Glucose Oscillations Can Activate an Endogenous Oscillator in Pancreatic Islets - Submission to PLOS Journals

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Abstract

Pancreatic islets manage elevations in blood glucose level by secreting insulin into the bloodstream in a pulsatile manner. Pulsatile insulin secretion is governed by islet oscillations such as bursting electrical activity and periodic Ca^{2+} entry in β-cells. In this report, we demonstrate that although islet oscillations are lost by fixing a glucose stimulus at a high concentration, they may be recovered by subsequently converting the glucose stimulus to a sinusoidal wave. We predict with mathematical modeling that the sinusoidal glucose signal's ability to recover islet oscillations depends on its amplitude and period, and we confirm our predictions by conducting experiments with islets using a microfluidics platform. Our results suggest a mechanism whereby oscillatory blood glucose levels recruit non-oscillating islets to enhance pulsatile insulin output from the pancreas. Our results also provide support for the main hypothesis of the Dual Oscillator Model, that a glycolytic oscillator endogenous to islet β-cells drives pulsatile insulin secretion.

Author Summary

A global shift throughout the last century toward excessive nutrient intake relative to energy expenditure has fueled a dramatic increase in the incidence of diabetes in humans. The epidemic is primarily of type 2 diabetes, a disease characterized by the inability of the body to effectively control blood glucose levels. Insulin plays a key role in regulating blood glucose levels by restraining endogenous glucose output from the liver and promoting blood glucose uptake by tissue throughout the body. It is secreted in pulses by islets of Langerhans, endocrine cell aggregates dispersed throughout the pancreas. Loss of insulin pulsatility is an early event in the development of type 2 diabetes. Here, we demonstrate, with a combined modeling and experimental approach, that the loss of pulsatile insulin release that results from elevated glucose may be recovered by an

oscillatory glucose stimulus. Our results have potential implications for enhancing insulin pulsatility and therefore mitigating the development of type 2 diabetes.

Introduction

Pancreatic islets manage elevations in blood glucose level, such as after meals, by secreting insulin into the bloodstream, which enables the liver, muscles, and adipose tissue to take up glucose. Insulin is secreted from islet β-cells in a pulsatile manner governed by oscillations in intracellular Ca^{2+} concentration, which are caused by bursting electrical activity on the membrane [1]. Pulsatile insulin secretion enhances the role of the liver in glucose homeostasis [2–4], and insulin pulsatility is impaired in patients with type 2 diabetes [5–7]. In healthy subjects, blood insulin levels are oscillatory with a period of approximately 5 min in both the hepatic portal vein proximal to the pancreas [8–10] and peripheral arteries [11,12], indicating that the several hundred thousand islets in a rodent or human pancreas [13] synchronize to produce pulsatile insulin output to the portal vein.

In response to glucose, some islets exhibit fast Ca^{2+} oscillations with periods of less than 2 min, some exhibit slow Ca^{2+} oscillations with periods of greater than 2 min, and some exhibit compound Ca^{2+} oscillations that consist of episodes of fast oscillations interrupted with silent phases (see [14] and [15] for reviews). This variety of responses suggests that two oscillators cooperate in islets: a "slow oscillator" with period corresponding to that of slow Ca^{2+} oscillations or episodes of compound Ca^{2+} oscillations (> 2 min) and a "fast oscillator" with period corresponding to that of fast Ca^{2+} oscillations or intra-episode oscillations of compound Ca^{2+} oscillations (< 2 min). In this report, we focus on slow oscillations since, with a period similar to that of pulsatile insulin secretion [16], they are a potential target for enhancing pulsatility.

At low concentrations of glucose, the islet slow oscillator is inactive and electrical activity is absent, which results in no more than basal insulin secretion. When glucose is continuously increased, the slow oscillator activates at a low threshold (≈ 5 mM), which results in slow or compound Ca^{2+} oscillations and pulsatile insulin secretion. As glucose is increased further, the slow oscillator deactivates although electrical activity may persist, which results in elevated intracellular Ca^{2+} and constant insulin secretion [17]. We found that slow oscillators are heterogeneous across islets by recording the glucose level at which slow Ca^{2+} oscillations were lost as we gradually increased external glucose (Fig. 1). Out of 34 islets tested, 21 islets exhibited oscillations with a period of 2 min or greater. Out of those 21 islets, the glucose level at which slow Ca^{2+} oscillations were lost varied from 11 to 17 mM, indicating that, *in vivo*, elevated blood glucose levels may trigger pulsatile insulin secretion from a sub-population of islets in the pancreas but continuous secretion from the remaining islets, which would attenuate insulin pulsatility.

Fig 1. Heterogeneity of islet slow oscillators.
The glucose concentration at which slow Ca^{2+} oscillations were lost as glucose was gradually increased. Out of 34 islets, ~~Of the~~ 21 islets ~~examined~~ exhbited Ca^{2+} oscillations with a period > 2 min. Of those 21 islets, the concentration at which slow Ca^{2+} oscillations were lost varied from 11 to 17 mM (mean: 12.90 mM, standard deviation: 2.47 mM).

In this report, we demonstrate that slow Ca^{2+} oscillations lost by fixing glucose at an elevated concentration may be recovered by subsequently oscillating glucose sinusoidally about that concentration~~. Furthermore, we demonstrate that the imposed glucose oscillations must have~~ with sufficiently large amplitude and period ~~to recover slow Ca^{2+} oscillations~~. Slow oscillations are often recovered after a delay or with a

period that differs significantly from that of the oscillatory glucose signal, indicating that the glucose signal activates an endogenous oscillator. Complex patterns of islet entrainment by sinusoidal glucose, including a 1:2 ratio between the frequencies of Ca^{2+} and imposed glucose oscillations, have previously been reported [18].

We previously demonstrated that blood glucose level fluctuations [19] could reflect interactions between the pancreas and the liver [20]. The liver plays a negative feedback role important to managing blood glucose levels. It removes glucose from the bloodstream when blood insulin is elevated (which is caused by elevated blood glucose) and releases glucose to the bloodstream when blood insulin level is low (which is caused by low blood glucose) [21]. Therefore, a sub-population of oscillating islets in the pancreas delivering an oscillatory insulin signal to the liver could establish blood glucose level oscillations of a similar period. ~~In this report, we demonstrate that blood glucose oscillations could, in turn,~~ that recruit non-oscillating islets to enhance insulin pulsatility. In this report, we demonstrate that a sub-population of slow-oscillating islets can recruit a sub-population of fast-oscillating islets when glucose delivery is put under the control of a model liver that supplies negative feedback to insulin secretion.

We use a mathematical model, the Dual Oscillator Model (DOM), to simulate experiments with islets and an *ex vivo* microfluidic platform to conduct experimental studies with islets. Our model simulations predict our experimental results and give further support to the main hypothesis of the DOM, that an endogenous glycolytic oscillator drives slow islet oscillations and pulsatile insulin secretion.

Methods

Chemicals and reagents

Potassium chloride (KCl), sodium chloride (NaCl), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), tricine, and penicillin-streptomycin were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Pluronic F-127 and fura-2-acetoxymethyl ester (fura-2-AM) were purchased from Life Technologies (Grand Island, NY, USA). Glucose (dextrose) was purchased from Thermo Fisher Scientific (Waltham, MA, USA). RPMI 1640 was purchased from Mediatech (Manassas, VA, USA). Gentamicin sulfate was purchased from Lonza (Basel, Switzerland). Poly(dimethylsiloxane) (PDMS) prepolymer (Sylgard 184) was purchased from Dow Corning (Midland, MI, USA). All solutions were made with Milli-Q (Millipore, Bedford, MA, USA) 18 MΩ cm deionized water.

Isolation and culture of islets of Langerhans

The isolation of mouse islets of Langerhans was performed under guidelines approved by the Florida State University Animal Care and Use Committee (protocol 1235). Islets of Langerhans were isolated in a similar manner as described before [20]. After isolation, islets were incubated at 37°C, 5% CO_2 in RPMI 1640 media containing 11 mM glucose, 10% calf serum, 100 units mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, and 10 μg mL^{-1} gentamicin. Islets were used within 5 days after isolation.

Microfluidic device

A microfluidic device was used to deliver different glucose concentrations to islets using the system described before [20]. Briefly, the device consisted of two inputs that were connected to 60 mL syringes that contained a balanced salt solution (BSS) composed of 2.4 mM $CaCl_2$, 125 mM NaCl, 1.2 mM $MgCl_2$, 5.9 mM KCl, and 25 mM tricine at pH 7.4 with either 3 or 20 mM glucose. The syringes were suspended above the microfluidic

device where the elevated heights produced a hydrostatic pressure that controlled the flow rates of the reagents in the device. A stepper motor controlled by a LabVIEW (National Instruments, Austin, TX) program was used to control the height of the syringe containing 20 mM glucose. The height of the other syringe was coupled to this syringe using a fixed length belt and pulley system. In this way, the height of a single syringe was controlled while the other syringe moved in an equal, but opposite, direction maintaining a constant total flow rate in the device. The difference in the heights of the two syringes caused different flow rates of the two glucose solutions to enter a 70 mm mixing channel prior to delivery to a 0.8 mm diameter chamber that housed 4-10 islets. By changing the heights of the two syringes, the concentration of glucose delivered to the islets could be changed in $\approx$ 30 s. In most experiments, the glucose level is initially held fixed at 11 mM or greater, and then sinusoidal oscillations are applied. We hold at these relatively high levels since our goal is to examine behavior near the upper threshold for slow oscillations.

Measurement of intracellular Ca^{2+}

The microfluidic device was fixed on the stage of a Nikon Eclipse Ti inverted microscope. A Lambda XL (Sutter Instruments, Novato, CA) system containing a lamp and appropriate filters was used for excitation of fura-2 at 340 nm and 380 nm. Fluorescent images were acquired with a 150 ms exposure every 20 s using a CCD (Cascade, Photometrics, Tucson, AZ, USA). The imaging system was controlled by Nikon NIS Elements software (Nikon, Melville, NY, USA). The ratio of fluorescence intensity excited at 340 nm to that at 380 nm (FCO_{340}/FCO_{380}) for all islets was obtained from the NIS software and converted to intracellular Ca^{2+} concentration using predetermined calibration values that were found by standard methods [20].

Loading of islets with fura-2 dye was performed by incubating islets in 2.5 μM fura-2-AM and 0.02% Pluronic F-127 in RPMI for 40 min at 37°C and 5% CO_2. Islets were then removed and placed in the microfluidic device where they were rinsed with BSS in 3 mM glucose for 5 min prior to the start of experiments.

Mathematical modeling

We use the Dual Oscillator Model (DOM) [1, 14] to simulate islet β-cells. A model schematic is depicted in Fig. 2. See S1 Model Equations for a full list of model equations and Table S2 Model Parameter Values for parameter values.

Fig 2. Schematic of the Dual Oscillator Model.
J: flux; GLUT: glucose transporter, GK: glucokinase, PFK: phosphofructokinase, PDH: pyruvate dehydrogenase, ANT: adenine nucleotide translocator, ER: endoplasmic reticulum, hyd: hydolysis, PMCA: plasma membrane Ca^{2+} ATPase. I: current; Ca(V): voltage-activated Ca^{2+} K(V): voltage-activated K^+, K(Ca): Ca^{2+}-activated K^+, K(ATP): ATP-sensitive K^+.

The central element of the DOM is a glycolytic subsystem, adapted from [22], that models the kinetics of the reaction catalyzed by the enzyme phosphofructokinase-1 (PFK1). Influx to the glycolytic subsystem is through glucose transporters (GLUT) that take up external glucose, G_e, to intracellular glucose, G_i. Efflux is through the mitochondrial pyruvate dehydrogenase (PDH) complex, which decarboxylates the final glycolytic product pyruvate to supply acetyl CoA to the citric acid cycle. The glycolytic model is a system of differential equations for the rates of concentration change of G_i and PFK1 substrate, fructose 6-phosphate (F6P), and product, fructose

1,6-bisphosphate (FBP):

$$dG_i/dt = J_{\mathrm{GLUT}}(G_e, G_i) - J_{\mathrm{GK}}(G_i)$$
$$d\mathrm{F6P}/dt \propto J_{\mathrm{GK}}(G_i) - J_{\mathrm{PFK}}(\mathrm{F6P}, \mathrm{FBP}, \mathrm{ATP})$$
$$d\mathrm{FBP}/dt \propto J_{\mathrm{PFK}}(\mathrm{F6P}, \mathrm{FBP}, \mathrm{ATP}) - J_{\mathrm{PDH}}(\mathrm{FBP})$$

derived by assuming the glucose 6-phosphate isomerase reaction upstream to PFK1 and the series of reactions downstream to PFK1 are at equilibrium [23, 24]. The glycolytic subsystem is coupled through ATP to a conductance-based model of electrical activity on the plasma membrane: a rise in glycolytic flux causes ATP production that reduces ATP-sensitive K^+(K(ATP)) membrane ion channel conductance. The concentration of cytosolic ATP increases as adenine nucleotide translocators (ANT) export ATP from the mitochondrial matrix to the cytosol and decreases with hydrolysis (hyd):

$$d\mathrm{ATP}/dt = J_{\mathrm{ANT}}(\mathrm{FBP}, \mathrm{ATP}, Ca_c) - J_{\mathrm{hyd}}(\mathrm{ATP}, Ca_c).$$

ATP hydrolysis powers pumping of cytosolic Ca^{2+}, Ca_c, across the endoplasmic reticulum (ER) and plasma membranes. The conductance-based model of membrane electrical activity, previously described in [25], includes voltage-activated Ca^{2+} (Ca(V)) and K^+(K(V)) currents, Ca^{2+}-activated K^+current (K(Ca)), and K(ATP) current as well as Ca^{2+} flux across the ER and plasma membranes.

Model equations were integrated using the backward differentiation formulas implemented by the CVODE software package. The model can be downloaded as freeware from `http://www.math.fsu.edu/~bertram/software/islet`.

Results

Glycolytic oscillations in the Dual Oscillator Model

Our model, the Dual Oscillator Model (DOM), of islet β-cells contains a glycolytic subsystem that can produce oscillations with a period of approximately 5 min. Glycolytic oscillations in β-cells lead to oscillations in the intracellular ATP concentration which, by acting on ATP-sensitive K^+ (K(ATP)) channels, cause bursting electrical activity on the membrane and oscillations in intracellular Ca^{2+} concentration (Fig. 3).

Fig 3. Slow oscillations in the Dual Oscillator Model.
In the DOM, glycolytic oscillations such as in the concentration of PFK product, FBP, (bottom) lead to bursting oscillations in membrane potential (top) and oscillations in intracellular Ca^{2+} concentration (middle).

Glycolytic oscillations are mediated by the autocatalytic activation of the enzyme PFK1 by its product, FBP. A rise in PFK1 substrate, F6P, from glucose consumption leads to FBP production, which positively feeds back onto PFK1 to increase its catalytic activity. Increased PFK1 catalytic activity eventually depletes F6P, leading to a crash in the FBP level and a recovery in F6P, completing one cycle of the oscillation. Ultimately, this mechanism coordinates sawtooth oscillations of F6P with pulses of FBP (Fig. 4A). Which glucose levels and which initial states of the glycolytic subsystem produce sustained oscillations? Fig. 4B depicts a bifurcation diagram for the asymptotic value of FBP with external glucose, G_e, as a bifurcation parameter. The minimum and maximum values of FBP for periodic states of the glycolytic subsystem ~~during oscillations~~ are represented by ~~solid~~ curves below and above the steady state curve, respectively, for $G_{\mathrm{low}} < G_e < G_{\mathrm{up}}$. Stable states are represented by solid curves, whereas unstable states are represented by dashed curves.

Fig 4. Glycolytic oscillations in the Dual Oscillator Model.
(A) Concurrent sawtooth F6P oscillations and FBP pulses produced by the glycolytic subsystem of the DOM. (B) Bifurcation diagram of the glycolytic subsystem. Sustained oscillations do not exist for low ($G_e < G_{\text{low}}$) or high ($G_e > G_{\text{up}}$) glucose, but can be triggered for an intermediate range ($G_{\text{low}} < G_e < G_{\text{up}}$). (C) Phase planes of the glycolytic subsystem within monostable (top) and bistable (bottom) glucose ranges. Initial states in the stable limit cycle (top and bottom, solid black curve) domain of attraction (top and bottom, white) trigger sustained oscillations. Initial states in the stable steady state (bottom, filled dot) domain of attraction (bottom, shaded gray) lead to transient, decaying oscillations. An unstable steady state (top, open dot) gains stability and gives rise to an unstable limit cycle (bottom, dashed curve) via a Hopf bifurcation as G_e increases past G_{mid}.

The glycolytic subsystem is at steady state with FBP constant for low ($G_e < G_{\text{low}}$) or high ($G_e > G_{\text{up}}$) glucose. It becomes oscillatory by, say, continuously increasing G_e from a low concentration to a concentration between G_{low} and G_{mid} or by continuously decreasing G_e from a high concentration to a concentration between G_{low} and G_{mid}. In either scenario, the steady state loses stability via a Hopf bifurcation, at $G_e = G_{\text{low}}$ or $G_e = G_{\text{mid}}$, and remains unstable for $G_{\text{low}} < G_e < G_{\text{mid}}$, (represented as ~~a~~ the dashed portion of the steady state curve in Fig. 4B~~) for $G_{\text{low}} < G_e < G_{\text{mid}}$~~.

The glycolytic subsystem can produce oscillations for an intermediate range of glucose ($G_{\text{low}} < G_e < G_{\text{up}}$) that extends above G_{mid} to a saddle node of periodics bifurcation at $G_e = G_{\text{up}}$. For $G_{\text{low}} < G_e < G_{\text{mid}}$, the system is monostable with co-existing unstable steady state and stable limit cycle (Fig. 4C, top), yet as G_e increases above G_{mid} so that $G_{\text{mid}} < G_e < G_{\text{up}}$, the system becomes bistable as the steady state gains stability and gives rise to an unstable limit cycle via the subcritical Hopf bifurcation at $G_e = G_{\text{mid}}$ (Fig. 4C, bottom).

All initial states in the stable limit cycle's domain of attraction trigger sustained oscillations. In the oscillatory, monostable range ($G_{\text{low}} < G_e < G_{\text{mid}}$), the domain of attraction is the whole state space except the unstable steady state, and in the bistable range ($G_{\text{mid}} < G_e < G_{\text{up}}$), it is the set of initial states outside the unstable limit cycle (depicted as a dashed line in Fig. 4C, bottom). In the bistable range, initial states in the stable steady state's domain of attraction (shaded gray in Fig. 4C, bottom) produce transient oscillations that decay to the stable steady state. The existence of a subcritical Hopf bifurcation at $G_e = G_{\text{mid}}$ is key to recovering slow Ca^{2+} oscillations with a sinusoidal glucose stimulus as we explain in the following sections.

The number and type of bifurcations of the glycolytic subsystem are robust with respect to increases in the maximal rate of GLUT transporter (V_{GLUT}) or maximal rate of glucokinase (V_{GK}). All of G_{low}, G_{mid}, and G_{up} are decreasing functions of V_{GLUT} and V_{GK}. For example, doubling the value of $V_{\text{GLUT}} = 0.01$ mM/ms used throughout this report decreases G_{mid} by 25.2% to 10.9 mM. Similarly, doubling the value of $V_{\text{GK}} = 0.01$ mM/ms used throughout this report decreases G_{mid} by 16.2% to 12.2 mM.

Sinusoidal variation in glucose can activate the glycolytic oscillator

In the glycolytic subsystem of the DOM, sustained oscillations are produced over an intermediate glucose range ($G_{\text{low}} < G_e < G_{\text{mid}}$), but they may be lost by increasing glucose to elevated concentrations ($G_e > G_{\text{mid}}$) (see Fig. 4B). We found that sustained oscillations can be recovered by subsequently varying G_e sinusoidally with sufficiently large amplitude and period.

To demonstrate the dependence on amplitude, we begin oscillating G_e with 0.5 mM

amplitude and 5 min period from the stable steady state at $G_e = 15$ mM above the
Hopf bifurcation at $G_{mid} \approx 14.5$ mM (Fig. 5A, top, $t < 60$ min). This results in small
FBP oscillations of the same period (Fig. 5A, bottom, $t < 60$ min), indicating the
glycolytic oscillator is not activated. At 60 min, we increase the amplitude of the
glucose oscillations to 1 mM so that now the glucose level crosses below G_{mid} (Fig. 5A,
top, $t > 60$ min). This produces oscillations in FBP that are much larger (Fig. 5A,
bottom, $t > 60$ min) indicating the glycolytic oscillator is activated. Thus, if the glucose
level is varied sinusoidally so that it crosses below G_{mid}, then the glycolytic oscillator
can be activated.

To demonstrate the dependence on period, we begin oscillating G_e with 1 min
period and 1 mM amplitude again from the stable steady state at $G_e = 15$ mM above
$G_{mid} \approx 14.5$ mM (Fig, 53B, top, $t < 60$ min). Although the glucose level crosses below
G_{mid}, the glycolytic oscillator is not activated. At 60 min, we increase the period of the
glucose oscillations to 5 min, which activates the glycolytic oscillator. Thus, even if the
glucose level is varied sinusoidally so that it crosses below G_{mid}, it must be varied with
sufficiently large period to activate the glycolytic oscillator.

We address the phenomena underlying these observations in the Discussion.

Fig 5. Model glycolytic oscillator recruitment.
A sinusoidal external glucose signal with baseline $G_e = 15$ mM, greater than the Hopf
bifurcation at $G_{mid} \approx 14.5$ mM, must have sufficiently large amplitude and period to
activate the DOM glycolytic oscillator. (A) External glucose oscillations started with 0.5
mM amplitude and 5 min period ($t < 60$ min) trigger large-amplitude FBP oscillations
only after the amplitude of glucose oscillations is increased to cross below $G_{mid} \approx 14.5$
mM ($t > 60$ min). (B) Although glucose oscillations started with 1 mM amplitude and 1
min period ($t < 60$ min) cross below G_{mid}, they trigger large-amplitude FBP oscillations
only after the period of glucose oscillations is increased to 5 min ($t > 60$ min).

Sinusoidal variation in glucose can initiate slow Ca^{2+} oscillations in the DOM

Next, using the full DOM rather than just the glycolytic subsystem, we simulate the
same glucose protocols as in Fig. 5 to examine the effect on cytosolic Ca^{2+}, Ca_c,
oscillations (Fig. 6). We begin oscillating G_e from the steady state at $G_e = 15$ mM,
above the Hopf bifurcation at $G_{mid} \approx 14.5$ mM (Fig. 6, $t < 60$ min). The glycolytic
oscillator is not activated and no slow Ca_c oscillations are produced when glucose
oscillations are started with 0.5 mM amplitude and 5 min period (Fig. 6A, $t < 60$ min)
or with 1 min period and 1 mM amplitude (Fig. 6B, $t < 60$ min). Instead, Ca_c is
elevated by a continuous spike train of action potentials carried by K(V) and Ca(V)
currents. The glycolytic oscillator activates when the amplitude of the glucose
oscillations started with 0.5 mM amplitude is increased to 1 mM (Fig. 6A, $t > 60$ min)
or the period of the glucose oscillations started with 1 min period is increased to 5 min
(Fig. 6B, $t > 60$ min). When the glycolytic oscillator activates, glycolytic oscillations
lead to oscillations in ATP level that, by modulating K(ATP) channels, recover bursting
electrical activity and slow Ca_c oscillations (Fig. 6, $t > 60$ min). Thus, activation of the
glycolytic oscillator underlies the recovery of slow Ca_c oscillations in the DOM, and an
oscillatory glucose signal that has sufficiently large amplitude and period can activate
the glycolytic oscillator and recover slow Ca_c oscillations.

Fig 6. Model islet slow oscillator recruitment.
A sinusoidal glucose stimulus with baseline $G_e = 15$ mM greater than the Hopf bifurcation
at $G_{\mathrm{mid}} \approx 14.5$ mM with sufficiently large amplitude and period activates the glycolytic
oscillator to recover slow Ca_c oscillations in the DOM. (A) Glucose oscillations started
with 0.5 mM amplitude and 5 min period ($t < 60$ min) trigger slow Ca_c oscillations
only after the amplitude of glucose oscillations is increased ($t > 60$ min) to activate
the glycolytic oscillator. (B) Glucose oscillations started with 1 min period and 1 mM
amplitude ($t < 60$ min) trigger slow Ca_c oscillations only after the period of glucose
oscillations is increased to 5 min ($t > 60$ min) to activate the glycolytic oscillator.

Sinusoidal variation in glucose can initiate slow Ca^{2+} oscillations in islets

We tested the model prediction that slow Ca^{2+} oscillations lost in high glucose could be
recovered by delivering a sinusoidal glucose signal with sufficiently large amplitude and
period. This is performed using our microfluidic platform described in Methods.

First, we tested the predicted dependence on amplitude (Fig. 7). After slow Ca^{2+}
oscillations were lost by increasing glucose to 15 mM, we began oscillating glucose with
0.5 mM amplitude and 5 min period for approximately 2 hr (Fig. 7, top, $t < 60$ min).
The last hour for two islets is depicted in Fig. 7 (top, $t < 60$ min). This did not initiate
slow, large-amplitude Ca^{2+} oscillations although small-amplitude oscillations are
apparent (Fig. 7, bottom, $t < 60$ min). At 60 min Next, we increased the amplitude of
the glucose oscillations to 1 mM (Fig. 7, top, $t > 60$ min). In one islet (Fig. 7A), this
triggered slow Ca^{2+} oscillations with large amplitude after an approximately 20 min
delay (Fig. 7, bottom, $t > 60$ min). In another islet (Fig. 7B), this triggered slow
large-amplitude Ca^{2+} oscillations with a period approximately twice that of the
imposed glucose signal (Fig. 7B, $t > 60$ min). Delayed initiation of Ca^{2+} oscillations
and a difference between G_e and Ca^{2+} oscillation periods are evidence that the glucose
signal activated an endogenous oscillator with an intrinsic stability and period and did
not merely force the islet back and forth between silent and active phases.
Increasing the amplitude of sinusoidal glucose oscillations after slow Ca^{2+}
oscillations had been lost as in Fig. 7 successfully recovered slow Ca^{2+} oscillations in
10 out of 15 islets.

**Fig 7. Islet slow oscillator recruitment and dependence on sinusoidal glucose
signal amplitude.**
As predicted by the DOM, an imposed sinusoidal glucose signal must have sufficiently
large amplitude to trigger slow Ca^{2+} oscillations in islets. Glucose oscillations started
with 0.5 mM amplitude and 5 min period about a 15 mM baseline concentration ($t < 60$
min) triggered slow Ca^{2+} oscillations only after the amplitude of the glucose oscillations
was increased to 1 mM ($t > 60$ min). (A) Slow Ca^{2+} oscillations were triggered with an
approximately 20 min delay, and (B) in another islet slow Ca^{2+} oscillations were triggered
with a period greater than that of the glucose signal, indicating that an endogenous
oscillator was activated.

Next, we tested the predicted dependence on period. After slow Ca^{2+} oscillations
were lost by increasing glucose to a high concentration, we began oscillating glucose
with 1 min period and 2 mM amplitude for 1 hr. (Fig. 8A, top, $t < 40$ min and Fig. 8B,
top, $t < 60$ min). The last 40 min for one islet are depicted in Fig. 8A (top, $t < 40$ min)
and the full hour for another islet is depicted in Fig. 8B (top, $t < 60$ min). An
amplitude of 2 mM, larger than in the simulations depicted in Fig. 6, was used in an
attempt to trigger slow Ca^{2+} oscillations despite the high-frequency glucose input.
However, slow, large-amplitude Ca^{2+} oscillations were triggered only after we increased

the period of glucose oscillations to 5 min (Fig. 8A, $t > 40$ min and Fig. 8B, $t > 60$ min). In one islet, slow Ca^{2+} oscillations were triggered after approximately a 40 min delay (Fig. 8A, $t > 40$ min), and in another islet, slow Ca^{2+} oscillations were triggered with a period nearly twice that of the glucose signal (Fig. 8B, $t > 60$ min). This is further evidence that the imposed glucose signal activated an endogenous oscillator.

Fig 8. Islet slow oscillator recruitment and dependence on sinusoidal glucose signal period.
As predicted by the model, an imposed sinusoidal glucose signal must have sufficiently large period to trigger slow Ca^{2+} oscillations. Glucose oscillations started with 1 min period and 2 mM amplitude about a high baseline concentration ($t < 60$ min) triggered slow Ca^{2+} oscillations only after the period of the glucose oscillations was increased to 5 min ($t > 60$ min). Slow Ca^{2+} oscillations were triggered with (A) an approximately 40 min delay and (B) a period greater than that of the glucose signal in another islet, both indicating an endogenous islet slow oscillator was activated.

What if the imposed glucose signal is even slower? The model predicts that slow Ca^{2+} oscillations could still be recovered. We tested this prediction (Fig. 9). After slow Ca^{2+} oscillations were lost by increasing glucose to a high concentration, oscillating glucose with 1 min period and 2 mM amplitude for 1 hr did not trigger slow Ca^{2+} oscillations in two islets (Fig. 9A, $t < 40$ min and Fig. 9B, $t < 20$ min), yet increasing the period of the glucose oscillations to 10 min triggered slow Ca^{2+} oscillations (Fig. 9A, $t > 40$ min and Fig. 9B, $t > 20$ min). In one islet (Fig. 9A), the slow oscillator was entrained to the glucose signal, evident from synchronization of the Ca^{2+} peaks and glucose nadirs (Fig. 9A, $t > 40$ min). In another islet (Fig. 9B), Ca^{2+} oscillations emerged with a period approximately half that of the glucose signal (Fig. 9B, $t > 20$ min). Thus, imposed glucose oscillations with a period as slow as 10 min can activate the islet slow oscillator.
~~Increasing the period of high-frequency sinusoidal glucose oscillations after slow Ca^{2+} oscillations had been lost as in Figs. 8 and 9 successfully recovered slow Ca^{2+} oscillations in 14 out of 26 islets.~~

Fig 9. Islet slow oscillator recruitment by sinusoidal glucose signals with 10 min period.
Imposed glucose oscillations with a period as slow as 10 min can activate the slow islet oscillator. (A) Slow Ca^{2+} oscillations were entrained by a sinusoidal glucose signal with 10 min period. (B) Slow Ca^{2+} oscillations were triggered with a period approximately half that of the imposed glucose signal in another islet.

In Fig. 10, we depict an experiment that exhibited most of the islet slow oscillator properties mentioned above in a single time course. We started by fixing external glucose at 15 mM (Fig. 10, top, $t < 30$ min) which triggered slow Ca^{2+} oscillations (Fig. 10, bottom, $t < 30$ min). We then stepped glucose up to 18 mM (Fig. 10, top, $30 < t < 50$ min), which terminated slow Ca^{2+} oscillations (Fig. 10, bottom, $30 < t < 50$ min), indicating that the islet slow oscillator's predicted G_{mid} value is less than 18 mM. We then began oscillating glucose with 1 mM amplitude and 5 min period (Fig. 10, top, $50 < t < 80$ min), which did not recover slow Ca^{2+} oscillations (Fig. 10, bottom, $50 < t < 80$ min), indicating further that G_{mid} is less than 17 mM, the minimum of the glucose oscillations during $50 < t < 80$ min. When we increased the amplitude of the glucose oscillations to 2 mM, slow Ca^{2+} oscillations were recovered with a period ~~nearly twice~~ approximately three times that of the glucose signal (Fig. 10, $80 < t < 180$ min), but similar to the period of Ca^{2+} oscillations during $t < 30$ min. Finally, when we decreased the period of glucose oscillations to 2 min, slow Ca^{2+} oscillations were eventually lost (Fig. 10, $t > 180$ min).

Fig 10. Islet slow oscillator recruitment and dependence on sinusoidal glucose signal amplitude and period.
After slow Ca^{2+} oscillations were lost by increasing external glucose concentration to 18 mM at $t = 30$ min, they were recovered at $t = 80$ min by oscillating glucose with sufficiently large amplitude and lost again after $t = 180$ min by decreasing the period of glucose oscillations.

In summary, slow Ca^{2+} oscillations were activated by glucose oscillations in 24 of 40 islets: in 9 of 11 islets by a G_e waveform with 1 mM amplitude and 5 min period, in 5 of 9 islets by a G_e waveform with 2 mM amplitude and 5 min period, and in 10 of 20 islets by a G_e waveform with 2 mM amplitude and 10 min period. Slow Ca^{2+} oscillations were activated in 10 of 15 islets in which the amplitude of the G_e waveform was stepped up (as in Fig. 7), and in 14 of 25 islets in which the period of the G_e waveform was stepped up (as in Figs. 8 and 9). The period of activated Ca^{2+} oscillations was closer to 5 min than to a multiple of 5 min in 15 of the 24 activated islets, regardless of the G_e waveform period: 8 of 14 islets by a G_e waveform with 5 min period and 7 of 10 islets by a G_e waveform with 10 min period. The effect of stepping the G_e waveform period down to 2 min after stepping the G_e waveform amplitude up to recover slow Ca^{2+} oscillations (as in Fig. 10) was studied in 10 islets. Of those 10 islets, slow Ca^{2+} oscillations were initially recovered in 5 islets, and of those 5 islets, slow oscillations were subsequently terminated by stepping down the G_e waveform period in 3 islets. In Fig. 11A, we plot the fraction of activated islets and the period of activated Ca^{2+} oscillations versus G_e waveform baseline, and in Fig. 11B, we plot histograms of the period of activated Ca^{2+} oscillations by G_e waveforms with 5 min or 10 min period.

Fig 11. Summary of islet slow oscillator recruitment by a sinusoidal glucose signal.
(A) The fraction of islets in which slow Ca^{2+} oscillations were activated and the period of activated Ca^{2+} oscillations versus G_e waveform baseline for the three combinations of G_e amplitude and period considered (1 mM/5 min, 2 mM/5 min, and 2 mM/10 min). For each amplitude and period combination, the activated fraction is generally higher for lower G_e waveform baselines, consistent with the existence of G_{mid}. Ca^{2+} oscillation periods varied from 2 min to 14 min (mean: 6.56 min, standard deviation: 2.99 min). (B) Histograms of the period of activated Ca^{2+} oscillations by G_e waveforms with 5 min (left) or 10 min (right) periods. Regardless of the glucose waveform period, the majority of activated Ca^{2+} oscillations had a period closer to 5 min than to a multiple of 5 min.

Slow-oscillating islets can recruit fast-oscillating islets through negative feedback

As mentioned above, a constant elevated glucose stimulus may produce pulsatile insulin secretion from only a sub-population of the pancreatic islets. That sub-population of oscillating islets may, through insulin-dependent hepatic control of blood glucose, establish blood glucose oscillations that recruit non-oscillating islets by the mechanism examined in the last section. We tested this hypothesis using a closed loop system, previously described in [20], consisting of the microfluidic device described in Methods and a model liver that controls glucose delivery to the islets in the device chamber (Fig. 12A). When the loop is closed, the insulin output from the islet population is modeled as a function of average intracelluluar Ca^{2+} concentration:

$$I_{avg} = \max(0, I_{slope}(Ca_{avg} - Ca_{thr})),$$

and the level of G_e delivered to the islets is continuously updated according to: 313

$$\frac{dG_e}{dt} = \frac{G_\infty(I_{avg}) - G_e}{\tau_G}$$

$$G_\infty(I_{avg}) = G_{min} + \frac{G_{max} - G_{min}}{1 + \exp[-(I_{avg} - I_{1/2})/S_G]}.$$

Notice, since the dose-response curve G_∞ is a decreasing function of I_{avg}, that this 314 supplies negative feedback to insulin secretion. Parameter values for these equations are 315 listed in S2 Model Parameter Values. 316

Fig. 12B depicts the G_e and Ca^{2+} time courses for 6 islets initially stimulated by a 317 constant 11 mM glucose signal ($t < 20$ min), then interacting in the closed loop system 318 ($t > 20$ min). The initial 11 mM glucose signal triggered slow Ca^{2+} oscillations in 4 319 islets and fast Ca^{2+} oscillations in 2 islets, indicating G_{mid} is greater than 11 mM in the 320 4 slow-oscillating islets but less than 11 mM in both fast-oscillating islets. Before the 321 loop was closed, Ca^{2+} oscillations were not synchronized among the population, 322 resulting in only small-amplitude oscillations in the average Ca^{2+} time course. When 323 the loop was closed, G_e oscillations with ≈ 3 mM amplitude emerged and recruited the 324 fast-oscillating islets, indicating G_{mid} is greater than 8 mM in both of the 325 fast-oscillating islets, the minimum concentration of G_e oscillations. When the loop was 326 closed, Ca^{2+} oscillations were synchronized among the population, resulting in 327 large-amplitude slow oscillations in the average Ca^{2+} time course. Thus, a 328 sub-population of islets that exhibit slow oscillations in response to an elevated glucose 329 concentration can recruit fast-oscillating islets through negative feedback to amplify the 330 average Ca^{2+} oscillation. We also found with the model that when 20 out of 25 islets in 331 a population, heterogeneous in their G_{mid} values, initially exhibit slow oscillations in 332 response to a constant 15 mM glucose signal, the other 5 islets could be recruited and 333 the average Ca^{2+} oscillation amplified when the loop was closed. 334

Fig 12. Recruitment of fast-oscillating islets by slow-oscillating islets through negative feedback.
(A) Schematic of a closed loop system in which glucose is delivered to islets in the chamber of a microfluidic device and a model liver controls glucose delivery. Average insulin, I_{avg}, modeled as a function of average intracellular Ca^{2+} Ca_{avg}, is used in a differential equation for G_e to continuously update the concentration of delivered glucose. (B) A population of 6 islets is initially delivered a constant 11 mM glucose signal ($t < 20$ min), then interacts in the closed loop ($t > 20$ min). When the loop was closed, 4 slow-oscillating islets synchronized and recruited 2 fast-oscillating islets to amplify the average Ca^{2+} oscillation.

Discussion 335

We have demonstrated that slow Ca^{2+} oscillations in pancreatic islets lost by fixing 336 glucose at an elevated concentration can be recovered by delivering a sinusoidal glucose 337 signal with sufficiently large amplitude and period. This was predicted by our model of 338 islet β-cells, which postulates that the sinusoidal glucose signal triggers an endogenous 339 glycolytic oscillator (Fig. 5) and that triggering of the glycolytic oscillator underlies 340 recovery of slow Ca^{2+} oscillations (Fig. 6). We obtained experimental support for these 341 predictions using a microfluidics platform (Figs. 7-11 ~~10~~). Importantly, Ca^{2+} 342 oscillations were often triggered after a delay or with a period significantly different 343 than that of a sinusoidal glucose signal, consistent with the activation of an endogenous 344 islet oscillator, rather than a situation in which the islet is forced back and forth 345

between inactive and active states. The data therefore provide further support for the key hypothesis of the Dual Oscillator Model: glycolytic oscillations endogenous to β-cells drive slow Ca^{2+} oscillations and pulsatile insulin secretion. The data also provide evidence for the existence of a subcritical Hopf bifurcation at the lower end ($G_e = G_{mid}$) of a glucose range of bistability ($G_{mid} < G_e < G_{up}$), in which oscillations may or may not occur. Furthermore, we have demonstrated, by coupling a model liver to microfluidic platform in a closed loop system, that a sub-population of islets, which respond to an elevated glucose stimulus with slow Ca^{2+} oscillations, can recruit the other islets to become slow oscillators and thereby amplify the population average Ca^{2+} oscillation (Fig. 12).

Finding that islet slow oscillators were heterogeneous (Fig. 1) was not surprising. The values of G_{low}, G_{mid}, and G_{up} for the glycolytic oscillator depend on reaction rates of the glycolytic enzymes in β-cells, and specifically on the kinetics of allosteric activation of PFK1 by its product, FBP. We expect that islets are subject to varying genetic and environmental factors by virtue of being heterogeneous in other respects, such as size and cell composition, and being dispersed throughout the pancreas. This would cause islet-to-islet variation in glycolytic enzymes' catalytic properties.

The prediction that a sinusoidal glucose signal must have sufficiently large amplitude to trigger slow oscillations is simple to explain in terms of the glycolytic oscillator. Starting from a steady state with $G_e > G_{mid}$, G_e oscillations with amplitude large enough to cross below G_{mid} lead the state of the glycolytic subsystem to the only stable structure that exists for $G_{low} < G_e < G_{mid}$: a limit cycle. But why must the sinusoidal glucose signal have sufficiently large period to trigger slow oscillations (Figs. 5B and 6B)? The answer in terms of the glycolytic oscillator is somewhat subtle and relies on the transition between monostable (Fig. 4C, top) and bistable (Fig. 4C, bottom) glucose ranges. In Fig. 13, we depict the state of the glycolytic subsystem, i.e. the phase point, trajectory (red curve in Fig. 13) in the F6P vs. FBP phase plane as G_e oscillations transition from 1 min period (Fig. 13, left) to 5 min period (Fig. 13, right) as in Fig. 5B, $55 < t < 75$ min (see S3 Video for an animated version of Fig. 13). The system alternates between bistable when $G_e > G_{mid} \approx 14.5$ mM (Fig. 13, top) and monostable when $G_e < G_{mid}$ (Fig. 13, bottom). When bistable, the system has a stable equilibrium surrounded by an unstable limit cycle (dashed curve in Fig. 13) which is itself surrounded by a stable limit cycle (solid black curve in Fig. 13). The unstable limit cycle is the separatrix between the domain of attraction (shaded gray in Fig. 13, top) of the stable equilibrium and the domain of attraction (white in Fig. 13 top and bottom) of the stable limit cycle.

Beginning from a steady state with $G_e > G_{mid}$ (Fig. 13, top left), the phase point is close and attracted to the stable equilibrium point. As G_e drops below G_{mid}, the equilibrium becomes unstable and it's domain of attraction vanishes (as the unstable limit cycle and equilibrium point coalesce), so the phase point momentarily remains close to the equilibrium point yet it is attracted to the stable limit cycle (Fig. 13, bottom left). The time spent below G_{mid} during a cycle of G_e oscillations with 1 min period is so brief that before the phase point can approach the stable limit cycle, the stable equilibrium's domain of attraction re-emerges and traps the phase point as G_e increases past G_{mid}. That is, the speed of the phase point trajectory is slow compared to the speed at which the stable equilibrium's domain of attraction vanishes and re-emerges by G_e oscillations with 1 min period, and therefore the phase point cannot escape the stable equilibrium's domain of attraction. The situation changes when the period of G_e oscillations increases to 5 min (Fig. 13, right). Now, when G_e drops below G_{mid} the phase point travels a significant distance from the unstable equilibrium towards the stable limit cycle (Fig. 13, bottom center). As G_e increases past G_{mid}, the stable equilibrium's domain of attraction re-emerges and transiently traps the phase

point (Fig. 13, top center). However, after a few cycles, the phase point escapes the
stable equilibrium's domain of attraction and approaches the stable limit cycle (Fig. 13,
top right). Afterwards, the phase point continues to follow the stable limit cycle
(Fig. 13, bottom right), producing the glycolytic oscillations that result in slow Ca^{2+}
oscillations in our model and, presumably, in islets.

Fig 13. Phase plane analysis of glycolytic oscillator recruitment and dependence on sinusoidal glucose signal period.
The glycolytic subsystem transitions between bistable (top) and monostable (bottom)
as G_e oscillations cross the Hopf bifurcation at $G_{mid} \approx 14.5$ mM. When bistable, a
stable equilibrium is surrounded by an unstable limit cycle (dashed curve, top), which is
surrounded by a stable limit cycle (solid black curve, top and bottom); when monostable,
an unstable equilibrium is surrounded by a stable limit cycle. The state of the glycolytic
subsystem, i.e. the phase point, trajectory (red curve) is trapped by rapid alternation
between disappearance and re-emergence of the stable equilibrium's domain of attraction
(shaded gray) when G_e oscillates with 1 min period (left) but not when when G_e oscillates
with 5 min period (right) (see S3 Video for an animated version of this figure.)

The ability to trigger the glycolytic oscillator with glucose oscillations requires that
(1) the baseline glucose level is above G_{mid}, (2) the oscillation amplitude is large enough
to cross below G_{mid}, and (3) the oscillation period is large enough that the phase point
can escape the stable equilibrium's domain of attraction. Satisfying these three
conditions simultaneously can be difficult, and this likely accounts for the imperfect rate
of successfully recovering slow Ca^{2+} oscillations in our experiments (10 out of 15 for the
protocol of Fig. 7 and 14 out of 25 for the protocols of Figs. 8 and 9). On the other
hand, requirement (2) may not strictly be necessary since inherent noise may perturb
the phase point out of the stable equilibrium's domain of attraction when G_e is in the
bistable range ($G_{mid} < G_e < G_{up}$) yet never crosses below G_{mid}. Another factor that
would contribute to the imperfect success rate is that the extent of bistability will likely
exhibit islet-to-islet heterogeneity, and may be quite small in some islets. If the bistable
region is too small, then one would not expect a glucose waveform to trigger sustained
slow oscillations.

In our model, the slow Ca^{2+} oscillations triggered by an oscillatory G_e signal are
stable and therefore persist indefinitely, in the absence of noise or other external stimuli,
if G_e oscillations are removed and G_e is held at the signal's baseline concentration.
However, we found that when noise is incorporated into the maximal rate of glucose
uptake (V_{GLUT}) and the baseline G_e concentration is in the bistable range
($G_{mid} < G_e < G_{up}$), glycolytic oscillations are terminated by noise perturbing the state
of the glycolytic subsystem into it's stable steady state's domain of attraction. We
tested whether Ca^{2+} oscillations persist when G_e oscillations are removed in 15 islets, of
which slow Ca^{2+} oscillations were recovered in 11 islets by a G_e waveform with 2 mM
amplitude: 4 of 6 islets by a G_e waveform with baseline 13 mM, 3 of 4 islets by a G_e
waveform with 15 mM baseline, and 4 of 5 islets by a G_e waveform with 16 mM
baseline. Of those 11 islets, slow Ca^{2+} oscillations did not persist for longer than one
period after G_e oscillations were removed. As mentioned above, we observed that
stepping the period of a G_e waveform that recovered Ca^{2+} oscillations down to 2 min
terminated Ca^{2+} oscillations (as in Fig. 10) in 3 of 5 islets. In the model, we found that
if Ca^{2+} oscillations were recovered by an oscillatory G_e signal with baseline in the
bistable range, stepping the period or amplitude down may or may not terminate the
recovered Ca^{2+} oscillations. Whether or not the transition terminates the recovered
oscillations depends on the value of G_{mid}.

Are there alternate explanations for the activation of Ca^{2+} oscillations by G_e
oscillations? The most obvious alternative, which we refer to as the *push-pull*

mechanism, is that the glucose waveform moves the system back and forth between periods when the cell spikes continuously and periods when it is silent, which does not require an endogenous oscillator at all. If this was the case, however, the period of the Ca^{2+} oscillations should equal the period of the glucose waveform. Yet, our data show the Ca^{2+} oscillations that result from the glucose waveform can be faster ~~(Fig. 9)~~ or slower ~~(Figs. 7, 8, and 10)~~ than the waveform (Fig. 11B). This is inconsistent with the push-pull mechanism. A second alternate explanation is that islets have an endogenous oscillator, but it is not the proposed glycolytic oscillator. This could be true as long as the oscillator has a similar bifurcation structure. However, we are unaware of what this alternate endogenous oscillator could be, while there is now ample direct evidence for a glycolytic oscillator in islet β-cells [26–28].

The findings presented here have potential physiological implications. If glucose levels sensed by islets are oscillatory, and of sufficiently large amplitude and period, then some islets that would not secrete insulin in a pulsatile manner could be recruited to do so, and this would be beneficial to glucose homeostasis. Few glucose measurements have been made with the temporal resolution needed to detect oscillations with period 5 min or less. In one study in humans, blood sampling done every minute revealed oscillations in glucose of ≈ 0.25 mM from nadir to peak [29]. These oscillations are smaller than those imposed in the current study, but they could be amplified by the islets themselves, which take up and metabolize glucose. Indeed, *in vitro* studies of mouse islets made with glucose-sensing electrodes showed fast (≈ 15 sec) or slow (≈ 3 min) intra-islet glucose oscillations even at a static bath glucose level. These oscillations ranged from 0.2 mM in the case of fast oscillations to 3 mM in the case of slow oscillations, from nadir to peak [30]. The amplitude of these latter oscillations is similar to that of the glucose oscillations imposed in the current study. ~~Measurements of plasma glucose in humans reveal small oscillations with period on the order of 5 min [19, 29]. We have previously shown that these could be due to the liver, which creates blood glucose oscillations in response to insulin oscillations output from the pancreas [20]. Our results demonstrate that these glucose oscillations can help to recruit non-oscillating islets so that a larger fraction of the islet population is in an oscillatory state. This would increase the pulsatile insulin secretion that has been shown to be beneficial to glucose homeostasis.~~

Supporting Information

S1 Model Equations

The Dual Oscillator Model equations are as follows.

Glycolysis:

$$dG_i/dt = J_{\text{GLUT}} - J_{\text{GK}}$$

$$d\text{F6P}/dt = (1 + k_{\text{GPI}})^{-1}(J_{\text{GK}} - J_{\text{PFK}})$$

$$d\text{FBP}/dt = (1 + k_{\text{LG}})^{-1}(J_{\text{PFK}} - J_{\text{PDH}})$$

$$J_{\text{GLUT}} = V_{\text{GLUT}} \left(\frac{G_e}{G_e + k_{\text{GLUT}}^{\text{GLC}}} - \frac{G_i}{G_i + k_{\text{GLUT}}^{\text{GLC}}} \right)$$

$$J_{\text{GK}} = V_{\text{GK}} \frac{G_i^{h_{\text{GK}}^{\text{GLC}}}}{G_i^{h_{\text{GK}}^{\text{GLC}}} + k_{\text{GK}}^{h_{\text{GK}}^{\text{GLC}}}}$$

$$J_{\text{PFK}} = V_{\text{PFK}} \frac{(1 - k_{\text{PFK}})w_{1110} + k_{\text{PFK}} \sum_{i,j,l \in \{0,1\}} w_{ij1l}}{\sum_{i,j,k,l \in \{0,1\}} w_{ijkl}}$$

$$w_{ijkl} = \frac{\left(\frac{\text{AMP}}{K_{\text{PFK}}^{\text{AMP}}} \right)^i \left(\frac{\text{FBP}}{K_{\text{PFK}}^{\text{FBP}}} \right)^j \left(\frac{\text{F6P}^2}{K_{\text{PFK}}^{\text{F6P}}} \right)^k \left(\frac{\text{ATP}^2}{K_{\text{PFK}}^{\text{ATP}}} \right)^l}{f_{\text{AMP}}^{ik} f_{\text{FBP}}^{jk} f_{\text{MT}}^{il} f_{\text{BT}}^{jl} f_{\text{ATP}}^{kl}}$$

$$J_{\text{PDH}} = k_{\text{PDH}} \sqrt{\text{FBP}}$$

ATP Production/Hydrolysis:

$$d\text{ATP}/dt = J_{\text{ANT}} - J_{hyd}$$

$$J_{\text{ANT}} = V_{\text{ANT}} \text{ADP} \exp\left[\left(k_{\text{ANT}} + k_{\text{ANT}}^{\text{PDH}} \frac{J_{\text{PDH}}}{J_{\text{PDH}} + V_{\text{ANT}}^{\text{PDH}}} \right) \left(1 - \frac{Ca_c}{k_{\text{ANT}}^{Ca}} \right) \right]$$

$$J_{hyd} = (k_{hyd,bas} + k_{hyd}^{Ca} Ca_c)\text{ATP}$$

$$\text{AMP} = \text{ADP}^2/\text{ATP}$$

$$\text{ATP} + \text{ADP} + \text{AMP} = A_{tot}$$

Ionic Currents:

$$dV/dt = -(I_{Ca(V)} + I_{K(V)} + I_{K(Ca)} + I_{K(\text{ATP})})/C_m$$

$$dn_{K(V)}/dt = (n_{K(V),\infty} - n_{K(V)})/\tau_{K(V)}$$

$$dCa_c/dt = k_{cyt}^{Ca}(J_{\text{PM}} - J_{\text{ER}})$$

$$dCa_{\text{ER}}/dt = k_{\text{ER}}^{Ca} J_{\text{ER}}$$

$$I_{i(s)} = g_{i(s)}n_{i(s)} \cdot (V - V_i), \quad i(s) \in \{Ca(V), K(V), K(Ca), K(\text{ATP})\}$$

$$n_{i(s),\infty} = [1 + (k_{i(s)}/r(s))^{h_{i(s)}}]^{-1}, \quad i(s) \in \{Ca(V), K(V), K(Ca)\}$$

$$n_{i(s)} = n_{i(s),\infty} \text{ for } i(s) \in \{Ca(V), K(V)\}$$

$$r(s) = \begin{cases} e^s & \text{if } s = V \\ s & \text{otherwise} \end{cases}$$

$$n_{K(\text{ATP})} = \frac{0.08\left(1 + 2\frac{\text{MgADP}^-}{kdd}\right) + 0.89\left(\frac{\text{MgADP}^-}{kdd}\right)^2}{\left(1 + \frac{\text{MgADP}^-}{kdd}\right)^2\left(1 + \frac{\text{ADP}^{3-}}{ktd} + \frac{\text{ATP}^{4-}}{ktt}\right)}$$

$$\text{MgADP}^- = 0.0165\text{ADP}$$

$$\text{ADP}^{3-} = 0.135\text{ADP}$$

$$\text{ADP}^{4-} = 0.05\text{ATP}$$

$$J_{\text{PM}} = -[I_{Ca(V)}/(2F) + k_{\text{PM}}Ca_c]$$

$$J_{\text{ER}} = k_{\text{ER},in}Ca_c - k_{\text{ER},out}(Ca_{\text{ER}} - Ca_c)$$

Model parameter values are listed in S2 Model Parameter Values. 474

S2 Model Parameter Values 475

Parameter	Value	Parameter	Value	Parameter	Value
V_{GLUT}	0.01 M/s	V_{GK}	0.01 M/s	V_{PFK}	0.01 M/s
k_{PDH}	0.001 M/s	$k_{\text{GLUT}}^{\text{GLC}}$	13 mM	k_{GK}	13 mM
$h_{\text{GK}}^{\text{GLC}}$	4	k_{GPI}	3.3	k_{PFK}	0.06
$K_{\text{PFK}}^{\text{AMP}}$	30 μM	$K_{\text{PFK}}^{\text{FBP}}$	1 μM	$K_{\text{PFK}}^{\text{F6P}}$	50 μM
$K_{\text{PFK}}^{\text{ATP}}$	0.1 μM	f_{AMP}	0.02	f_{FBP}	0.2
f_{MT}	20	f_{BT}	20	f_{ATP}	20
k_{LG}	0.3	V_{ANT}	1 s^{-1}	$k_{\text{hyd},bas}$	3.3×10^{-3} s^{-1}
k_{hyd}^{Ca}	0	k_{ANT}	1	$k_{\text{ANT}}^{\text{PDH}}$	2.2
$V_{\text{ANT}}^{\text{PDH}}$	0.05 M/s	k_{ANT}^{Ca}	3.5×10^{-4} μM	A_{tot}	3 mM
V_{Ca}	25 mV	V_K	-75 mV	$g_{Ca(V)}$	1 nS
$g_{K(V)}$	2.7 nS	$g_{K(Ca)}$	50 pS	$g_{K(\text{ATP})}$	25.5 nS
$v_{Ca(V)}$	-20 mV	$v_{K(V)}$	-16 mV	$k_{K(Ca)}$	0.5 μM
$h_{Ca(V)}$	0.08	$h_{K(V)}$	0.2	$h_{K(Ca)}$	2
$\tau_{K(V)}$	20 ms	C_m	5.3 pF	kdd	17 μM
ktd	26 μM	ktt	1 μM	k_{cyt}^{Ca}	0.01
k_{ER}^{Ca}	0.01	$k_{\text{ER},in}$	0.46 s^{-1}	$k_{\text{ER},out}$	0.02 s^{-1}
k_{PM}	0.23 s^{-1}	I_{slope}	1 A.U./nM	Ca_{thr}	0.22 μM
τ_G	50 s	G_{min}	8 mM	G_{max}	14 mM
$I_{1/2}$	30 A.U.	S_G	50 A.U.		

S2 Table. Model parameter values.
Parameter values of the Dual Oscillator Model used for all simulations and parameter values for the closed loop system used in Fig. 12.

S3 Video

S3 Video. Phase plane animation of glycolytic oscillator recruitment and dependence on sinusoidal glucose signal period.
An animated version of Fig. 13. The glycolytic subsystem transitions between bistable and monostable as G_e oscillations cross the Hopf bifurcation at $G_{\mathrm{mid}} \approx 14.5$ mM. When bistable, a stable equilibrium is surrounded by an unstable limit cycle (dashed curve), which is surrounded by a stable limit cycle (solid black curve); when monostable, an unstable equilibrium is surrounded by a stable limit cycle. The state of the glycolytic subsystem, i.e. the phase point, trajectory (red curve) is trapped by rapid alternation between disappearance and re-emergence of the stable equilibrium's domain of attraction (shaded gray) when G_e oscillates with 1 min period (left) but not when when G_e oscillates with 5 min period (right).

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