Ca^{2+} Release or Ca^{2+} Entry, that is the Question: What Governs Ca^{2+} Oscillations in Pancreatic Beta Cells?

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

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

[*]Corresponding author: Arthur Sherman, 12A South Drive, Room 4007, National Institutes of Health, Bethesda, MD 20892-5621, arthurs@niddk.nih.gov

Keywords:
calcium oscillations, bursting electrical activity, calcium induced calcium release, endoplasmic reticulum, ryanodine receptors, IP3 receptors

Running Head:
Ca^{2+} Release or Ca^{2+} Entry: That is the Question

Abstract:

The standard model for Ca^{2+} oscillations in insulin-secreting pancreatic beta cells centers on Ca^{2+} entry through voltage-activated Ca^{2+} channels. These work in combination with ATP-28 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 beta 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 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.

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 Ca^{2+} fluctuations and oscillations in islets of Langerhans observed in pancreatic slices from mice and argue that this study "demands an updated model of beta 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 Ca^{2+} and takes ER Ca^{2+} 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 Ca^{2+} is most often monitored using Fura-2, a high affinity Ca^{2+} 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 Ca^{2+} sensing dye that detects faster events and does not saturate in response to high Ca^{2+} transients. It is proposed that fast Ca^{2+} events due to ER Ca^{2+} 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 Ca^{2+} oscillations, which found little evidence to support a major role for 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-month-old male Swiss-Webster mice (25-35g) in accordance with the regulations of the University of Michigan Committee on the Use and Care of Animals (UCUCA). A previously described (6) and 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 hour in a medium containing 11 mM glucose before imaging.

Modeling:

Mathematical models were simulated using the `ode15s` function in Matlab 2022b (Natick, Massachusetts: The MathWorks Inc.). Program code defining the models in both Matlab and xppaut (http://www.math.pitt.edu/~bard/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-2 AM 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 ~ 1 + G + S + (1 | mouse) + (1 | 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 ~ 1 + G + (1 | mouse) + (1 | islet)

or

X ~ 1 + S + (1 | mouse) + (1 | islet).

500 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 seconds (7) and slow bursting, with a period of 3 – 10 minutes (8-11). Fura-2 is 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 ultra-short 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 Ca^{2+} 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 vs 8 mM glucose in (12) and 6 – 9 mM vs 11 mM in (13).

Fast Ca^{2+} transients to near 10 μM have been proposed to reflect nanodomains very near the mouth of voltage-activated Ca^{2+} channels (VACCs) of the beta cell plasma membrane. These localized Ca^{2+} transients are likely important for insulin secretion, as a subset of insulin vesicles (the readily or immediately releasable pools) are tightly colocalized to the Ca^{2+} channels (14-18). They also may play a role in inactivating the Ca^{2+} channels (19, 20) and activating large-conductance, voltage-dependent Ca^{2+}-activated (BK) potassium channels (21, 22). While these processes (which occur on a ms-sec time scale) shape beta-cell action potentials, they are unlikely to play a critical role in the slow Ca^{2+} oscillations governed by bursts of action potentials (time scale tens of seconds to minutes) described in detail below.

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 co-released 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 Ca^{2+} 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 Ca^{2+} influx, not Ca^{2+} release (32-34).

Figure 1 summarizes results obtained in our own experiments using either Fura-2AM or Calbryte-520 AM to monitor islet Ca^{2+} oscillations. Representative whole islet recording of Fura-2AM (A) and Calbryte 520 AM (B) 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 panels 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 Ca^{2+} 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 (see discussion of plateau fraction below and Fig. 1). We therefore do not consider it plausible that different mechanisms control islet oscillations in 6 vs 10 mM glucose.

While we agree that the ER can be depleted of free Ca^{2+} 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 (37). Since experimental studies of islet Ca^{2+} signaling last tens of minutes or longer, the initial filling state of the ER is unlikely to influence measurements of steady-state free Ca^{2+} oscillations. We address below the nature of the initial transient rise of Ca^{2+} seen in response to a stepwise increase in glucose, another important question.

Taken together, the six main points raised above 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 Ca^{2+} is the driver of islet oscillations, as detailed below.

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

This does not mean, however, that CICR or ER Ca^{2+} release more generally do not occur in beta cells. Indeed, Ravier et al (37) provide evidence (see their Fig. 5) that Ca^{2+} release events can occur in response to strong depolarizing stimulation with KCl or tolbutamide. During those events ER Ca^{2+} fell as cytosolic Ca^{2+} rose. Most importantly, repetitive Ca^{2+} releases did not occur in parallel with cytosolic Ca^{2+} oscillations in their studies. We are also not saying that ryanodine or IP3 receptors cannot play a role in T2D or potentially be useful drug targets for treating disease. However, these are entirely different questions from how oscillations are generated by beta 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 Ca^{2+} reuptake and release, not just Ca^{2+} 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 (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 Ca^{2+} 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 Ca^{2+} release is the main driver of Ca^{2+} oscillations.

In contrast, the findings in (6, 30, 45, 46) are fully compatible with the standard model in which rises in cytosolic Ca^{2+} are mainly due to Ca^{2+} entry through VACCs. Fast oscillations are more affected by ER Ca^{2+} 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 Ca^{2+} concentration is reduced (50, 51) (see next section for more about modeling of SOC in beta cells). SOC can link ER Ca^{2+} status to plasma membrane electrical activity and concomitant Ca^{2+} influx, so it was surprising that despite their emphasis on the importance of ER Ca^{2+} 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 Ca^{2+} 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 Postić et al.

Postić et al. propose that the role of VACCs in beta 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 Ca^{2+}-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 Ca^{2+} (52). This was demonstrated experimentally by using K(Ca) channels as a reporter for cytosolic Ca^{2+}, as shown in Fig. 3A, 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 Ca^{2+}, and almost all of the Ca^{2+} rise results from ER release (52).

In contrast, published recordings in which V_m and Ca^{2+} are measured simultaneously show that this is not the case in beta cells (6, 8, 53). A model of beta-cells similar to the Li-Rinzel model was proposed many years ago for beta cells (54), but objections were immediately raised based on the voltage-dependent nature of islet Ca^{2+} 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 sub-threshold concentration to > 7 mM, a first phase of intense action potential firing from a maintained plateau is observed that lasts a few minutes prior to the beginning of steady-state bursting. Postić et al. assert that this first phase is due to ER Ca^{2+} release, a hypothesis previously proposed in (56). If this were true, one would expect then that lowering ER below its 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 prior to 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. While 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 beta cells, we believe that any alternative model based on intracellular Ca^{2+} release will fail to account for beta-cell Ca^{2+} data.

<u>Can the observations of Postić et al. be explained by the standard model?</u>

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 in (58) and later developments covered in (59-61). 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 (62-65) 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 beta-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 does 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 to 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, while 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 beta 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 Figs, 4B and 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 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 Ca^{2+} 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 Ca^{2+} oscillations in 8 mM glucose (their Fig. 4), we did not find this in our experiments. Fig. 7 shows a representative recording of steady-state islet Ca^{2+} 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 Ca^{2+} oscillations support the hypothesis that Ca^{2+} influx, and not ryanodine-receptor mediated Ca^{2+} release, mediates islet Ca^{2+} oscillations. Nonetheless, the diagram in Fig. 4B predicts that that blockage of RyR can push the cells below threshold in 8G by reducing SOC if at rest RyRs contribute to Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} transients they observed were eventually largely abolished. They attributed this slow action to the time required for the ER Ca^{2+} to drain, since there was no longer a way to restore ER Ca^{2+} when VACC-mediated Ca^{2+} influx to the cell was blocked by isradipine. Once the ER was sufficiently drained of Ca^{2+} their interpretation was that the ER Ca^{2+} release events would stop.

The literature is somewhat inconclusive regarding the effects of L-type channel blockers on islet Ca^{2+} activity, with some published experiments showing that Ca^{2+} 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 Ca^{2+} channel blockers (85, 86). Fig. 8 shows that in our hands steady-state islet Ca^{2+} oscillations in 11 mM glucose were rapidly and reversibly 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 minutes; this is less of a problem in experiments with isolated islets. In addition, it is known that beta cells also have VACCs that are non-L type and hence would be untouched by dihydropyridine blockers; these can contribute to Ca^{2+} influx as well (85, 87, 88).

Postić et al. also blocked Ca^{2+} entry using diazoxide, a K(ATP) channel opener that hyperpolarizes the cell and should deactivate all VACCs, not just L-type ones (their Figs. S4-2, S4-3). 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 Ca^{2+} oscillations, reducing Ca^{2+} to baseline levels (see for example Fig. 5 in (25)). As Postić et al. point out, in high glucose the ER is expected to be replete with Ca^{2+} and therefore should be well able to mediate Ca^{2+} induced Ca^{2+} release. In our experiments, and those of many others, sustained islet Ca^{2+} oscillations are never observed in diazoxide even when the islet is depolarized with high KCl to maintain Ca^{2+} 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 Ca^{2+} release on cytosolic Ca^{2+} 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 Ca^{2+} and glucose metabolism(82, 89); it can explain oscillations in cAMP (90, 91); it can be used to study the implications of heterogeneity of beta-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 beta cells have been taken (5).

Conclusions

We thus conclude that the standard model is not significantly challenged by the slice data presented in 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 Ca^{2+} release in beta cells is worthwhile. However, as we pointed out above, 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 Figure 9 in (38)). We cannot rule out that some model not previously considered would be more consistent with what we know about beta 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 beta cell pathogenesis in pancreatic slices from human type 1 diabetes organ donors (95, 96). Compared to 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 beta cell function. Another flawed premise is that Ca^{2+} release from the ER is responsible for the cytosolic Ca^{2+} events that evoke insulin secretion. Again, there is a large body of evidence and theory supporting the consensus view that Ca^{2+} influx through voltage-gated Ca^{2+} channels is the primary way in which intracellular Ca^{2+} is raised to the level where the exocytosis of insulin granules can occur. The ER plays many important roles in the beta cell, but it is not the driver of electrical activity and islet Ca^{2+} 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

R.B. was partially supported by National Science Foundation Grant DMS 1853342. L.S.S. was partially supported by National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Grants RO1 DK46409 and U01 DK127747. P.A.F. and A.S.S. were supported by the Intramural Research Program of the NIDDK.

Disclosures

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

Contributions

P.A.F. and A. S. S. performed mathematical simulations and analysis; B. T. and C. L. carried out experiments in the Satin Lab; P.A.F., A. S. S., B. T. and C. L. prepared figures; A.S.S., L.S.S., and R.B. provided resources and supervision; P.A.F., R.B., L.S.S., B. T., and A.S.S. edited and revised manuscript; P.A.F., R.B., L.S.S., and A.S.S. approved the final version of the manuscript.

Figure Legends

Fig. 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 (mean$\pm$sd): 6 mM G, 8.1$\pm$2.4 (Fura), 7.7$\pm$2.3 (Calbryte); 8 mM G: 5.4$\pm$2.4 (Fura), 5.2$\pm$1.2 (Calbryte). Plateau Fractions: 6 mM G: 0.47$\pm$0.12 (Fura), 0.46$\pm$0.13 (Calbryte); 8 mM G: 0.64$\pm$0.14 (Fura), 0.56$\pm$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 3 mice for Fura-2AM and 14 islets from 3 mice for Calbryte 520 AM. 30/58 islets in Fura-2AM and 7/14 islets in Calbryte 520 AM did not have oscillations in 6 mM glucose and were not included in the analysis.

Fig. 2: Simulations with a version of the Li-Rinzel model for ER-driven Ca^{2+} oscillations (40) show that cytosolic and ER Ca^{2+} 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. IP3 concentration in (B) shows the border between oscillations (with period color-coded) and no oscillations (grey region).

Fig. 3: During ER-driven Ca^{2+} oscillations in pituitary gonadotrophs, membrane potential and cytosolic Ca^{2+} are out of phase. Experimental records reprinted from (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+}. Panel C shows 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.

Fig. 4: Simulations with the integrated oscillator model (IOM) (59) for coupled oscillations in membrane potential, cytosolic and ER Ca^{2+}, and ATP/ADP ratio, as extended in (82). The model has been supplemented with a store-operated Ca^{2+} (SOC) current to represent effects of ER Ca^{2+} on membrane potential. (A) Starting in 6 mM glucose, no oscillations are seen, but increasing ER Ca^{2+} 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) The two-parameter diagram shows regions of oscillation as ER leak rate and glucose concentration are varied (color indicates oscillation period; grey area denotes space where no oscillations occur).

Fig. 5: Simulations with the IOM as in Fig. 4. (A) Blocking the SERCA pump under subthreshold conditions can trigger Ca^{2+} oscillations. After raising glucose, SERCA blockade is no longer required to maintain the oscillations that were triggered initially by CPA. The two-parameter

plane shown is as in Fig. 4 but showing regions of oscillations as SERCA rate and glucose are varied.

Fig. 6: Experimental confirmation of the prediction shown in Fig. 5 using the reversible SERCA blocker CPA. Representative image showing that the acute application of 25 µM CPA induces cytosolic Ca^{2+} 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 3 different mice.

Fig. 7: High dose ryanodine did not block oscillations in 8 mM glucose. Representative recordings showing steady state islet Ca^{2+} oscillations recorded in 8 mM glucose before and after acute application of 100 µM ryanodine (Ryan). These data support the hypothesis that Ca^{2+} influx, and not ryanodine receptor-mediated Ca^{2+} release, mediates islet Ca^{2+} oscillations. Data shown are representative of 37 islets isolated from 3 mice.

Fig. 8: A representative recording showing the acute application of 10 µM nifedipine (Nif) reversibly inhibited cytosolic Ca^{2+} oscillations in 11mM glucose in an isolated mouse islet. The response shown is representative of 25/27 islets isolated from 2 different mice.

References

1. **Postic S, Sarikas S, Pfabe J, Pohorec V, Krizancic Bombek L, Sluga N, Skelin Klemen M, Dolensek J, Korosak D, Stozer A, Evans-Molina C, Johnson JD, and Slak Rupnik M**. High-resolution analysis of the cytosolic Ca(2+) events in beta cell collectives in situ. *Am J Physiol Endocrinol Metab* 324: E42-E55, 2023.
2. **Idevall-Hagren O, and Tengholm A**. Metabolic regulation of calcium signaling in beta cells. *Semin Cell Dev Biol* 103: 20-30, 2020.
3. **Martin F, and Soria B**. Glucose-induced [Ca2+]i oscillations in single human pancreatic islets. *Cell Calcium* 20: 409-414, 1996.
4. **Quesada I, Todorova MG, Alonso-Magdalena P, Beltra M, Carneiro EM, Martin F, Nadal A, and Soria B**. Glucose induces opposite intracellular Ca2+ concentration oscillatory patterns in identified alpha- and beta-cells within intact human islets of Langerhans. *Diabetes* 55: 2463-2469, 2006.
5. **Riz M, Braun M, and Pedersen MG**. Mathematical modeling of heterogeneous electrophysiological responses in human beta-cells. *PLoS Comput Biol* 10: e1003389, 2014.
6. **Zhang M, Goforth P, Bertram R, Sherman A, and Satin L**. The Ca2+ dynamics of isolated mouse beta-cells and islets: implications for mathematical models. *Biophys J* 84: 2852-2870, 2003.
7. **Ashcroft FM, and Rorsman P**. Electrophysiology of the pancreatic beta-cell. *Prog Biophys Mol Biol* 54: 87-143, 1989.
8. **Beauvois MC, Merezak C, Jonas JC, Ravier MA, Henquin JC, and Gilon P**. Glucose-induced mixed [Ca2+]c oscillations in mouse beta-cells are controlled by the membrane potential and the SERCA3 Ca2+-ATPase of the endoplasmic reticulum. *Am J Physiol Cell Physiol* 290: C1503-1511, 2006.
9. **Nunemaker CS, Zhang M, Wasserman DH, McGuinness OP, Powers AC, Bertram R, Sherman A, and Satin LS**. Individual mice can be distinguished by the period of their islet calcium oscillations: is there an intrinsic islet period that is imprinted in vivo? *Diabetes* 54: 3517-3522, 2005.
10. **Liu YJ, Tengholm A, Grapengiesser E, Hellman B, and Gylfe E**. Origin of slow and fast oscillations of Ca2+ in mouse pancreatic islets. *J Physiol* 508 (Pt 2): 471-481, 1998.
11. **Li J, Shuai HY, Gylfe E, and Tengholm A**. Oscillations of sub-membrane ATP in glucose-stimulated beta cells depend on negative feedback from Ca(2+). *Diabetologia* 56: 1577-1586, 2013.
12. **Glynn E, Thompson B, Vadrevu S, Lu S, Kennedy RT, Ha J, Sherman A, and Satin LS**. Chronic Glucose Exposure Systematically Shifts the Oscillatory Threshold of Mouse Islets: Experimental Evidence for an Early Intrinsic Mechanism of Compensation for Hyperglycemia. *Endocrinology* 157: 611-623, 2016.
13. **Nunemaker CS, Bertram R, Sherman A, Tsaneva-Atanasova K, Daniel CR, and Satin LS**. Glucose modulates [Ca2+]i oscillations in pancreatic islets via ionic and glycolytic mechanisms. *Biophys J* 91: 2082-2096, 2006.
14. **Barg S, Ma X, Eliasson L, Galvanovskis J, Gopel SO, Obermuller S, Platzer J, Renstrom E, Trus M, Atlas D, Striessnig J, and Rorsman P**. Fast exocytosis with few Ca(2+) channels in insulin-secreting mouse pancreatic B cells. *Biophys J* 81: 3308-3323, 2001.

15. **Bokvist K, Eliasson L, Ammala C, Renstrom E, and Rorsman P**. Co-localization of L-type Ca2+ channels and insulin-containing secretory granules and its significance for the initiation of exocytosis in mouse pancreatic B-cells. *EMBO J* 14: 50-57, 1995.
16. **Rutter GA, Tsuboi T, and Ravier MA**. Ca2+ microdomains and the control of insulin secretion. *Cell Calcium* 40: 539-551, 2006.
17. **Pertusa JA, Sanchez-Andres JV, Martin F, and Soria B**. Effects of calcium buffering on glucose-induced insulin release in mouse pancreatic islets: an approximation to the calcium sensor. *J Physiol* 520 Pt 2: 473-483, 1999.
18. **Gandasi NR, Yin P, Riz M, Chibalina MV, Cortese G, Lund PE, Matveev V, Rorsman P, Sherman A, Pedersen MG, and Barg S**. Ca2+ channel clustering with insulin-containing granules is disturbed in type 2 diabetes. *J Clin Invest* 127: 2353-2364, 2017.
19. **Sherman A, Keizer J, and Rinzel J**. Domain model for Ca2(+)-inactivation of Ca2+ channels at low channel density. *Biophys J* 58: 985-995, 1990.
20. **Peterson BZ, DeMaria CD, Adelman JP, and Yue DT**. Calmodulin is the Ca2+ sensor for Ca2+ -dependent inactivation of L-type calcium channels. *Neuron* 22: 549-558, 1999.
21. **Prakriya M, and Lingle CJ**. Activation of BK channels in rat chromaffin cells requires summation of Ca(2+) influx from multiple Ca(2+) channels. *J Neurophysiol* 84: 1123-1135, 2000.
22. **Montefusco F, Tagliavini A, Ferrante M, and Pedersen MG**. Concise Whole-Cell Modeling of BK(Ca)-CaV Activity Controlled by Local Coupling and Stoichiometry. *Biophys J* 112: 2387-2396, 2017.
23. **Dolensek J, Stozer A, Skelin Klemen M, Miller EW, and Slak Rupnik M**. The relationship between membrane potential and calcium dynamics in glucose-stimulated beta cell syncytium in acute mouse pancreas tissue slices. *PLoS One* 8: e82374, 2013.
24. **Lewandowski SL, Cardone RL, Foster HR, Ho T, Potapenko E, Poudel C, VanDeusen HR, Sdao SM, Alves TC, Zhao X, Capozzi ME, de Souza AH, Jahan I, Thomas CJ, Nunemaker CS, Davis DB, Campbell JE, Kibbey RG, and Merrins MJ**. Pyruvate kinase controls signal strength in the insulin secretory pathway. *Cell Metab* 32: 736-750 e735, 2020.
25. **Merrins MJ, Poudel C, McKenna JP, Ha J, Sherman A, Bertram R, and Satin LS**. Phase analysis of metabolic oscillations and membrane potential in pancreatic islet beta-cells. *Biophys J* 110: 691-699, 2016.
26. **Pedersen MG, Mosekilde E, Polonsky KS, and Luciani DS**. Complex patterns of metabolic and Ca(2)(+) entrainment in pancreatic islets by oscillatory glucose. *Biophys J* 105: 29-39, 2013.
27. **Nunemaker CS, and Satin LS**. Comparison of metabolic oscillations from mouse pancreatic beta cells and islets. *Endocrine* 25: 61-67, 2004.
28. **Dahlgren GM, Kauri LM, and Kennedy RT**. Substrate effects on oscillations in metabolism, calcium and secretion in single mouse islets of Langerhans. *Biochim Biophys Acta* 1724: 23-36, 2005.
29. **Tarasov AI, Semplici F, Li D, Rizzuto R, Ravier MA, Gilon P, and Rutter GA**. Frequency-dependent mitochondrial Ca(2+) accumulation regulates ATP synthesis in pancreatic beta cells. *Pflugers Arch* 465: 543-554, 2013.
30. **Gilon P, Arredouani A, Gailly P, Gromada J, and Henquin JC**. Uptake and release of Ca2+ by the endoplasmic reticulum contribute to the oscillations of the cytosolic Ca2+

concentration triggered by Ca2+ influx in the electrically excitable pancreatic B-cell. *J Biol Chem* 274: 20197-20205, 1999.

31. **Marinelli I, Thompson BM, Parekh VS, Fletcher PA, Gerardo-Giorda L, Sherman AS, Satin LS, and Bertram R**. Oscillations in K(ATP) conductance drive slow calcium oscillations in pancreatic beta-Cells. *Biophys J* in press: 2022.

32. **Cha CY, Nakamura Y, Himeno Y, Wang J, Fujimoto S, Inagaki N, Earm YE, and Noma A**. Ionic mechanisms and Ca2+ dynamics underlying the glucose response of pancreatic beta cells: a simulation study. *J Gen Physiol* 138: 21-37, 2011.

33. **Fridlyand LE, Ma L, and Philipson LH**. Adenine nucleotide regulation in pancreatic beta-cells: modeling of ATP/ADP-Ca2+ interactions. *Am J Physiol Endocrinol Metab* 289: E839-848, 2005.

34. **Silva JR, Cooper P, and Nichols CG**. Modeling K,ATP--dependent excitability in pancreatic islets. *Biophys J* 107: 2016-2026, 2014.

35. **Fernandez J, and Valdeolmillos M**. Synchronous glucose-dependent [Ca(2+)](i) oscillations in mouse pancreatic islets of Langerhans recorded in vivo. *FEBS Lett* 477: 33-36, 2000.

36. **Valdeolmillos M, Gomis A, and Sanchez-Andres JV**. In vivo synchronous membrane potential oscillations in mouse pancreatic beta-cells: lack of co-ordination between islets. *J Physiol* 493 (Pt 1): 9-18, 1996.

37. **Ravier MA, Daro D, Roma LP, Jonas JC, Cheng-Xue R, Schuit FC, and Gilon P**. Mechanisms of control of the free Ca2+ concentration in the endoplasmic reticulum of mouse pancreatic beta-cells: interplay with cell metabolism and [Ca2+]c and role of SERCA2b and SERCA3. *Diabetes* 60: 2533-2545, 2011.

38. **Bertram R, and Sherman A**. Filtering of calcium transients by the endoplasmic reticulum in pancreatic beta-cells. *Biophys J* 87: 3775-3785, 2004.

39. **Li YX, Keizer J, Stojilkovic SS, and Rinzel J**. Ca2+ excitability of the ER membrane: an explanation for IP3-induced Ca2+ oscillations. *Am J Physiol* 269: C1079-1092, 1995.

40. **Li YX, and Rinzel J**. Equations for InsP3 receptor-mediated [Ca2+]i oscillations derived from a detailed kinetic model: a Hodgkin-Huxley like formalism. *J Theor Biol* 166: 461-473, 1994.

41. **Koster JC, Permutt MA, and Nichols CG**. Diabetes and insulin secretion: the ATP-sensitive K+ channel (K ATP) connection. *Diabetes* 54: 3065-3072, 2005.

42. **Ashcroft FM, Puljung MC, and Vedovato N**. Neonatal Diabetes and the K(ATP) Channel: From Mutation to Therapy. *Trends Endocrinol Metab* 28: 377-387, 2017.

43. **Gloyn AL, Weedon MN, Owen KR, Turner MJ, Knight BA, Hitman G, Walker M, Levy JC, Sampson M, Halford S, McCarthy MI, Hattersley AT, and Frayling TM**. Large-scale association studies of variants in genes encoding the pancreatic beta-cell KATP channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. *Diabetes* 52: 568-572, 2003.

44. **Li YX, Rinzel J, Keizer J, and Stojilkovic SS**. Calcium oscillations in pituitary gonadotrophs: comparison of experiment and theory. *Proc Natl Acad Sci U S A* 91: 58-62, 1994.

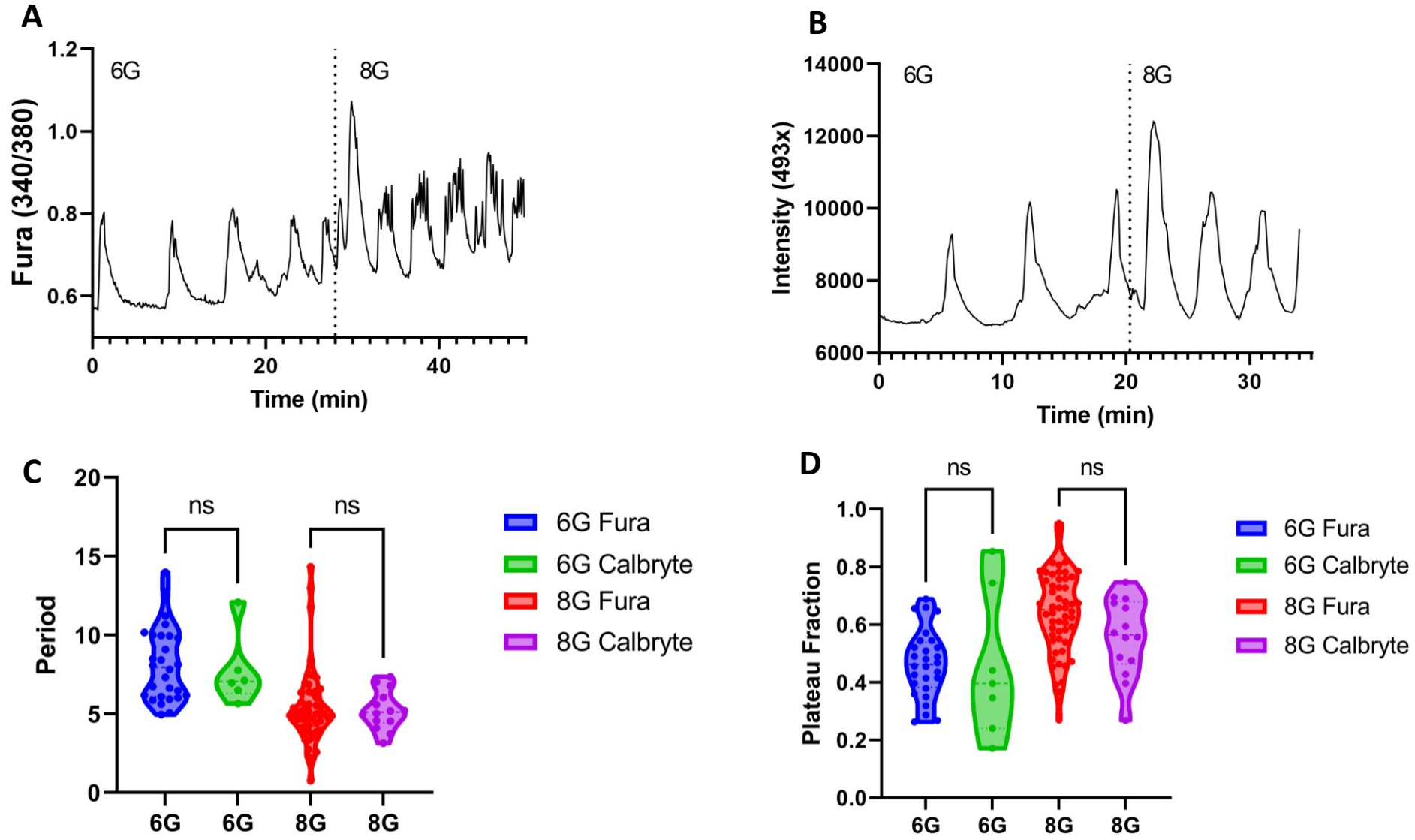
45. **Arredouani A, Guiot Y, Jonas JC, Liu LH, Nenquin M, Pertusa JA, Rahier J, Rolland JF, Shull GE, Stevens M, Wuytack F, Henquin JC, and Gilon P**. SERCA3 ablation does not impair insulin secretion but suggests distinct roles of different sarcoendoplasmic reticulum Ca(2+) pumps for Ca(2+) homeostasis in pancreatic beta-cells. *Diabetes* 51: 3245-3253, 2002.

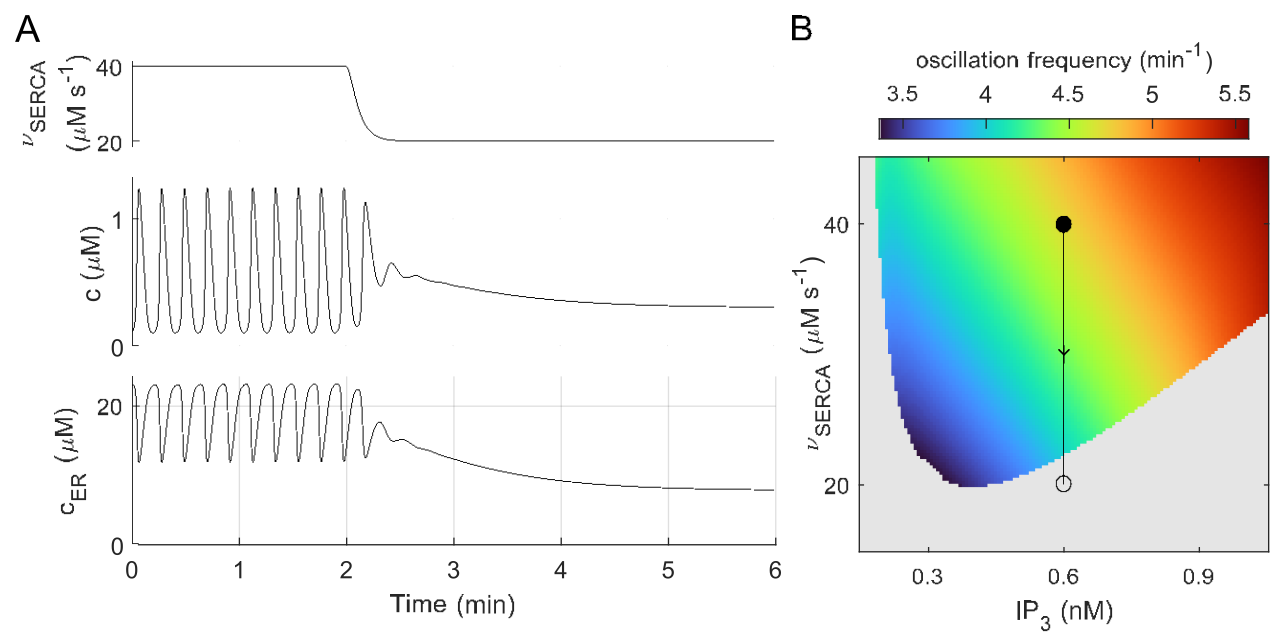
46. **Arredouani A, Henquin JC, and Gilon P**. Contribution of the endoplasmic reticulum to the glucose-induced [Ca(2+)](c) response in mouse pancreatic islets. *Am J Physiol Endocrinol Metab* 282: E982-991, 2002.
47. **Mears D, Sheppard NF, Jr., Atwater I, Rojas E, Bertram R, and Sherman A**. Evidence that calcium release-activated current mediates the biphasic electrical activity of mouse pancreatic beta-cells. *J Membr Biol* 155: 47-59, 1997.
48. **Ader M, Stefanovski D, Kim SP, Richey JM, Ionut V, Catalano KJ, Hucking K, Ellmerer M, Van Citters G, Hsu IR, Chiu JD, Woolcott OO, Harrison LN, Zheng D, Lottati M, Kolka CM, Mooradian V, Dittmann J, Yae S, Liu H, Castro AV, Kabir M, and Bergman RN**. Hepatic insulin clearance is the primary determinant of insulin sensitivity in the normal dog. *Obesity (Silver Spring)* 22: 1238-1245, 2014.
49. **Bertram R, and Sherman A**. A calcium-based phantom bursting model for pancreatic islets. *Bull Math Biol* 66: 1313-1344, 2004.
50. **Park CY, Hoover PJ, Mullins FM, Bachhawat P, Covington ED, Raunser S, Walz T, Garcia KC, Dolmetsch RE, and Lewis RS**. STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. *Cell* 136: 876-890, 2009.
51. **Parekh AB, and Putney JW, Jr.** Store-operated calcium channels. *Physiol Rev* 85: 757-810, 2005.
52. **Fletcher PA, Sherman A, and Stojilkovic SS**. Common and diverse elements of ion channels and receptors underlying electrical activity in endocrine pituitary cells. *Mol Cell Endocrinol* 463: 23-36, 2018.
53. **Gilon P, Shepherd RM, and Henquin JC**. Oscillations of secretion driven by oscillations of cytoplasmic Ca2+ as evidences in single pancreatic islets. *J Biol Chem* 268: 22265-22268, 1993.
54. **Ammala C, Larsson O, Berggren PO, Bokvist K, Juntti-Berggren L, Kindmark H, and Rorsman P**. Inositol trisphosphate-dependent periodic activation of a Ca(2+)-activated K+ conductance in glucose-stimulated pancreatic beta-cells. *Nature* 353: 849-852, 1991.
55. **Cook DL**. Electrical bursting in islet beta cells. *Nature* 357: 28, 1992.
56. **Worley JF, 3rd, McIntyre MS, Spencer B, Mertz RJ, Roe MW, and Dukes ID**. Endoplasmic reticulum calcium store regulates membrane potential in mouse islet beta-cells. *J Biol Chem* 269: 14359-14362, 1994.
57. **Sluga N, Postic S, Sarikas S, Huang YC, Stozer A, and Slak Rupnik M**. Dual Mode of Action of Acetylcholine on Cytosolic Calcium Oscillations in Pancreatic Beta and Acinar Cells In Situ. *Cells* 10: 2021.
58. **Sherman A**. Contributions of modeling to understanding stimulus-secretion coupling in pancreatic beta-cells. *Am J Physiol* 271: E362-372, 1996.
59. **Bertram R, Satin LS, and Sherman AS**. Closing in on the mechanisms of pulsatile insulin secretion. *Diabetes* 67: 351-359, 2018.
60. **Bertram R, Sherman A, and Satin LS**. Metabolic and electrical oscillations: partners in controlling pulsatile insulin secretion. *Am J Physiol Endocrinol Metab* 293: E890-900, 2007.
61. **Felix-Martinez GJ, and Godinez-Fernandez JR**. Mathematical models of electrical activity of the pancreatic beta-cell: a physiological review. *Islets* 6: e949195, 2014.
62. **Atwater I, Ribalet B, and Rojas E**. Cyclic changes in potential and resistance of the beta-cell membrane induced by glucose in islets of Langerhans from mouse. *J Physiol* 278: 117-139, 1978.

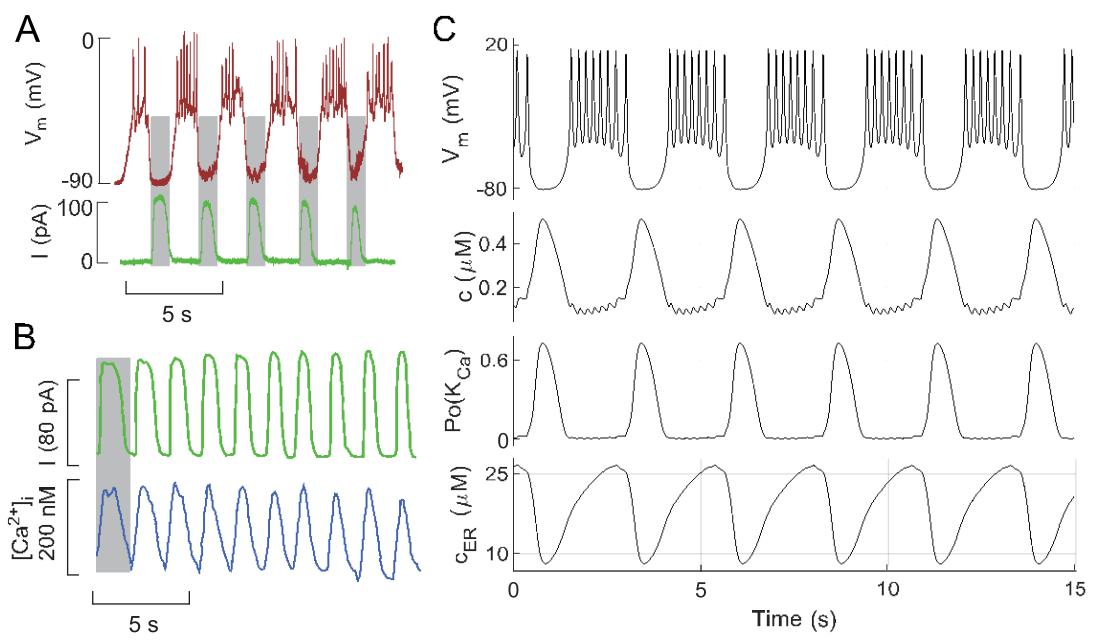
63. **Dean PM, and Matthews EK**. Electrical activity in pancreatic islet cells. *Nature* 219: 389-390, 1968.
64. **Dean PM, and Matthews EK**. Glucose-induced electrical activity in pancreatic islet cells. *J Physiol* 210: 255-264, 1970.
65. **Meissner HP, and Schmelz H**. Membrane potential of beta-cells in pancreatic islets. *Pflugers Arch* 351: 195-206, 1974.
66. **Chay TR, and Keizer J**. Minimal model for membrane oscillations in the pancreatic beta-cell. *Biophys J* 42: 181-190, 1983.
67. **Valdeolmillos M, Santos RM, Contreras D, Soria B, and Rosario LM**. Glucose-induced oscillations of intracellular Ca2+ concentration resembling bursting electrical activity in single mouse islets of Langerhans. *FEBS Lett* 259: 19-23, 1989.
68. **Chay TR**. Electrical bursting and luminal calcium oscillation in excitable cell models. *Biol Cybern* 75: 419-431, 1996.
69. **Chay TR**. Effects of extracellular calcium on electrical bursting and intracellular and luminal calcium oscillations in insulin secreting pancreatic beta-cells. *Biophys J* 73: 1673-1688, 1997.
70. **Akrouh A, Halcomb SE, Nichols CG, and Sala-Rabanal M**. Molecular biology of K(ATP) channels and implications for health and disease. *IUBMB Life* 61: 971-978, 2009.
71. **Ashcroft FM, Harrison DE, and Ashcroft SJ**. Glucose induces closure of single potassium channels in isolated rat pancreatic beta-cells. *Nature* 312: 446-448, 1984.
72. **Cook DL, and Hales CN**. Intracellular ATP directly blocks K+ channels in pancreatic B-cells. *Nature* 311: 271-273, 1984.
73. **Rinzel J, Chay TR, Himmel D, and Atwater I**. Prediction of the glucose-induced changes in membrane ionic permeability and cytosolic Ca2+ by mathematical modeling. *Adv Exp Med Biol* 211: 247-263, 1986.
74. **Tarasov AI, Griffiths EJ, and Rutter GA**. Regulation of ATP production by mitochondrial Ca(2+). *Cell Calcium* 52: 28-35, 2012.
75. **Civelek VN, Deeney JT, Kubik K, Schultz V, Tornheim K, and Corkey BE**. Temporal sequence of metabolic and ionic events in glucose-stimulated clonal pancreatic beta-cells (HIT). *Biochem J* 315 (Pt 3): 1015-1019, 1996.
76. **Rorsman P, and Ashcroft FM**. Pancreatic beta-Cell Electrical Activity and Insulin Secretion: Of Mice and Men. *Physiol Rev* 98: 117-214, 2018.
77. **Chow RH, Lund PE, Loser S, Panten U, and Gylfe E**. Coincidence of early glucose-induced depolarization with lowering of cytoplasmic Ca2+ in mouse pancreatic beta-cells. *J Physiol* 485 (Pt 3): 607-617, 1995.
78. **Keizer J, and Magnus G**. ATP-sensitive potassium channel and bursting in the pancreatic beta cell. A theoretical study. *Biophys J* 56: 229-242, 1989.
79. **Denton RM**. Regulation of mitochondrial dehydrogenases by calcium ions. *Biochim Biophys Acta* 1787: 1309-1316, 2009.
80. **Magnus G, and Keizer J**. Model of beta-cell mitochondrial calcium handling and electrical activity. II. Mitochondrial variables. *Am J Physiol* 274: C1174-1184, 1998.
81. **Detimary P, Gilon P, and Henquin JC**. Interplay between cytoplasmic Ca2+ and the ATP/ADP ratio: a feedback control mechanism in mouse pancreatic islets. *Biochem J* 333 (Pt 2): 269-274, 1998.

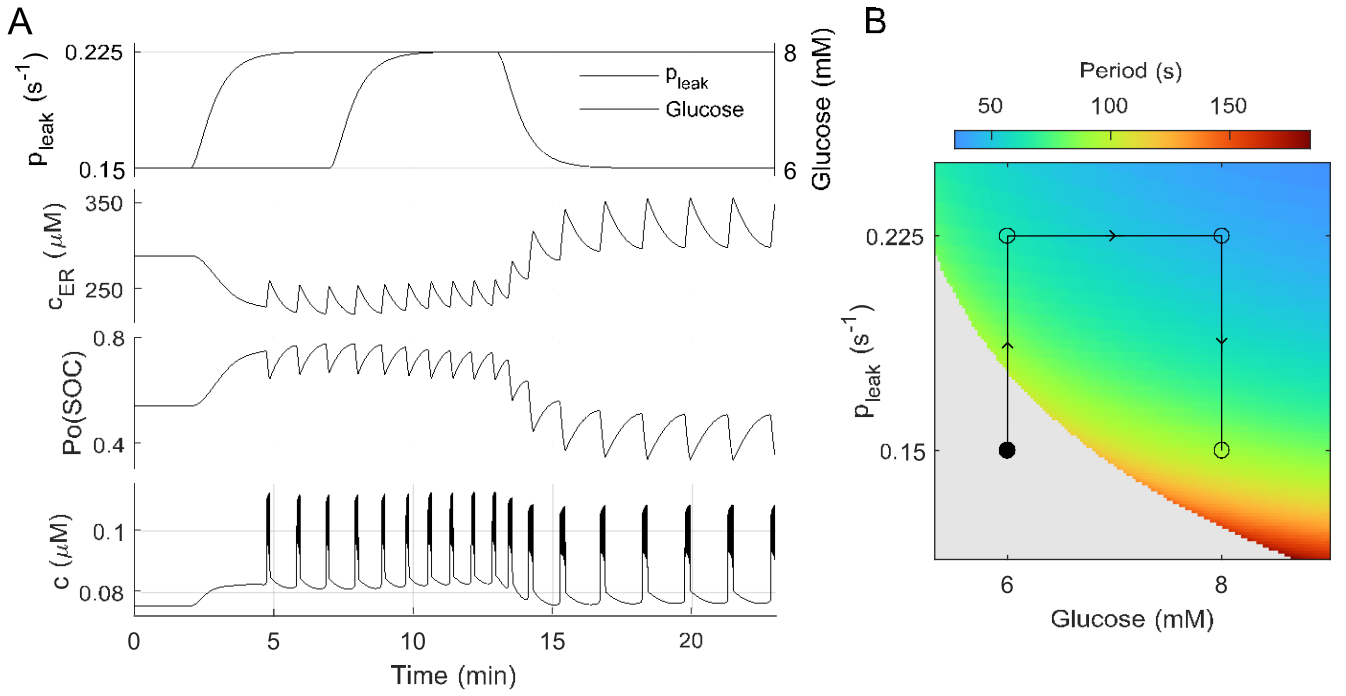
82. **Fletcher PA, Marinelli I, Bertram R, Satin LS, and Sherman AS**. Pulsatile Basal Insulin Secretion Is Driven by Glycolytic Oscillations. *Physiology (Bethesda)* 37: 0, 2022.
83. **Zhang IX, Ren J, Vadrevu S, Raghavan M, and Satin LS**. ER stress increases store-operated Ca(2+) entry (SOCE) and augments basal insulin secretion in pancreatic beta cells. *J Biol Chem* 295: 5685-5700, 2020.
84. **Tanna B, Welch W, Ruest L, Sutko JL, and Williams AJ**. The interaction of a neutral ryanoid with the ryanodine receptor channel provides insights into the mechanisms by which ryanoid binding is modulated by voltage. *J Gen Physiol* 116: 1-9, 2000.
85. **Schulla V, Renstrom E, Feil R, Feil S, Franklin I, Gjinovci A, Jing XJ, Laux D, Lundquist I, Magnuson MA, Obermuller S, Olofsson CS, Salehi A, Wendt A, Klugbauer N, Wollheim CB, Rorsman P, and Hofmann F**. Impaired insulin secretion and glucose tolerance in beta cell-selective Ca(v)1.2 Ca2+ channel null mice. *EMBO J* 22: 3844-3854, 2003.
86. **Wollheim CB, and Sharp GW**. Regulation of insulin release by calcium. *Physiol Rev* 61: 914-973, 1981.
87. **Thompson B, and Satin LS**. Beta-Cell Ion Channels and Their Role in Regulating Insulin Secretion. *Compr Physiol* 11: 1-21, 2021.
88. **Tuluc P, Theiner T, Jacobo-Piqueras N, and Geisler SM**. Role of High Voltage-Gated Ca(2+) Channel Subunits in Pancreatic beta-Cell Insulin Release. From Structure to Function. *Cells* 10: 2021.
89. **Satin LS, Butler PC, Ha J, and Sherman AS**. Pulsatile insulin secretion, impaired glucose tolerance and type 2 diabetes. *Mol Aspects Med* 42: 61-77, 2015.
90. **Landa LR, Jr., Harbeck M, Kaihara K, Chepurny O, Kitiphongspattana K, Graf O, Nikolaev VO, Lohse MJ, Holz GG, and Roe MW**. Interplay of Ca2+ and cAMP signaling in the insulin-secreting MIN6 beta-cell line. *J Biol Chem* 280: 31294-31302, 2005.
91. **Peercy BE, Sherman AS, and Bertram R**. Modeling of glucose-induced cAMP oscillations in pancreatic beta cells: cAMP rocks when metabolism rolls. *Biophys J* 109: 439-449, 2015.
92. **Dwulet JM, Ludin NWF, Piscopio RA, Schleicher WE, Moua O, Westacott MJ, and Benninger RKP**. How Heterogeneity in Glucokinase and Gap-Junction Coupling Determines the Islet [Ca(2+)] Response. *Biophys J* 117: 2188-2203, 2019.
93. **Cappon G, and Pedersen MG**. Heterogeneity and nearest-neighbor coupling can explain small-worldness and wave properties in pancreatic islets. *Chaos* 26: 053103, 2016.
94. **Dwulet JM, Briggs JK, and Benninger RKP**. Small subpopulations of beta-cells do not drive islet oscillatory [Ca2+] dynamics via gap junction communication. *PLoS Comput Biol* 17: e1008948, 2021.
95. **Panzer JK, Hiller H, Cohrs CM, Almaca J, Enos SJ, Beery M, Cechin S, Drotar DM, Weitz JR, Santini J, Huber MK, Muhammad Fahd Qadir M, Pastori RL, Dominguez-Bendala J, Phelps EA, Atkinson MA, Pugliese A, Caicedo A, Kusmartseva I, and Speier S**. Pancreas tissue slices from organ donors enable in situ analysis of type 1 diabetes pathogenesis. *JCI Insight* 5: 2020.
96. **Gosak M, Yan-Do R, Lin H, MacDonald PE, and Stozer A**. Ca2+ Oscillations, Waves, and Networks in Islets From Human Donors With and Without Type 2 Diabetes. *Diabetes* 71: 2584-2596, 2022.

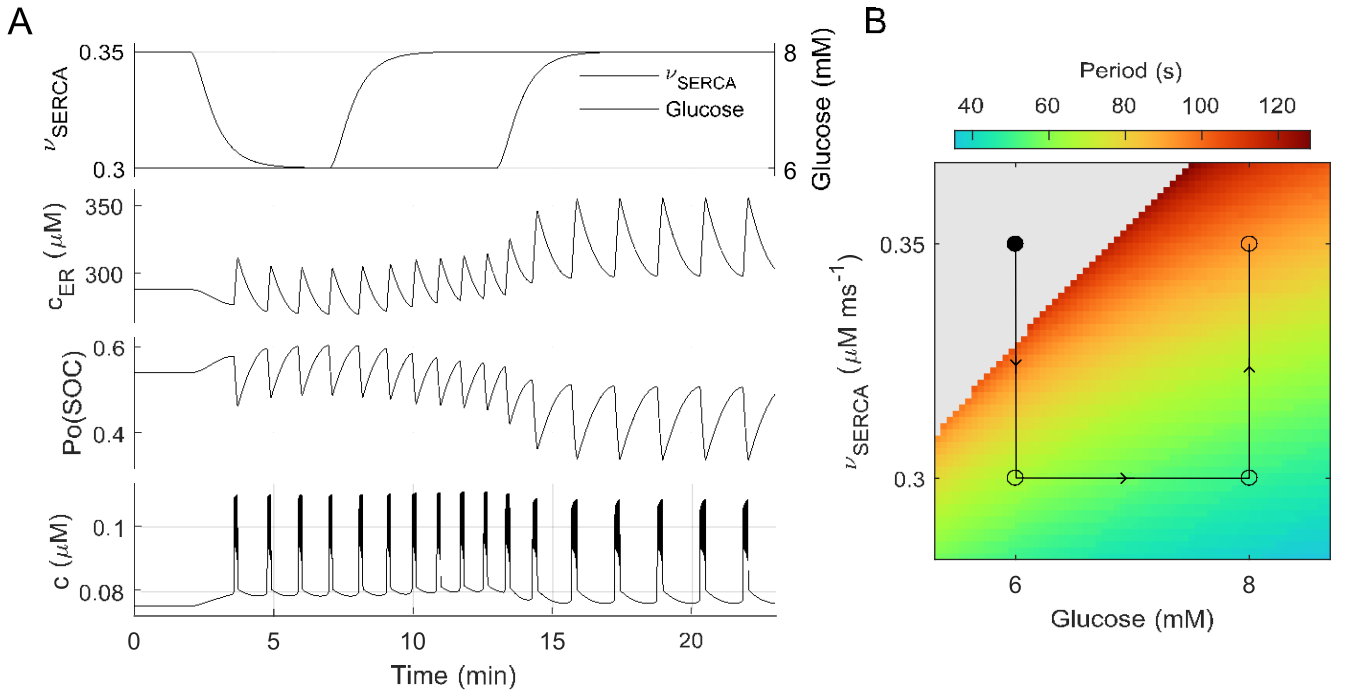
Fig. 1 Fura vs Calbryte

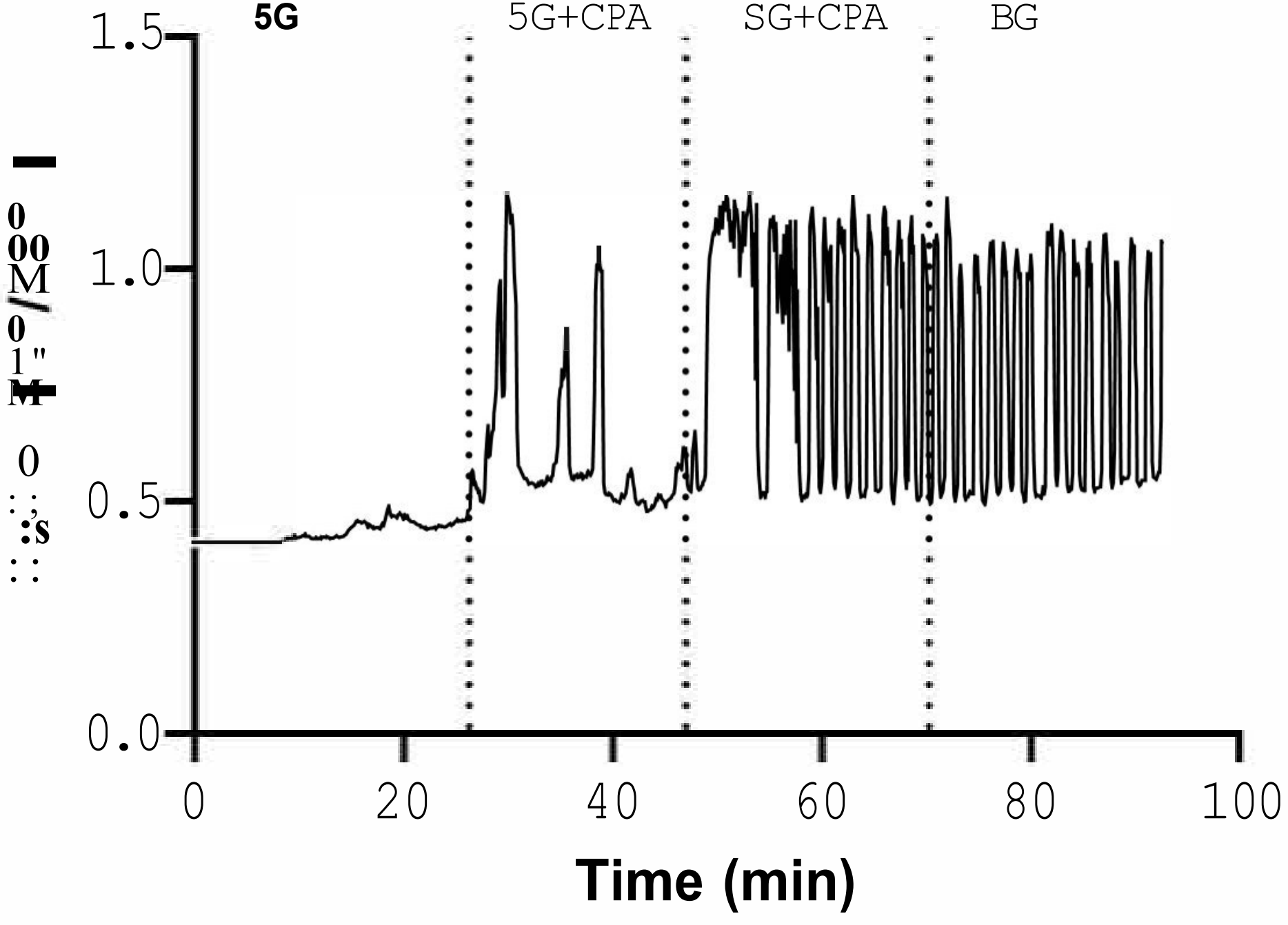


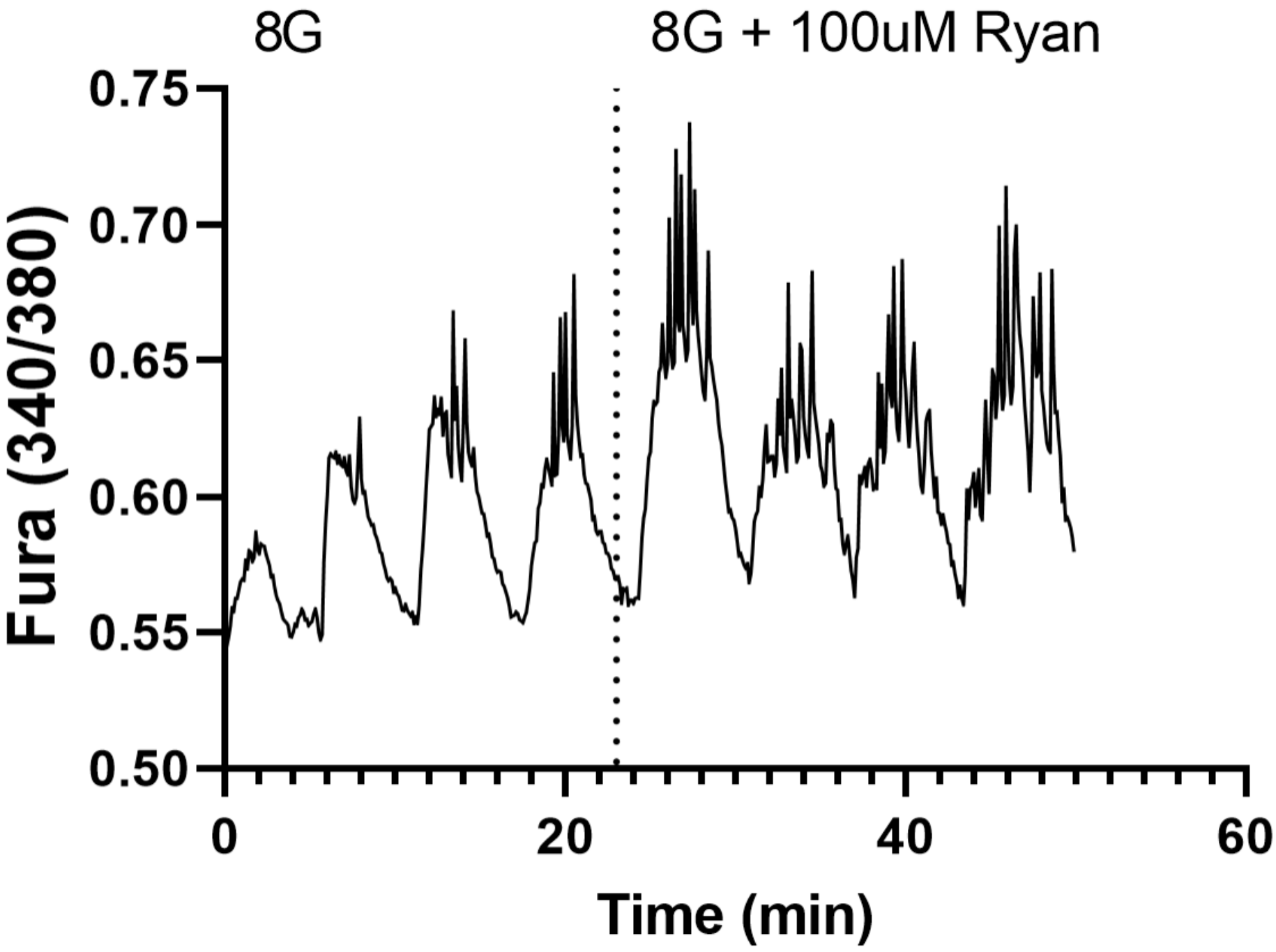

A
ν_{SERCA} (μM s^{-1})
40
20
c (μM)
1
0
c_{ER} (μM)
20
0
0
1
2
3
4
5
6
Time (min)
B
oscillation frequency (min^{-1})
3.5
4
4.5
5
5.5
ν_{SERCA} (μM s^{-1})
40
20
0.3
0.6
0.9
IP$_3$ (nM)

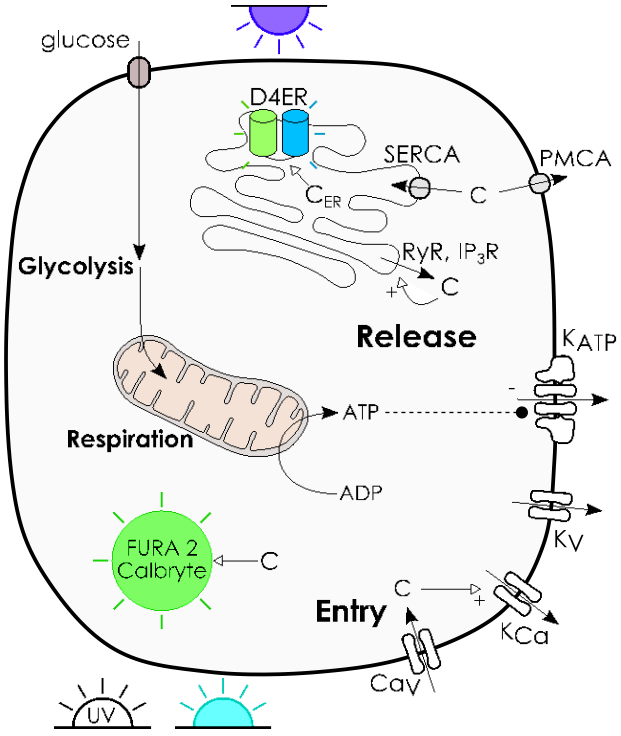

A
0
-90
V_m (mV)
100
0
I (pA)
5 s
B
I (80 pA)
$[Ca^{2+}]_i$
200 nM
5 s
C
20
-80
V_m (mV)
0.4
0.2
c (μM)
0.6
0
$Po(K_{Ca})$
25
10
c_{ER} (μM)
0
5
10
15
Time (s)

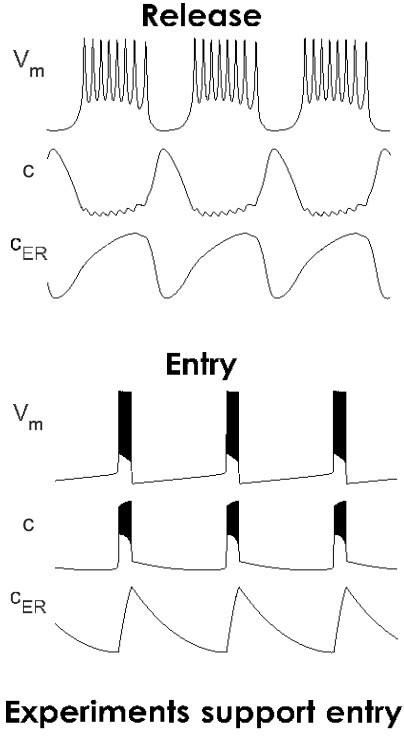

A
B
p_{leak} (s^{-1})
0.225
0.15
8
6
Glucose (mM)
p_{leak}
Glucose
c_{ER} (μM)
350
250
Po(SOC)
0.8
0.4
c (μM)
0.1
0.08
0
5
10
15
20
Time (min)
Period (s)
50
100
150
0.225
0.15
p_{leak} (s^{-1})
6
8
Glucose (mM)


A
B
ν_{SERCA}
Glucose
Glucose (mM)
c_{ER} (μM)
Po(SOC)
c (μM)
Time (min)
Period (s)
ν_{SERCA} (μM ms^{-1})
Glucose (mM)

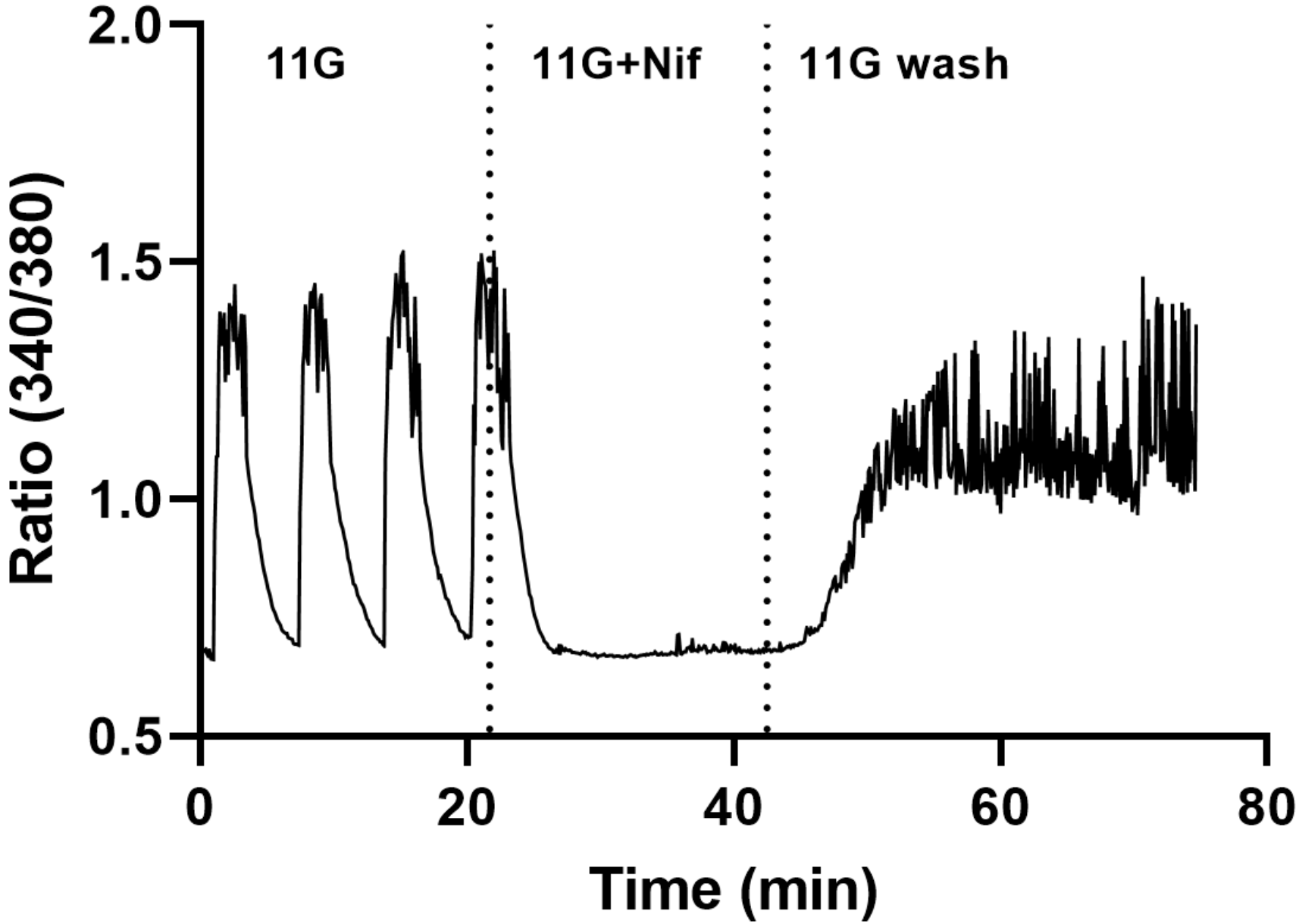

5G
5G+CPA
SG+CPA
BG
1.5
1.0
0.5
0.0
0
20
40
60
80
100
Time (min)


8G
8G + 100uM Ryan
0.75
0.70
0.65
0.60
0.55
0.50
Fura (340/380)
0
20
40
60
Time (min)





Experiments support entry


11G
11G+Nif
11G wash
2.0
1.5
1.0
0.5
Ratio (340/380)
0
20
40
60
80
Time (min)