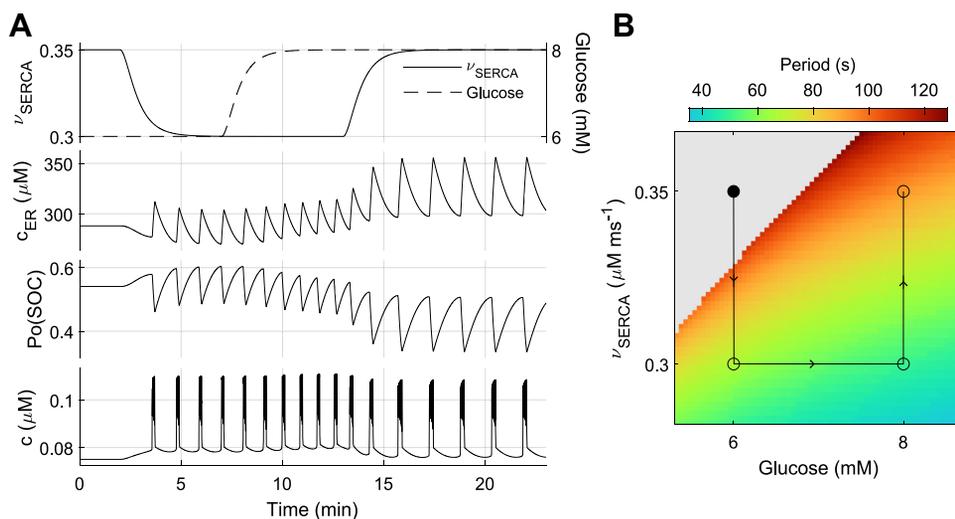
Figure 5. Simulations with the integrated oscillator model (IOM) as in Fig. 4. *A*: blocking the SERCA pump under subthreshold conditions can trigger Ca²⁺ oscillations. After raising glucose, SERCA blockade is no longer required to maintain the oscillations that were triggered initially by cyclopiazonic acid (CPA). *B*: two-parameter plane shown is as in Fig. 4 but showing regions of oscillations as SERCA rate and glucose are varied.
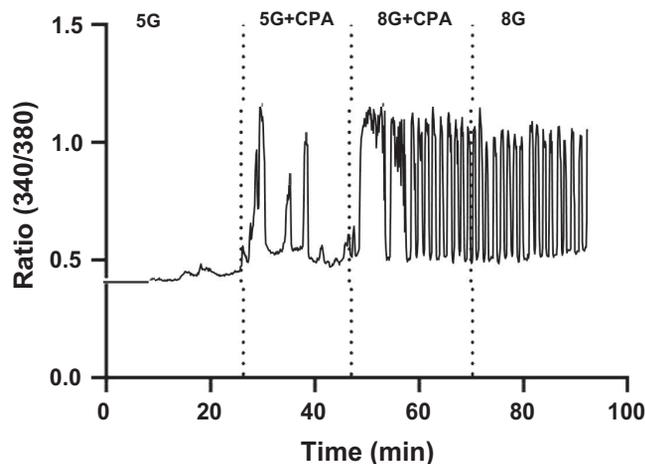
Figure 6. Experimental confirmation of the prediction shown in Fig. 5 using the reversible SERCA blocker cyclopiazonic acid (CPA). Representative image showing that the acute application of 25 µM CPA induces cytosolic Ca²⁺ oscillations in 5 mM glucose in an isolated mouse islet. Upon increasing the glucose concentration to 8 mM while in the continued presence of CPA, the oscillation frequency and plateau fraction increased (not shown). Oscillations persisted after washing off CPA. Representative responses are from 30/38 islets isolated from three different mice.

blocked by addition of the L-type calcium channel blocker nifedipine in isolated mouse islets.

We think the slow response observed by Postić et al. does not reflect a problem with the standard model but is more likely a sign of slow solution exchange in the pancreas slice preparation, where the effects of solution changes they observed never occurred in less than 3 min; this is less of a problem in experiments with isolated islets. In addition, it is known that β cells also have VACCs that are non-L type and hence would be untouched by dihydropyridine blockers; these can contribute to Ca²⁺ influx as well (85, 87, 88).

Postić et al. also blocked Ca²⁺ entry using diazoxide, a K(ATP) channel opener that hyperpolarizes the cell and should deactivate all VACCs, not just L-type ones (their Supplemental
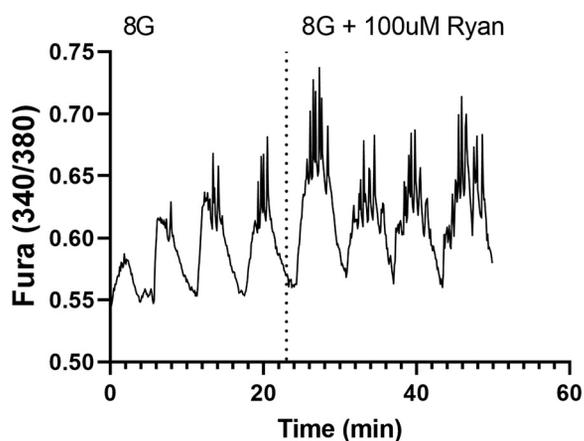
Figure 7. High-dose ryanodine did not block oscillations in 8 mM glucose. Representative recordings showing steady-state islet Ca²⁺ oscillations recorded in 8 mM glucose before and after acute application of 100 µM ryanodine (Ryan). These data support the hypothesis that Ca²⁺ influx, and not ryanodine receptor-mediated Ca²⁺ release, mediates islet Ca²⁺ oscillations. Data shown are representative of 37 islets isolated from three mice.
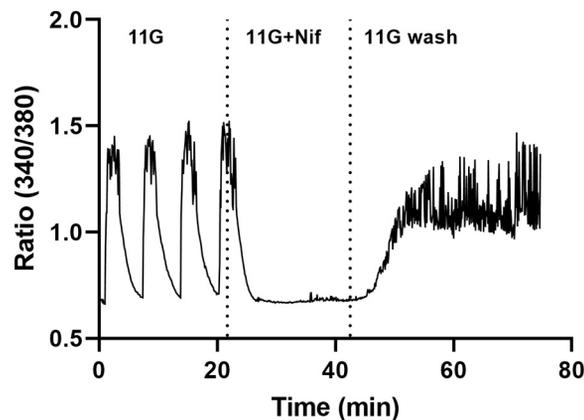
Figure 8. A representative recording showing the acute application of 10 µM nifedipine (Nif) reversibly inhibited cytosolic Ca²⁺ oscillations in 11 mM glucose in an isolated mouse islet. The response shown is representative of 25/27 islets isolated from two different mice.

Fig. S4, 2 and 3). They again highlight the slow and incomplete response to diazoxide they observed in their system. However, we believe this too is a sign of slow solution exchange, as in our hands, diazoxide added to islets exposed to suprathreshold glucose rapidly and completely abolishes Ca²⁺ oscillations, reducing Ca²⁺ to baseline levels (see for example Fig. 5 in Ref. 25). As Postić et al. point out, in high glucose the ER is expected to be replete with Ca²⁺ and therefore should be well able to mediate Ca²⁺-induced Ca²⁺ release. In our experiments, and those of many others, sustained islet Ca²⁺ oscillations are never observed in diazoxide even when the islet is depolarized with high KCl to maintain Ca²⁺ entry.

In sum, the standard model can well account for the key observations made by Postić et al., using their preparation and their glucose levels. In addition, the standard model can successfully account for a wide array of other findings made by many groups. To cite just a few examples, it can account for the effects of ER Ca²⁺ release on cytosolic Ca²⁺ and membrane potential (49); it can elucidate the role of K(ATP) channel mutations in human diseases (34); it has shown how exocytosis is coupled to Ca²⁺ and glucose metabolism (82, 89); it can explain oscillations in cAMP (90, 91); it can be used to study the implications of heterogeneity of β-cell ion channels and gap junctional coupling in islets (92–94); and it can explain the diversity of oscillation patterns on time scales ranging from seconds to minutes (59). The first steps of extending the model to human β cells have been taken (5).

CONCLUSIONS

We thus conclude that the standard model is not significantly challenged by the slice data presented by Postić et al. and is in fact well able to account for many observations that their alternative model fails on. We do not, however, claim that current versions of the standard model account for everything or have no need for improvement.

We do feel that better understanding the role of intracellular Ca²⁺ release in β cells is worthwhile. However, as we pointed out earlier, these ideas have been proposed previously, albeit not subjected to the discipline of mathematical modeling to see if they hold together in a quantitative and

consistent way. We ourselves have attempted to fill this gap by modeling CICR as a possible mechanism for islet oscillations and found that it led to counterfactual predictions, most notably that the ER would empty during the active phase of bursting (see Fig. 9 in Ref. 38). We cannot rule out that some model not previously considered would be more consistent with what we know about β cells and welcome renewed attempts by others to flesh out the ideas in Postić et al. in a quantitative, mathematical form. We must say that we are not, however, optimistic that this can be made to work, as we have discussed herein.

We acknowledge that the novel pancreatic slice preparation is a valid and useful tool, especially for the study of β cell pathogenesis in pancreatic slices from human type 1 diabetes organ donors (95, 96). Compared with slices, fully isolated islets from human donors often do not survive isolation in good condition for physiological studies.

However, we are concerned that the alternative model of Postić et al taken from slice studies is based on the premise that the isolated islet is not a reliable physiological model. In fact, a multitude of studies show that the islet has been and continues to be a most valuable tool for investigating β cell function. Another flawed premise is that Ca²⁺ release from the ER is responsible for the cytosolic Ca²⁺ events that evoke insulin secretion. Again, there is a large body of evidence and theory supporting the consensus view that Ca²⁺ influx through voltage-gated Ca²⁺ channels is the primary way in which intracellular Ca²⁺ is raised to the level where the exocytosis of insulin granules can occur. The ER plays many important roles in the β cell, but it is not the driver of electrical activity and islet Ca²⁺ oscillations in this system.

The standard model for islet oscillations was developed over decades based on mutual interactions between theorists and experimentalists, and the number of findings that can be explained with the model has grown considerably over time. It should not be discarded lightly.

GRANTS

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

L.S.S. and A.S.S. conceived and designed research; B.T. and C.L. performed experiments; P.A.F., B.T., C.L., and A.S.S. analyzed data; B.T., C.L., R.B., L.S.S., and A.S.S. interpreted results of experiments; P.A.F. and B.T. prepared figures; A.S.S. drafted manuscript; P.A.F., R.B., L.S.S., and A.S.S. edited and revised manuscript; P.A.F., R.B., L.S.S., and A.S.S. approved final version of manuscript.

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