Using Mathematical Modeling and Experiments to Understand the Mechanism of Pulsatile Insulin Secretion

Richard Bertram
Department of Mathematics
and
Programs in Neuroscience and Molecular Biophysics
Florida State University
Tallahassee, FL 32306

Collaborators

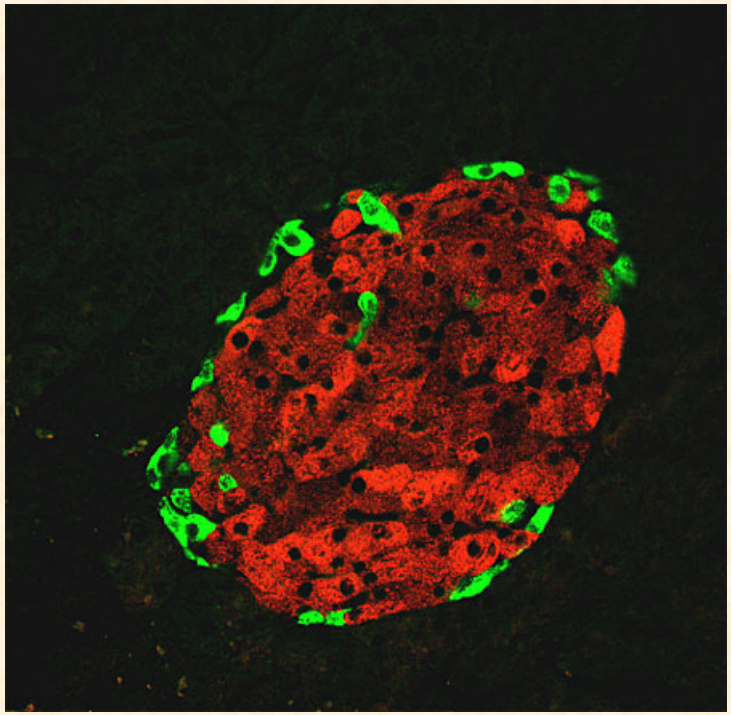
Mathematical	Experimental
Artie Sherman	**Les Satin**
Morten Gram Pedersen	Craig Nunemaker
Paul Smolen	Min Zhang
Krasi Tsaneva-Atanasova	Dan Luciani
Bernard Fendler	

Funding

National Science Foundation, Division of Mathematical Sciences

What is an Islet of Langerhans?

Coupled cluster of hormone-secreting cells. These clusters are located in the pancreas. Human pancreas has about 1 million islets.

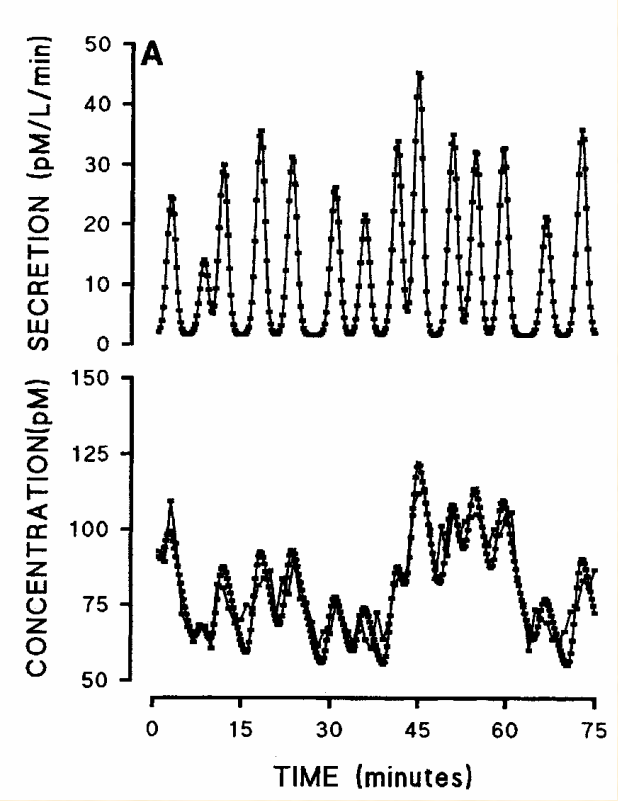


Courtesy of Rohit Kulkarni

Immunostained for glucagon (green) and insulin (red)

Insulin Secretion is Pulsatile

Porksen et al.,
AJP, 273:E908,
1997



deconvoluted

measured

Peripheral insulin measurements in the blood of humans exhibits oscillations, suggesting that insulin is secreted in a pulsatile manner.

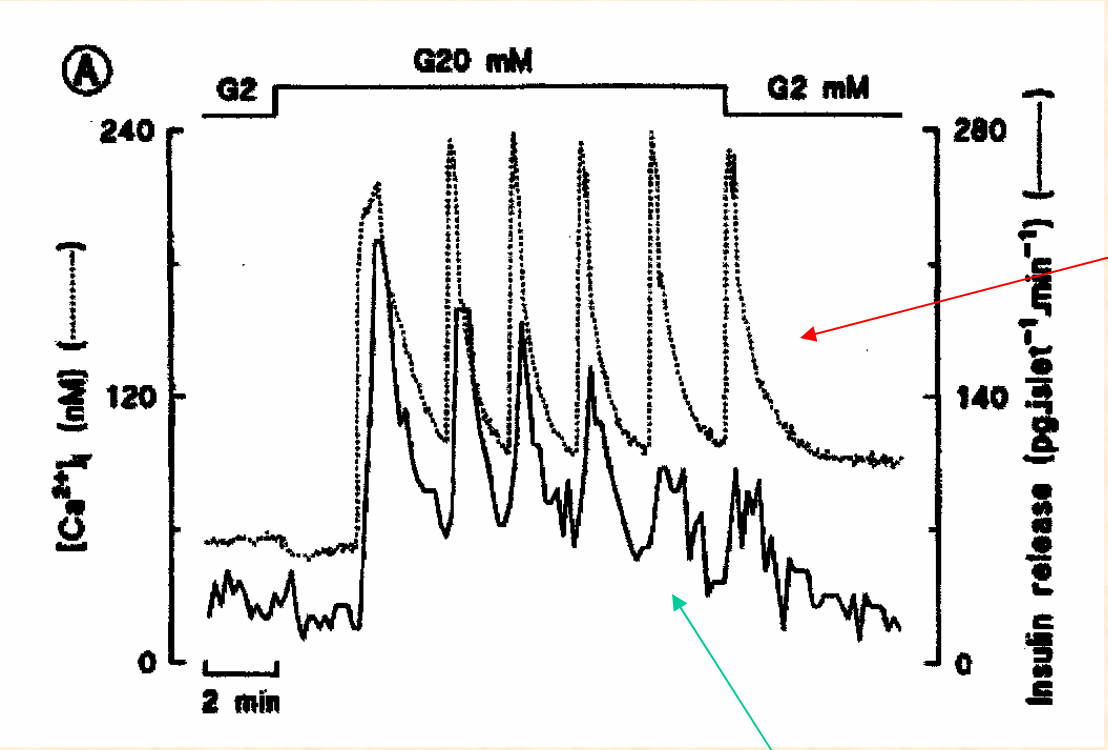
Central Questions:

1. What is the biophysical mechanism for pulsatile insulin secretion from an islet?

2. How do the many islets in a pancreas synchronize their activity?

Islets are Electrically Excitable

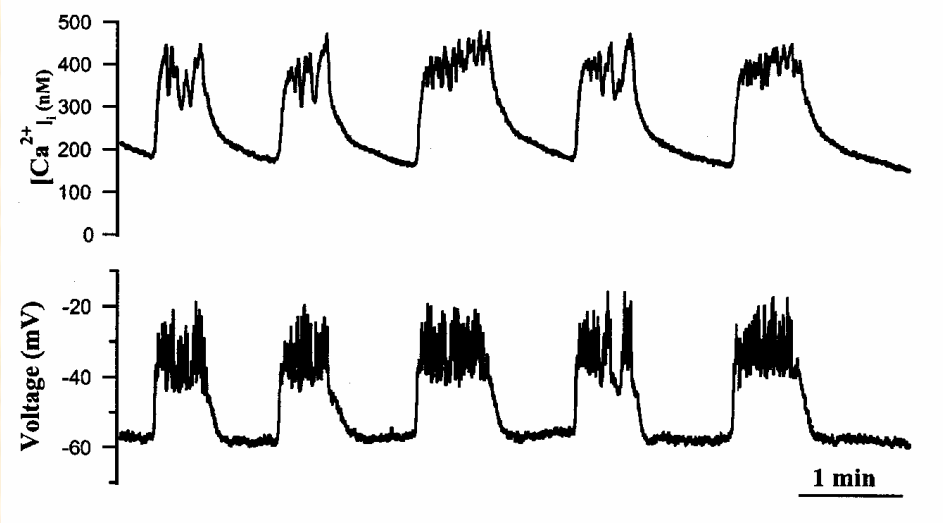
Islets are like nerve cells in that they produce electrical impulses. During an upstroke of an impulse Ca^{2+} enters the cells, causing insulin to be released.



Gilon et al., JBC, 268:22265, 1993

Islets Have Characteristic Patterns of Electrical Activity

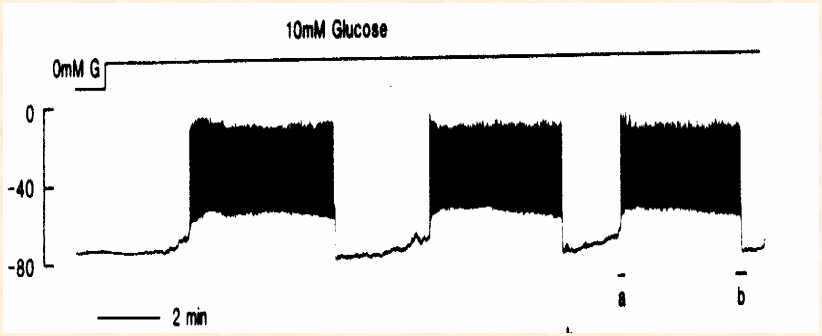
Fast Bursting Oscillations



Simultaneous fast Ca^{2+} and voltage measurements from a mouse islet in 11.1 mM glucose. From Zhang et al., *Biophys. J.*, 84:2852, 2003

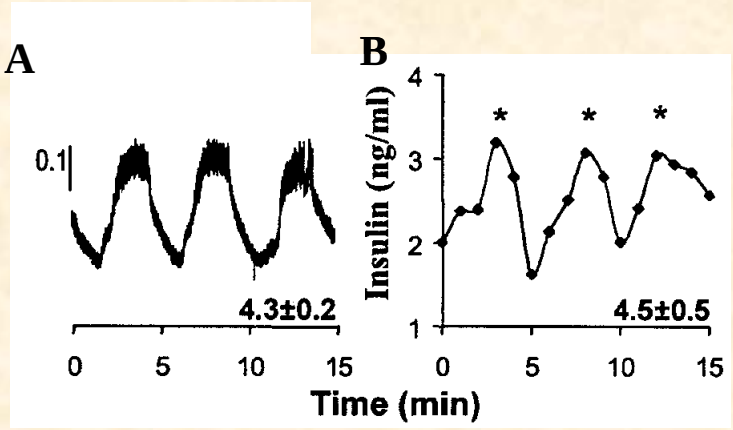
Slow Bursting Oscillations

Slow oscillations of Ca^{2+} and voltage from an islet...



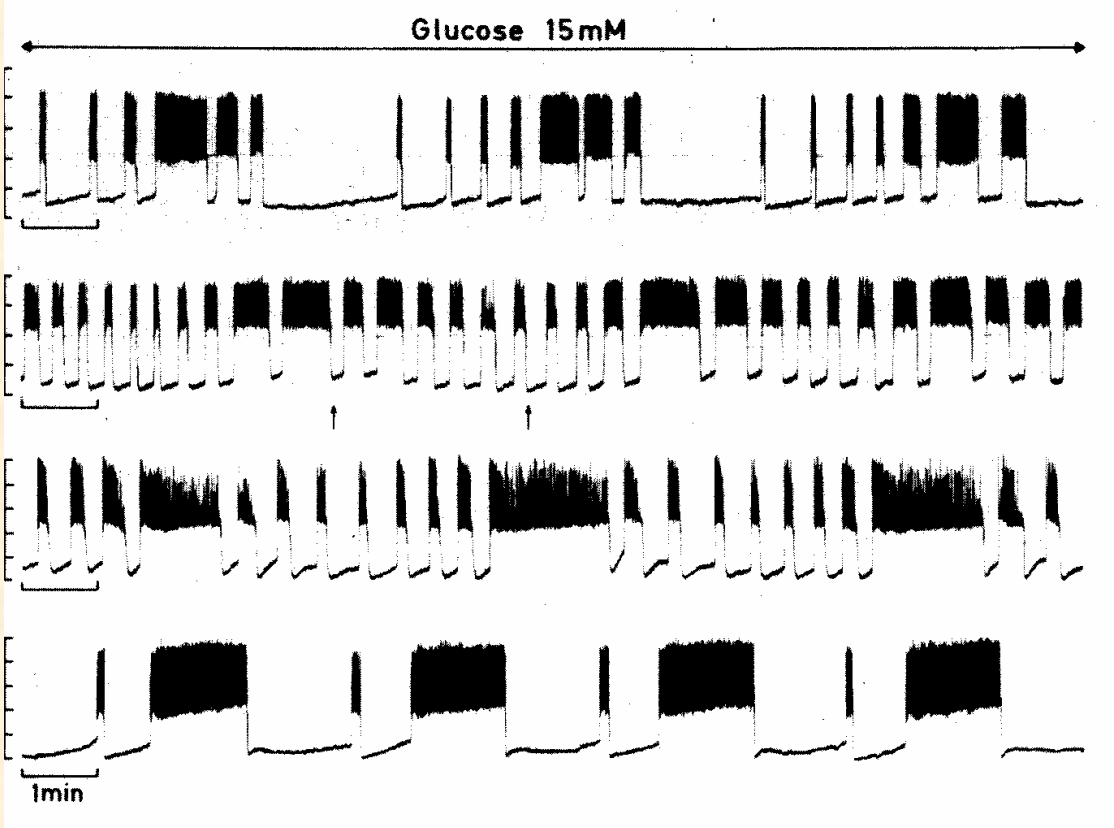
Smith et al., *FEBS Lett*., 261-187, 1990

Zhang et al., *Biophys. J*., 84:2852, 2003



…have period similar to slow insulin oscillations measured from a mouse in vivo (Nunemaker et al., Diabetes, 54:3517, 2005)

Compound Bursting Oscillations

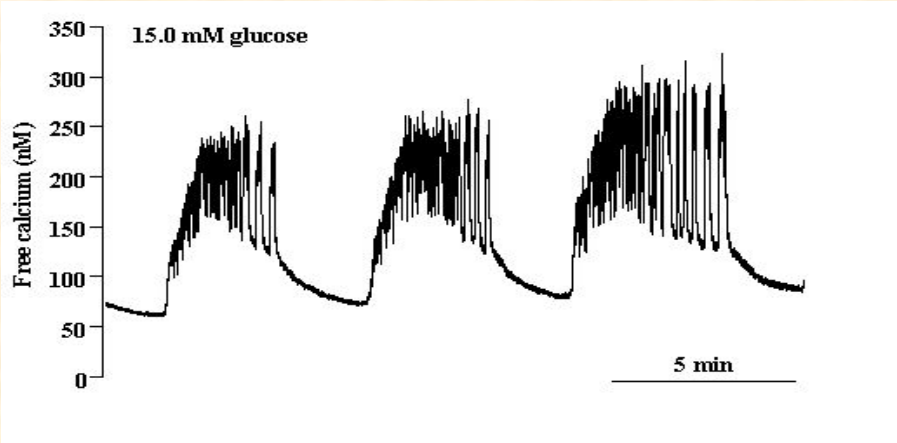


Henquin et al., Eur. *J. Physiol*., 393:322, 1982

Bursting oscillations superimposed on a slow wave of activity

More Evidence of Compound Oscillations

Measurements of intracellular Ca^{2+} also reveal compound oscillations.



Compound Ca^{2+} oscillations in an islet
(Zhang et al., *Biophys. J.*, 84:2852, 2003)

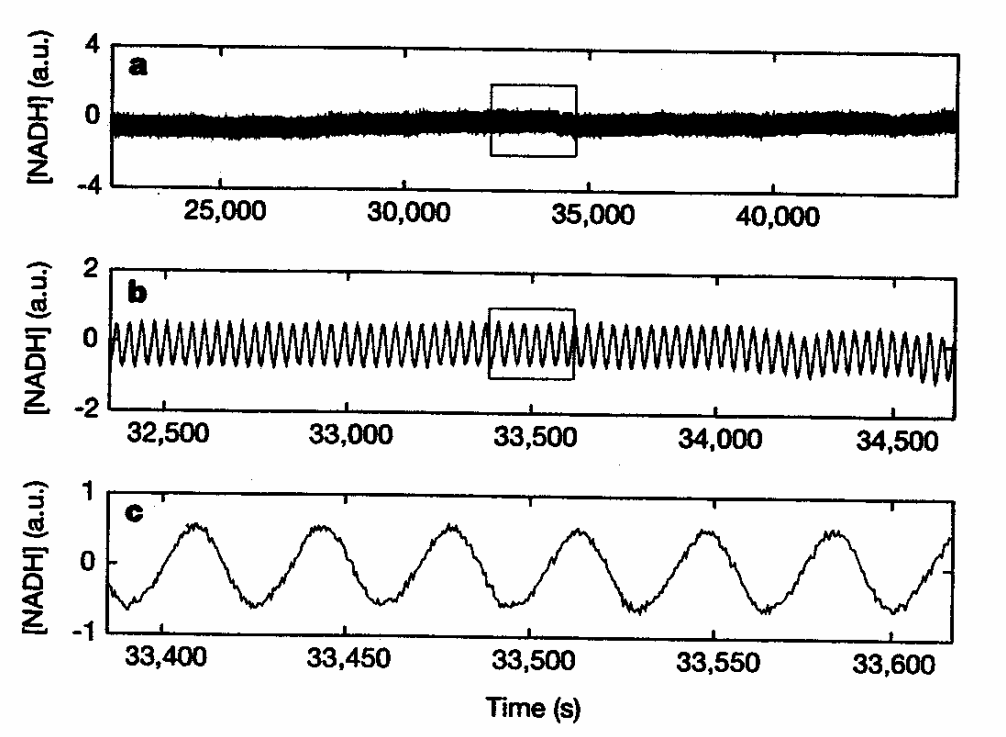
Goal: Develop a Mathematical Model That Can Reproduce the Various Patterns of Activity

The Dual Oscillator Model

Central Hypothesis

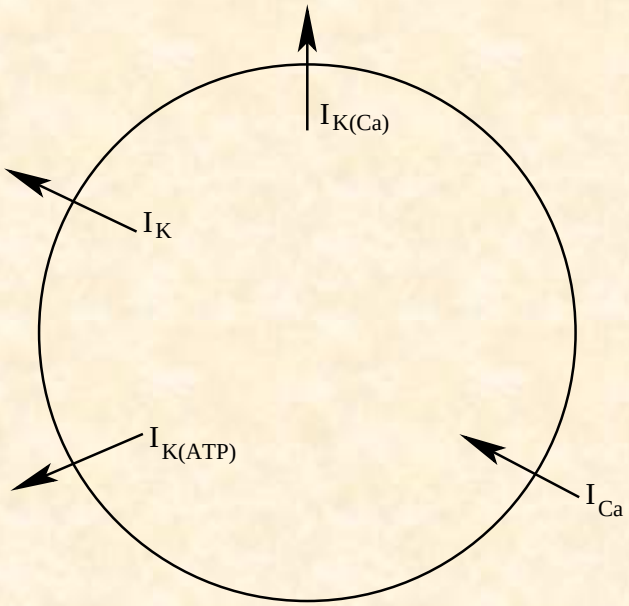
Fast, slow, and compound oscillations can all be produced by a mechanism that includes Ca^{2+} feedback onto ion channels (for the fast component of the oscillation) and glycolytic oscillations (for the slow component). This mechanism is the basis for our recent **Dual Oscillator Model** (DOM) for β-cell activity.

Glycolytic Oscillations in Yeast



Dano et al., Nature, 402:320-322, 1999

Electrical Component of the DOM



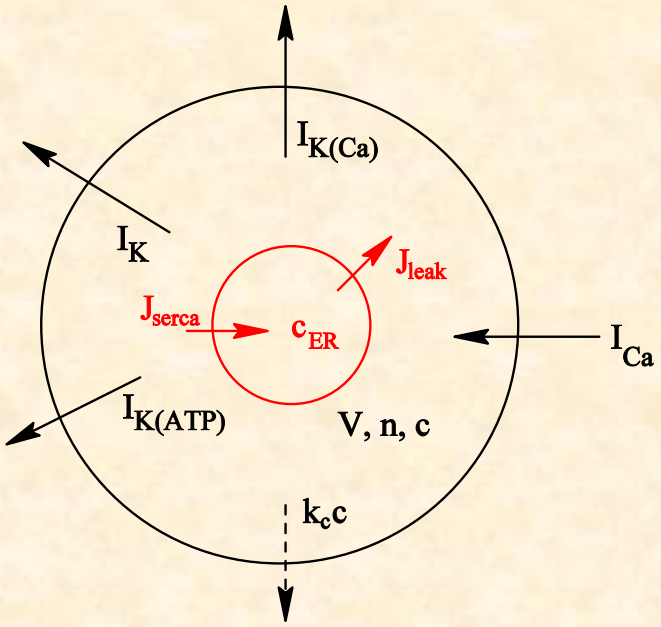
$$\dot{V} = -(I_{Ca} + I_K + I_{K(Ca)} + I_{K(ATP)})/C_m$$

$$\dot{n} = (n_\infty(V) - n)/\tau_n$$

Voltage equation reflects Kirchoff's current law

Second equation describes dynamics of the K^+ activation variable *n*. This depends on the voltage.

Electrical/Calcium Components of the DOM



$$\dot{V} = -(I_{Ca} + I_K + I_{K(Ca)} + I_{K(ATP)})/C_m$$

$$\dot{n} = (n_\infty(V) - n)/\tau_n$$

$$\dot{c} = f(J_{leak} - J_{serca} - \alpha I_{Ca} - k_c c)$$

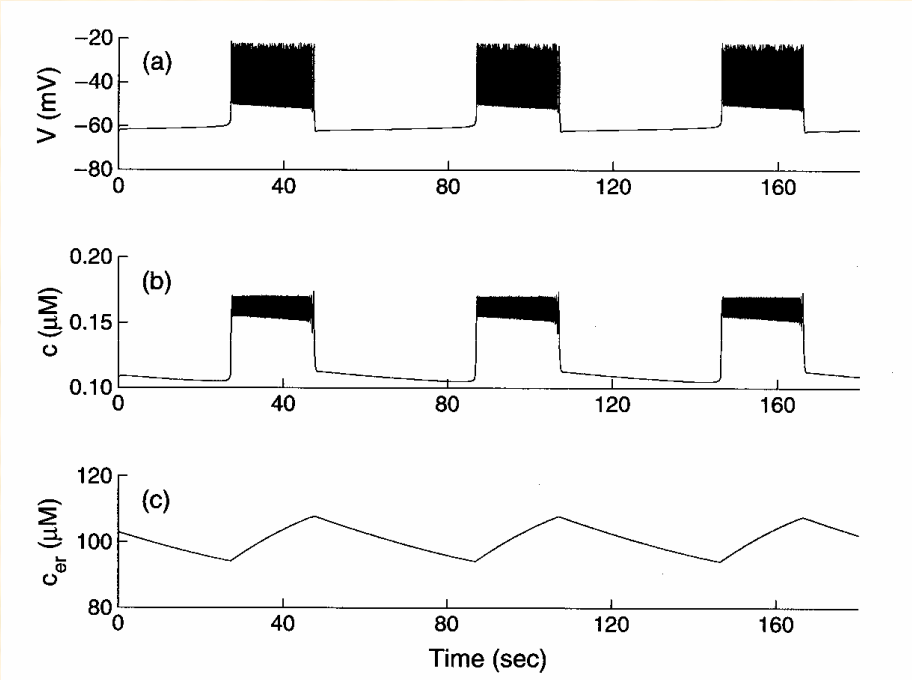
$$\dot{c}_{ER} = f_{ER}(V_{cyt}/V_{ER})(J_{serca} - J_{leak})$$

ER is the Endoplasmic Reticulum

Ca^{2+} enters the cell through L-type Ca^{2+} channels. The free cytosolic Ca^{2+} activates K(Ca) channels. Thus, there is mutual feedback between the electrical and Ca^{2+} components.

Fast Oscillations with the DOM

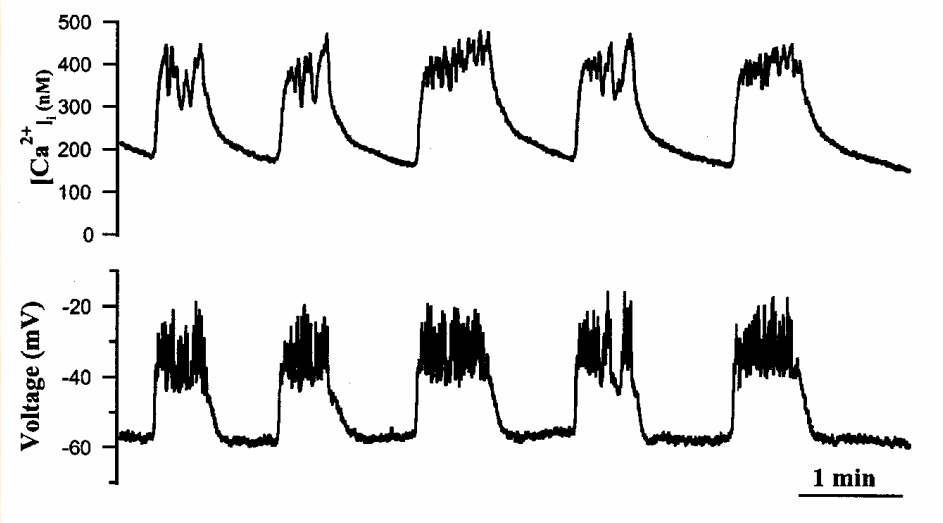
When glycolysis is non-oscillatory, the DOM produces fast bursting oscillations, due to the electrical/calcium components of the model.



Bertram and Sherman, BMB, 66:1313, 2004

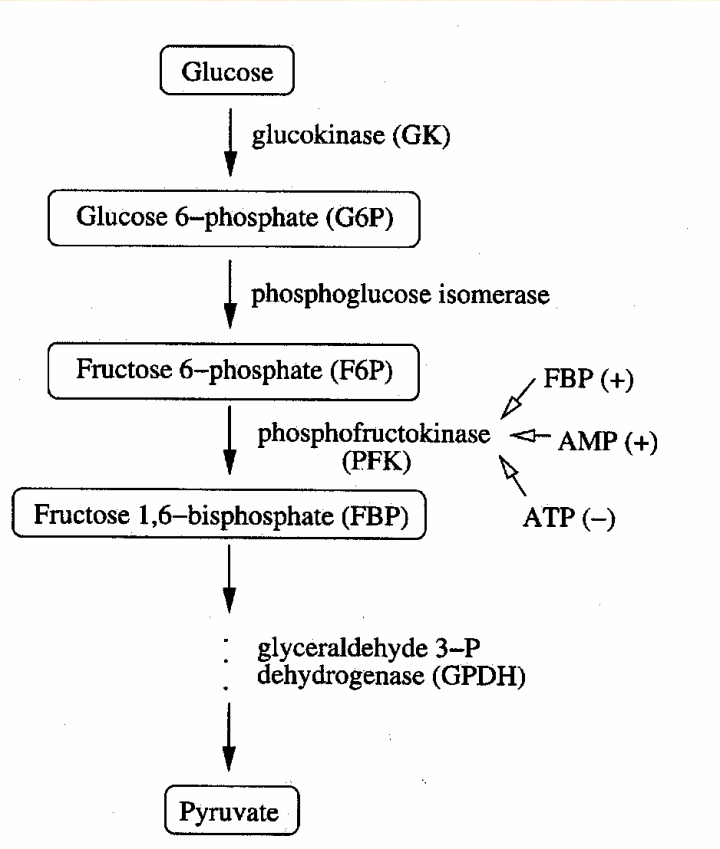
The ER acts as a slow Ca^{2+} filter, setting the period of bursting through its interaction with the cytosol.

Fast Oscillations in Islets



Simultaneous fast Ca^{2+} and voltage measurements from a mouse islet in 11.1 mM glucose. From Zhang et al., *Biophys. J.*, 84:2852, 2003

Glycolytic Component of the DOM



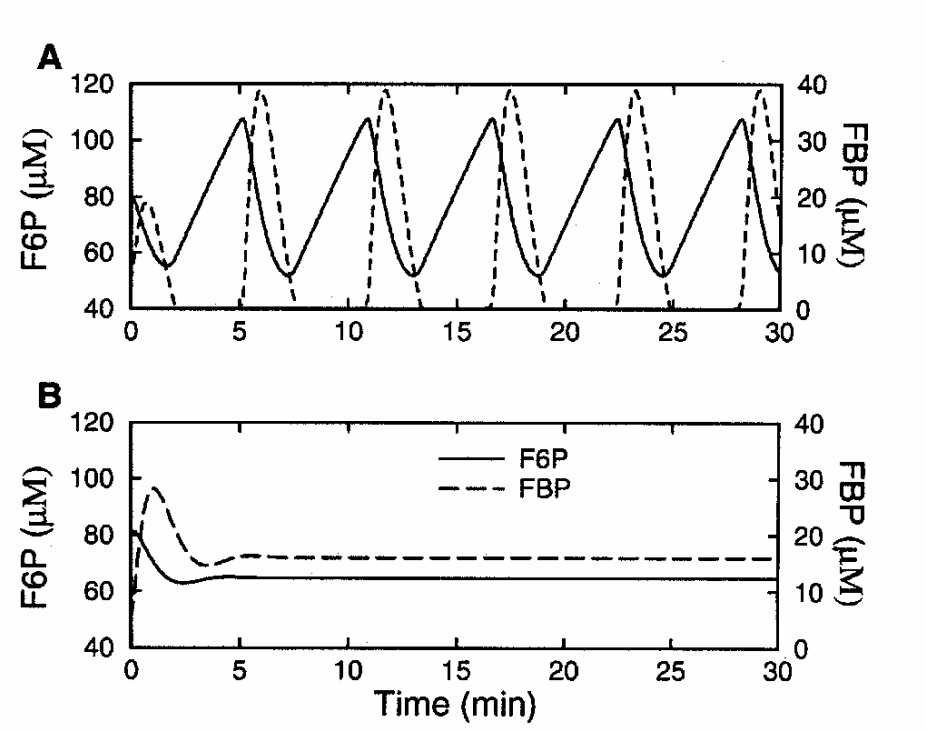
$$\frac{dF6P}{dt} = \lambda(J_{GK} - J_{PFK})$$

$$\frac{dFBP}{dt} = J_{PFK} - 0.5J_{GPDH}$$

Key feature: The product FBP feeds back positively onto the allosteric enzyme PFK (phosphofructokinase). Leads to oscillations due to substrate depletion.

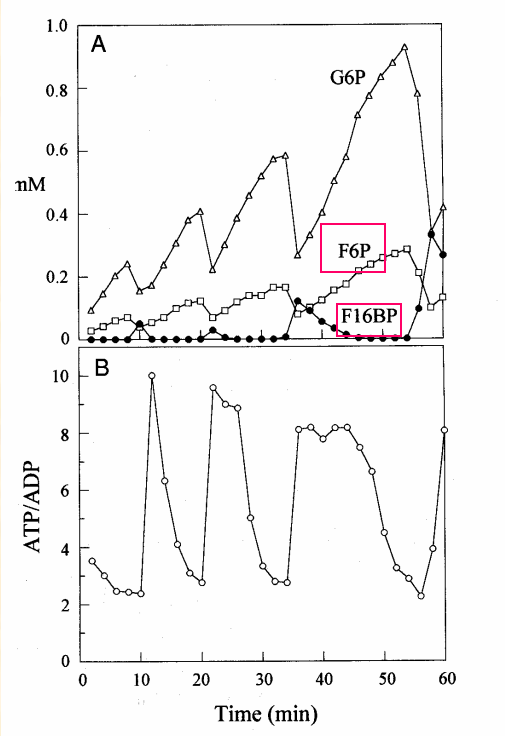
Glycolytic Oscillations Produced if Glucokinase Rate is in the Right Range

Solid-F6P, Dashed-FBP



(A) Intermediate J_{GK}
(B) High J_{GK}

Bertram et al., BJ, 87:3074, 2004

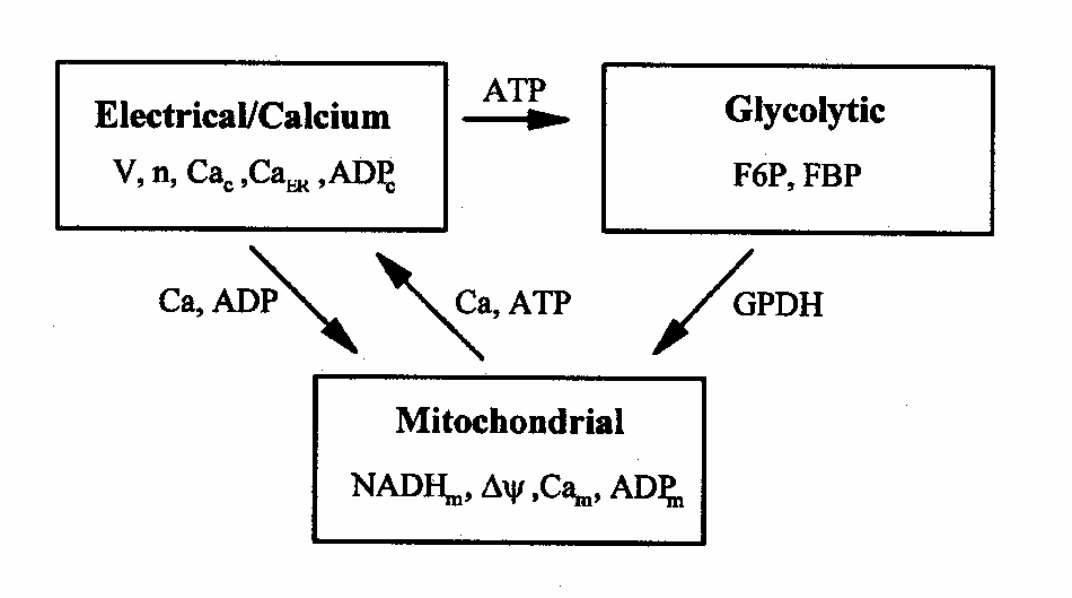


Glycolytic oscillations in muscle extracts (Tornheim, *Diabetes*, 46:1375, 1997)

Mitochondrial Component

Includes equations for mitochondrial NADH concentration, inner membrane potential, Ca^{2+} concentration, and ADP/ATP concentrations.

Final 3-compartment model:



Bertram et al.,
BJ, 92:1544, 2007

The Three Types of Activity can be Reproduced by the Model

No glycolytic oscillations

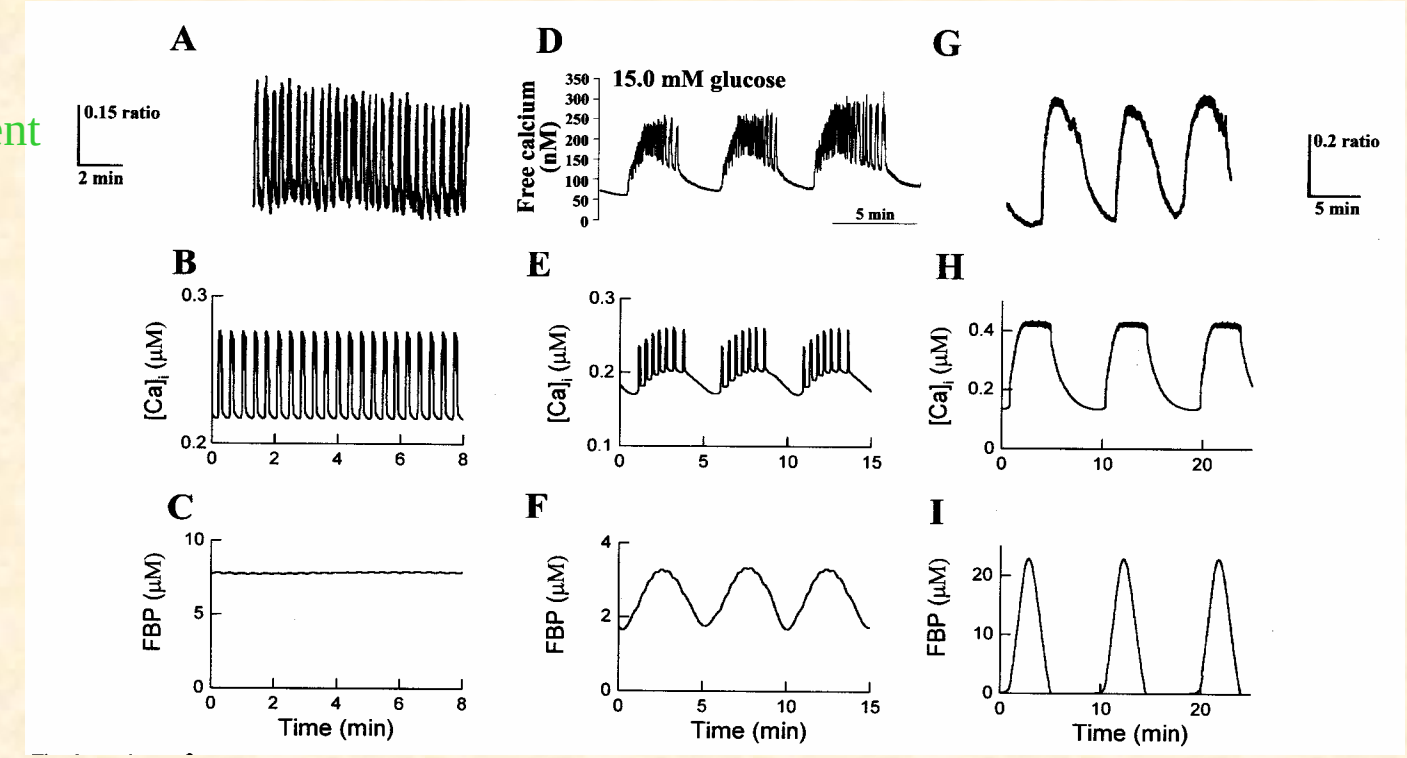
With glycolytic oscillations

With glycolytic oscillations

Experiment

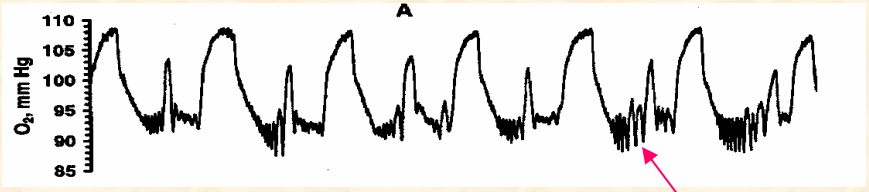
Model

Model



Bertram et al., *Am. J. Physiol.*, 293:E890, 2007

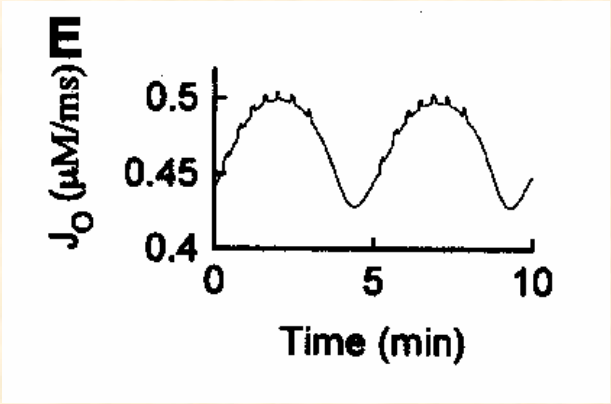
DOM Reproduces the Slow Rhythm and "Teeth" in O_2 Consumption



O_2 measured with O_2 electrode

Jung et al., *BBRC*, 259:331, 1999

Teeth in oxygen concentration



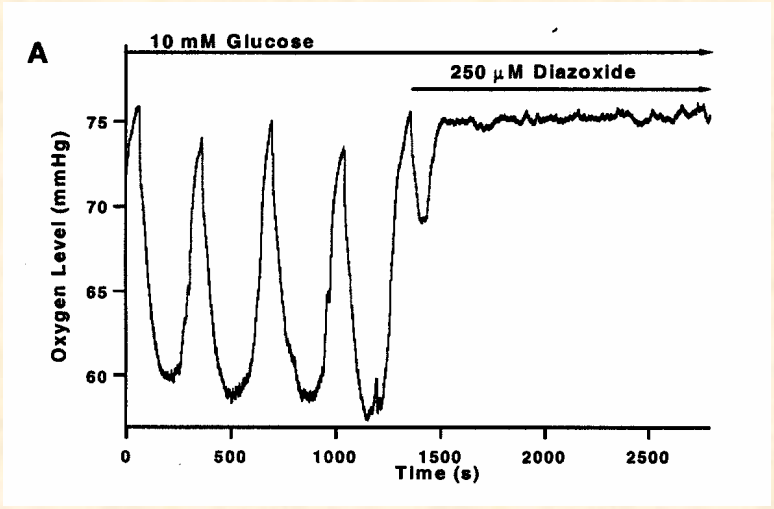
Teeth in oxygen consumption

Bertram et al., BJ, 92:1544, 2007

Terminating Bursting Terminates Metabolic Oscillations in Islets

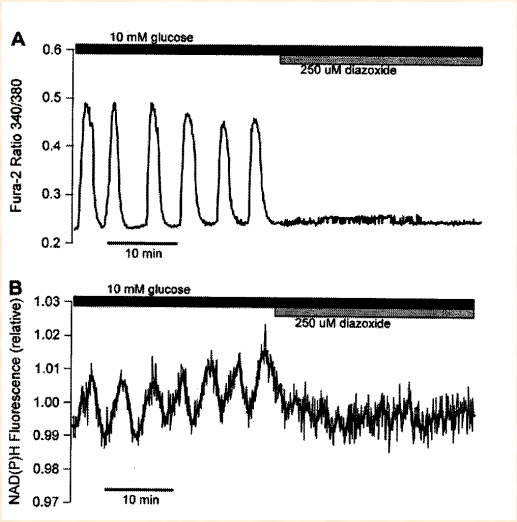
Diazoxide hyperpolarizes the islet, terminating electrical activity.

Oxygen



Kennedy et al., *Diabetes*, 51:S152, 2002

NADH



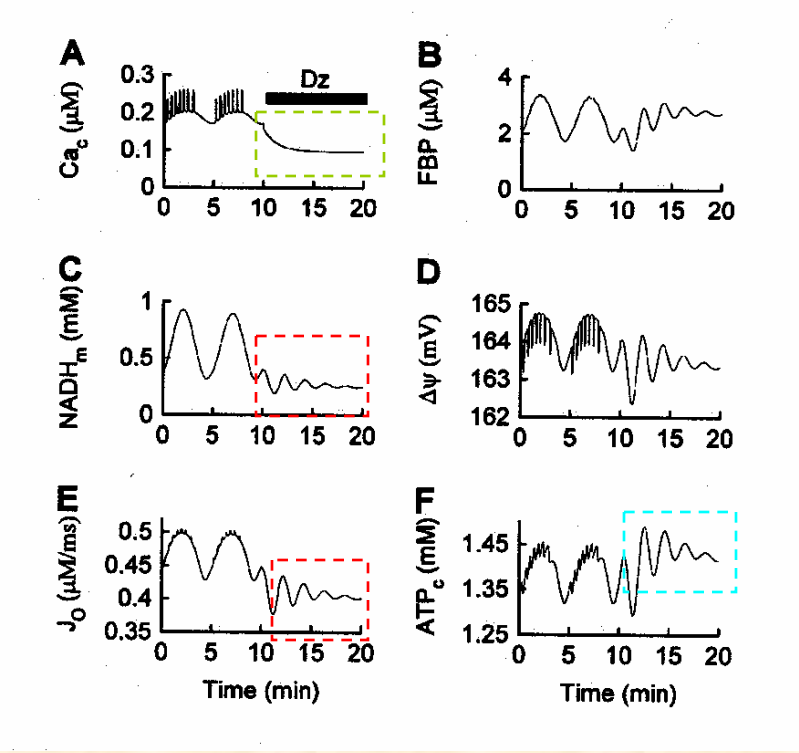
Bertram et al., BJ, 92:1544, 2007

Kennedy's Conclusion

Slow oscillations in metabolic variables are driven by Ca^{2+} feedback. That's why they stop when Ca^{2+} is constant at a low value.

Kennedy Data Consistent with the DOM

Opening K(ATP) channels with diazoxide (Dz) can terminate the oscillations in glycolysis, and thus the metabolic oscillations. Explains O_2 recordings from Kennedy's lab and our own NAD(P)H data.

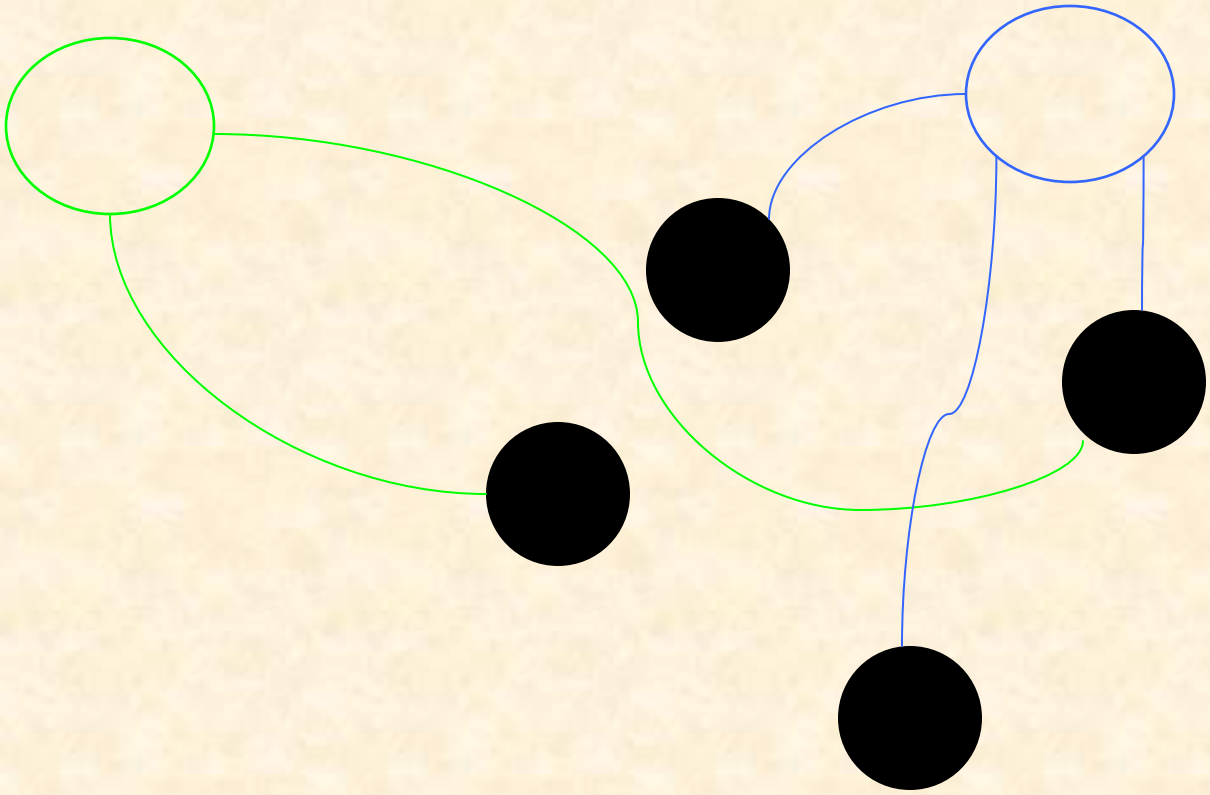


Bertram et al., BJ, 92:1544, 2007

1. Dz hyperpolarizes cell
2. Cytosolic Ca^{2+} concentration is reduced
3. Ca^{2+} pumps don't need to work as hard, so less ATP is utilized.
4. Cytosolic ATP level increases
5. The ATP inhibits PFK, terminating metabolic oscillations

How are Islets Synchronized?

Hypothesis: Islets are Entrained by Intrapancreatic Ganglia



The Idea and Evidence for It

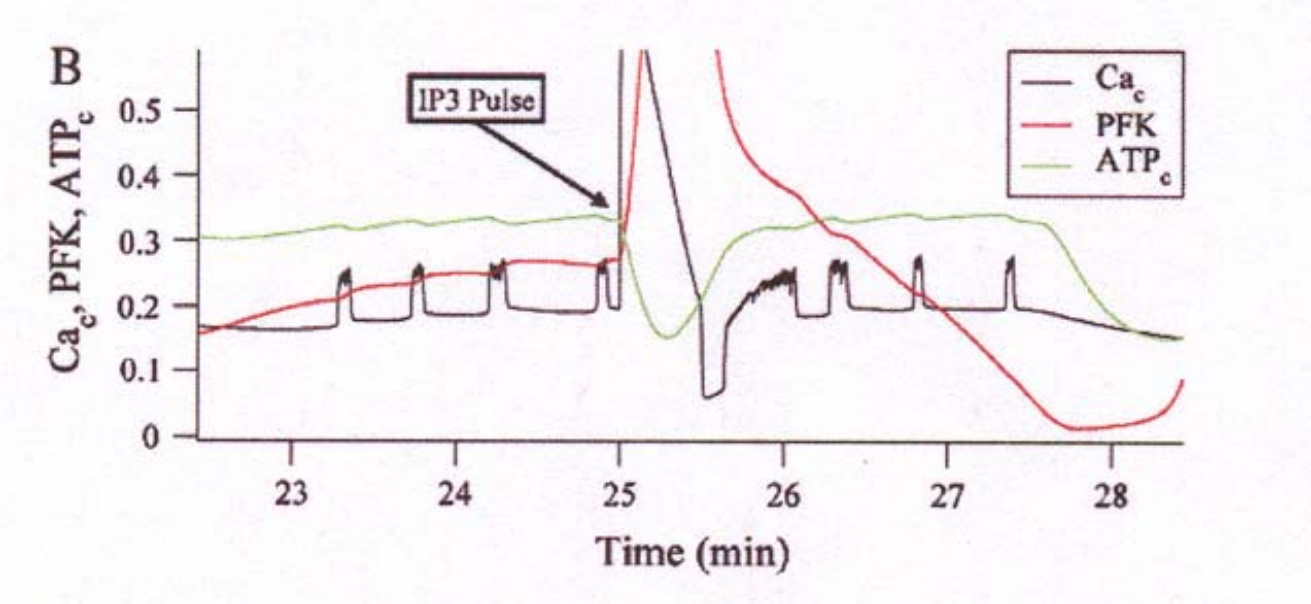
- Intrapancreatic ganglia send out axons that innervate islets

- The synapses onto islets release Acetylcholine (ACh) and islets have muscarinic ACh receptors

- Stimulation of the vagus nerve, which connects to ganglia, induces insulin release from islets

Idea: Ganglia send out periodic pulses that entrain the islet oscillators, thus synchronizing the islet population

What's the Mechanism of Action of ACh?

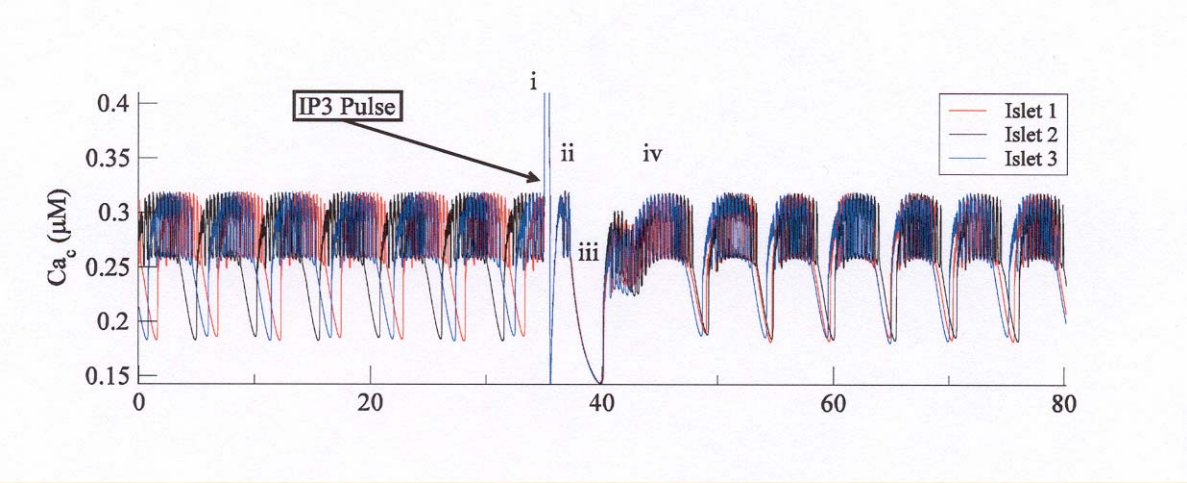


Zhang et al., BJ, 95:4676, 2008

The effect of a pulse of ACh is to perturb the glycolytic oscillator via transient stimulation of PFK.

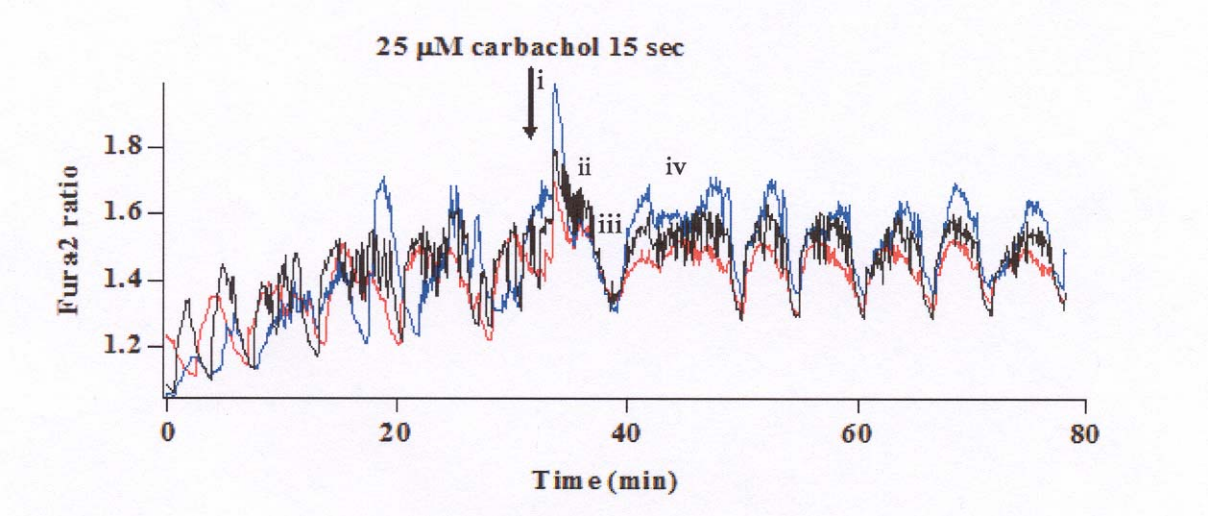
Modeling Suggests an *in vitro* Experiment

Experiment: Put some islets in a dish and measure cytosolic Ca^{2+} levels for each islet as oscillations proceed. Add a single large bolus of ACh to the bath and see if the islets synchronize.



Simulation with 3 model islets, following single pulse of ACh

The *in vitro* Experiment Matched the Prediction of the Model



Zhang et al., BJ, 95:4676, 2008

Three islets synchronized by a single pulse of the muscarinic agonist carbachol.

A More Realistic Scenario

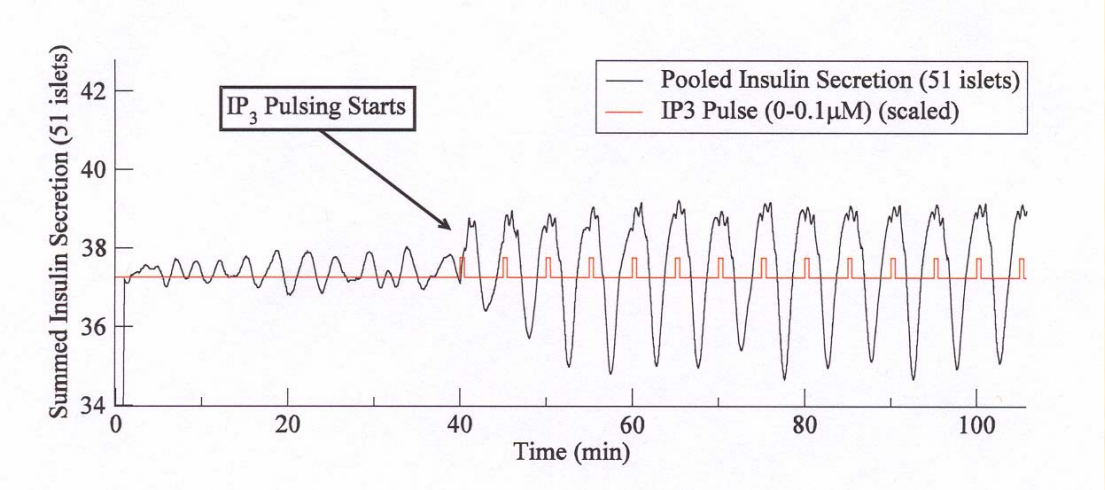
In an *in vivo* setting the ACh pulses will not be as large. Can a periodic train of small ACh pulses synchronize the islet population?

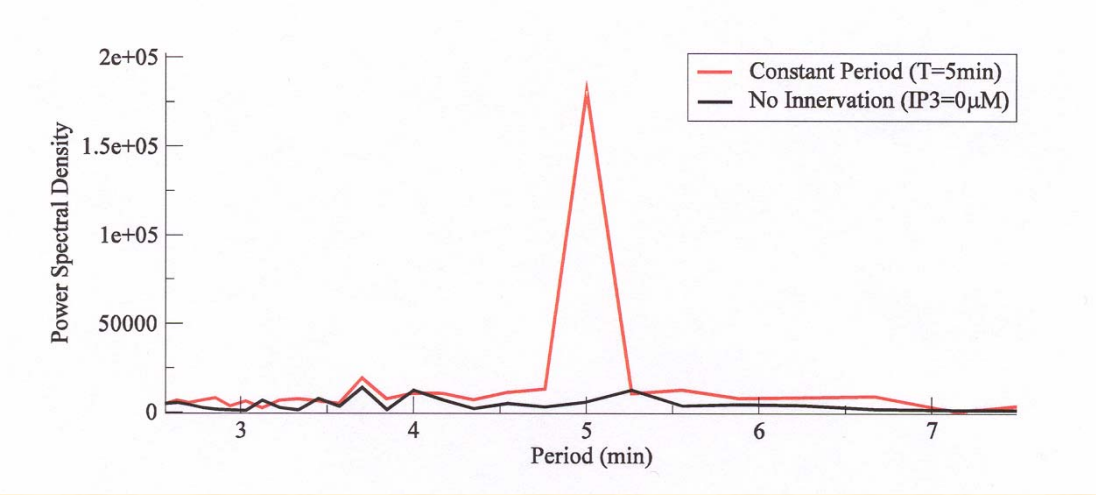
How should we measure synchronization for a large model islet population?

Each model islet has an insulin secretion variable. Sum this over the islet population. Synchronous oscillations will produce large-amplitude summed insulin oscillations.

Model Islets Synchronized by IP_3 Pulse Train

Time course of summed insulin secretion

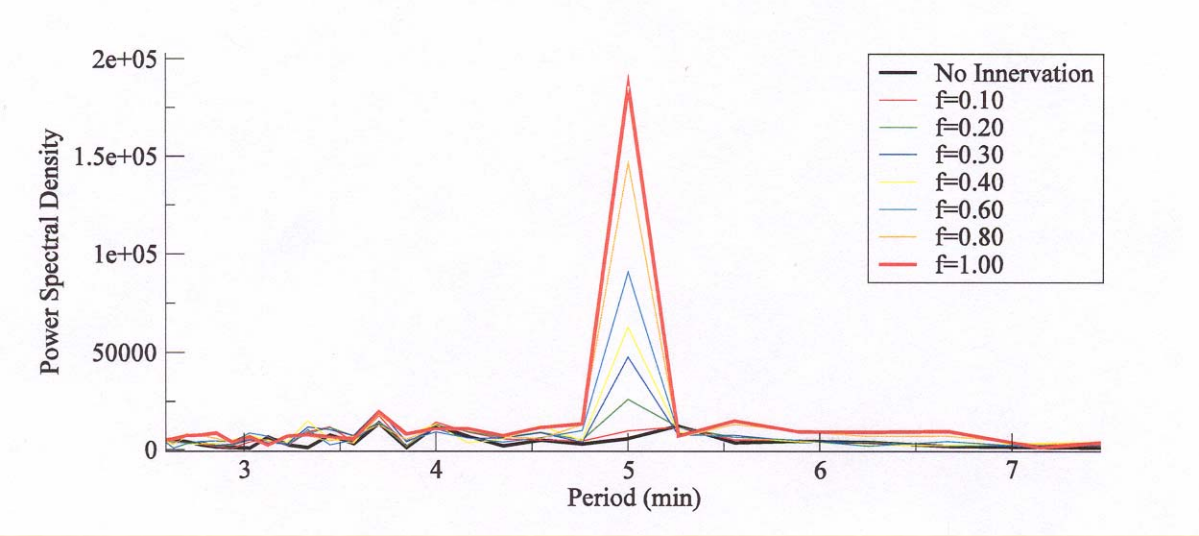




Power spectrum, with (red) and without (black) periodic IP_3 input

Synchronization Occurs with only a Small Fraction of Islet Innervation

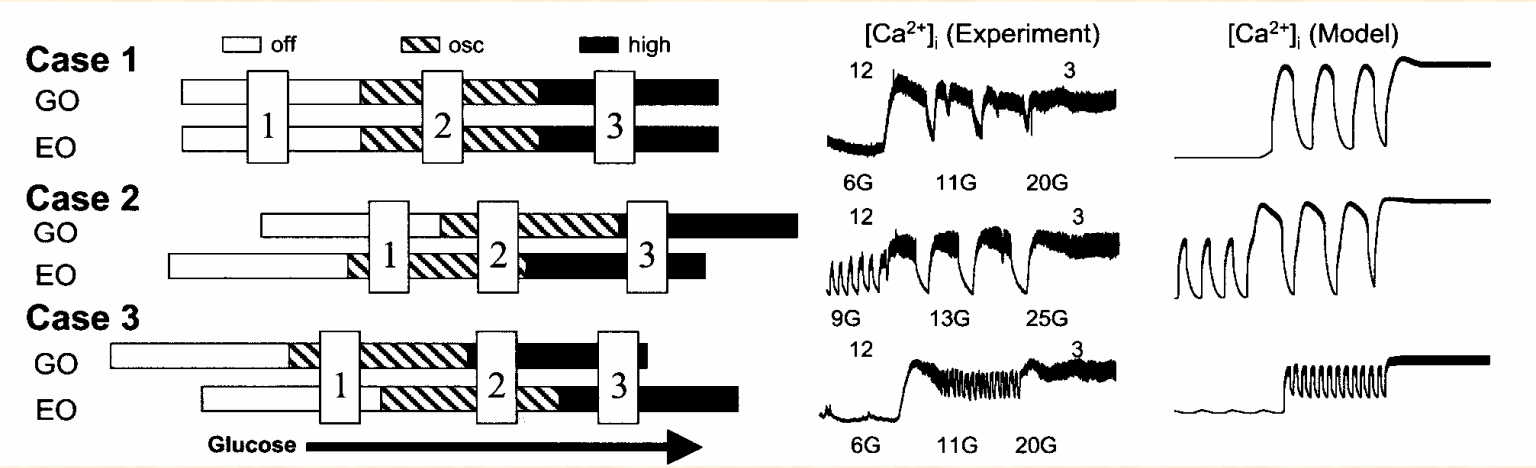
It is not known how many of the islets are innervated by ganglia. How effective is entrainment if only a fraction of islets are innervated?



Power spectrum from a population of 50 heterogeneous islets.

Thank You!

Glucose Response Can Be Explained with a Sliding Bar Diagram



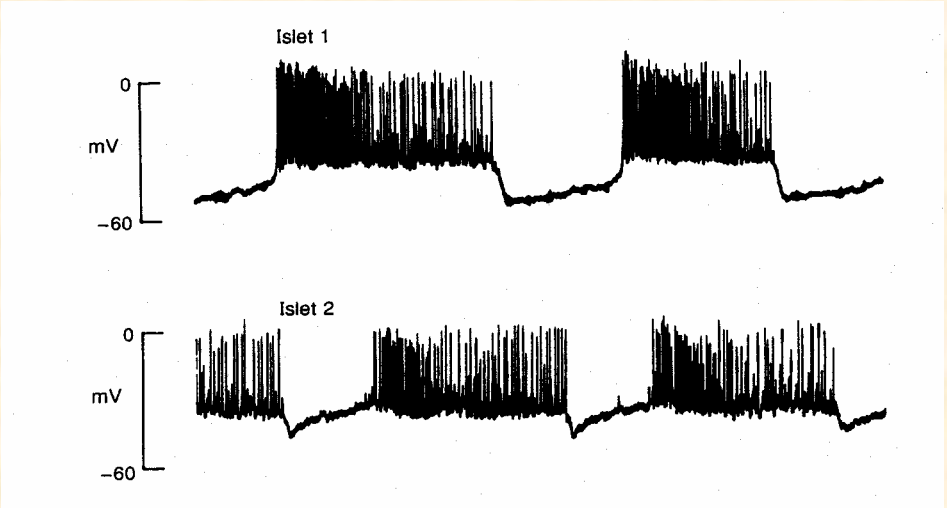
Bertram et al., *Am. J. Physiol.*, 293:E890, 2007

Model reproduces the islet response to changes in the glucose level, the primary hormone affecting islets.

In Vivo Bursting Found To Be Asynchronous

***In vivo* synchronous membrane potential oscillations in mouse pancreatic β-cells: lack of co-ordination between islets**

M. Valdeolmillos, A. Gomis and J. V. Sánchez-Andrés

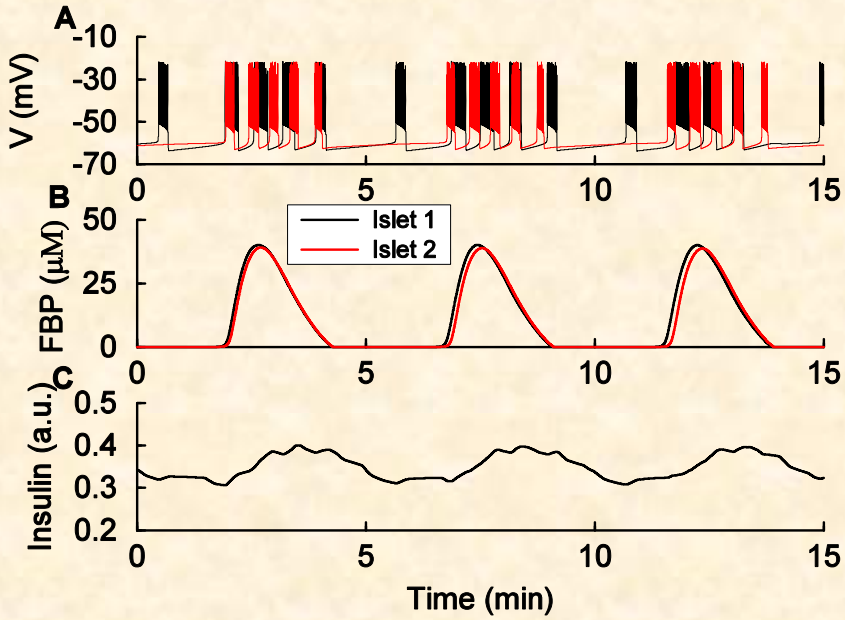


J. Physiol., 493:9, 1996

Electrical recordings from two islets *in vivo* show that islet bursting is not synchronized

Electrical Synchronization Not Required

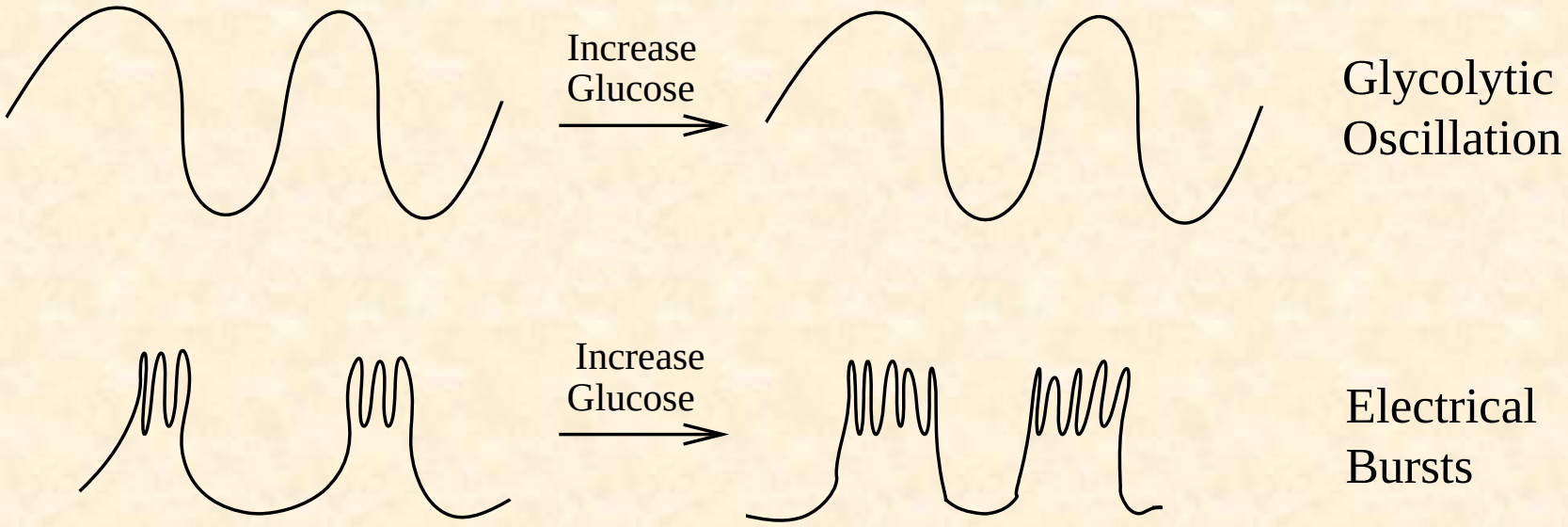
The DOM predicts that insulin secretion oscillations within an islet population can be synchronized even if the individual fast bursts are not synchronized. That is, only the glycolytic oscillations need be synchronized.



This can account for *in vivo* data from Valdeolmillos et al. (J. Physiol., 493:9, 1996) showing that bursting oscillations in two islets were not synchronized.

Why Compound Oscillations?

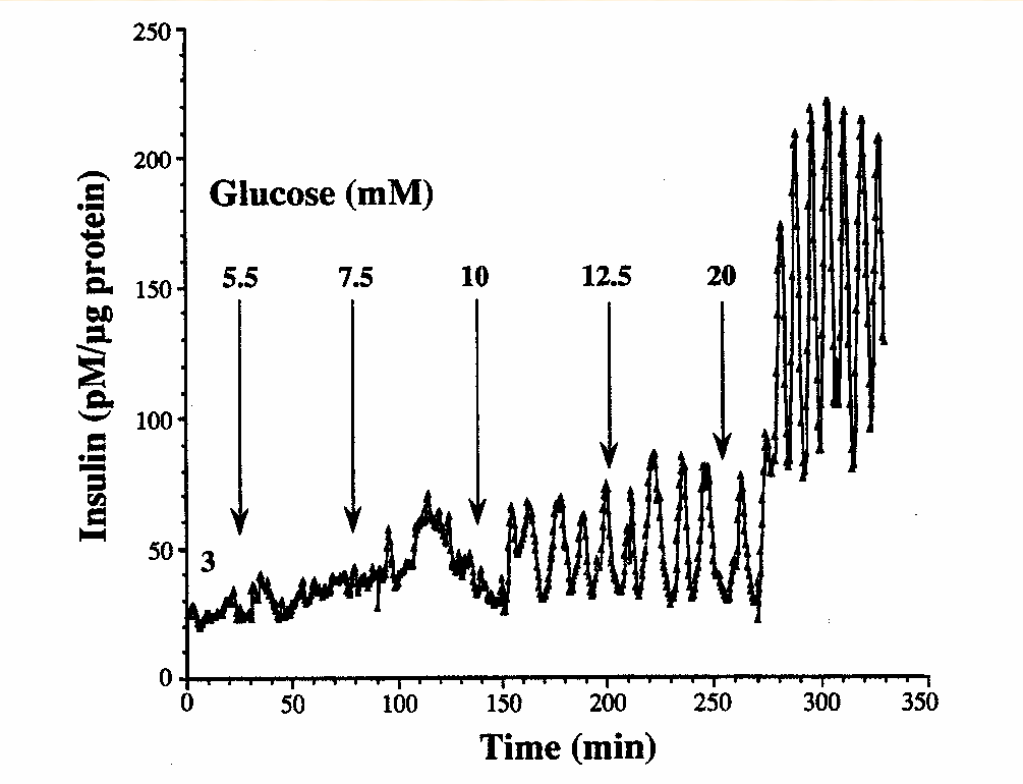
Metronome Hypothesis



The frequency of glycolytic oscillations in the DOM is only moderately sensitive to the glucose concentration. However, the plateau fraction (i.e., duty cycle) of the inner bursts is very sensitive to glucose. When glucose is increased, the plateau fraction increases. This amplifies the amplitude of the insulin oscillation, while the effect on frequency is smaller.

Increasing Glucose Primarily Increases Insulin Oscillation Amplitude

Perifused islets



Cunningham et al., *Am. J. Physiol.*, 271:E702, 1996

Data from several labs have shown that increasing glucose primarily increases the amplitude of insulin oscillations. The change in frequency is more modest.