MATHEMATICAL BIOLOGY: THE VIRTUAL CELL

# Bursting in pancreatic $\beta$-cells

Milton Nogueira

Correspondence:
m.nogueira.da.silva.junior@umail.leidenuniv.nl, Master student, Leiden University,
Full list of author information is available at the end of the article

**Abstract**

In this essay we will critically explore the results of the effects of extracellular calcium concentrations on the bursting phenomenon and cytosolic and luminal calcium oscillations in the context of the Chay's $\beta$-model for the pancreatic beta-cells.

## Introduction

So far we know that mathematical models have helped to unravel the underlying mechanisms involved in glucose-stimulated insulin secretion. These models are focused on understanding the oscillations in the electrical activity of pancreatic $\beta$-cells which are known to be involved into the process of insulin secretion. In fact, with the increase of intracellular glucose concentration one has that the ratio $ATP/ADP$ also increases due to cellular respiration, what causes ATP-sensitive potassium channels to stop sending $K^+$ out of the cell [6]. When these channels close, one has that the membrane depolarizes seeing that the potassium ATPases go on allowing $K^+$ to enter into the cell. As soon as a threshold for the cross membrane potential is attained, voltage-gated calcium ion channels are activated what causes the intracellular concentration of calcium to increase. This rapid increase triggers the process of exocytosis [7]. This whole process culminates into the bursting phenomenon, which is characterized by the in-phase burst of the cross membrane potential and cytosolic calcium oscillations.

In order to unravel the mechanism underlying this process, Chay and Keizer [3] formulated a model based on the paradigm that the oscillations in cellular electrical activity must be seen as a compromise between a fast positive feedback and a low negative feedback. Grounded on deductive-nomological and mechanistic accounts of explanation, i.e., the integration between mechanisms and their mathematical counterparts as an approach for modeling of biological phenomena, we regard the qualitative and quantitative description of a model as its fundamental properties [4]. More specifically, as for its qualitative description, we refer to the experimental evidences of the causal contributions of the mechanisms. Regarding its quantitative description, we refer to the down to minute generation of quantitative dynamic-details of the phenomena through the mathematical counterparts of the proposed mechanisms [1].

[1] In this hypothetical inductive-deductive cycle, we take falsifiability implicitly into account seeing that we are dealing with models that are certainly falsifiable. Hence, our set of criteria amounts to falsifiability, empirical consistency and predictive precision. However, we do not have any philosophical argument to support the sufficiency of these properties as to the analysis of the robustness of this sort of model.

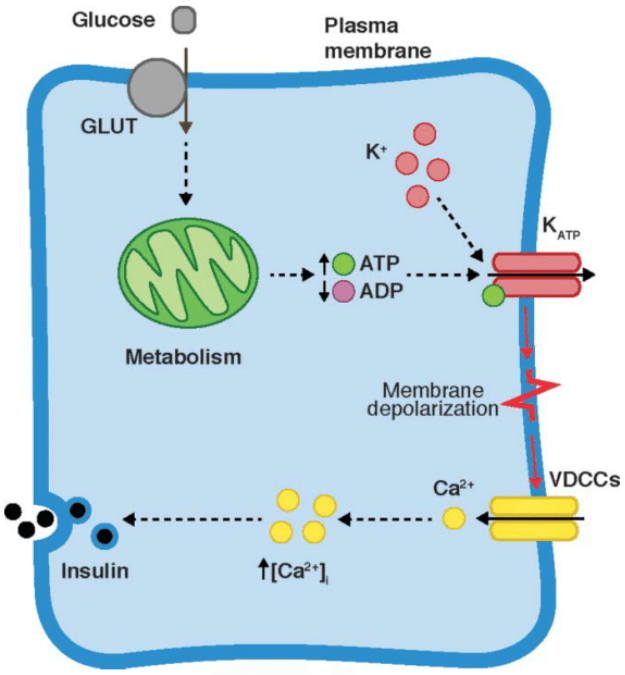


**Figure 1** This cartoon has been taken from [13]. Glucose-stimulated insulin secretion(GSIS): GLUT2 transports glucose into the cell what, through cell respiration, raises the ratio of $ATP/ADP$, which, in turn, deactivates ATP-sensitive potassium channels $K_{ATP}$ causing the membrane to depolarize. When this depolarization attains a threshold, the voltage-dependent calcium ion channels(VDCCs) are activated, which allows calcium to flow into the cell wherein it triggers the fusion of insulin-containing granules with plasma membrane culminating with the extrusion of insulin into the blood stream(exocytosis).

To simplify the wording, we refer to the later properties as *empirical consistency* and *predictive precision* respectively. Upon doing so, we are now able to use formal logic in our critical analysis of the described models. This essay is organized as follows. First, we introduce the Chay-Keizer model and we refer to its insights and drawbacks related to the bursting process. Secondly, we explain the Chay's $\beta$-model and we put it into perspective with the Chay-keizer model followed by a critical discussion of the simulations. Lastly, we conclude the essay by summarizing the analysis and by providing a concise discussion thereof.

## Chay-Keizer model: "the minimal model"

In 1983, translating the ideas displayed in the figure 1, Chay and Keizer [3] proposed a mechanism to explain the bursting process. This reads as follows. If the level of glucose concentration rises into the cytoplasm then the ratio $ATP/ADP$, owing to cellular respiration, also becomes higher therein, which, in turn, closes the ATP-sensitive potassium ion channels. Due to the sodium-potassium pumps, the cytoplasm becomes more positive giving rise to a depolarisation of the cross membrane potential what culminates with the activation of the voltage-gated calcium ion channels and this allows the inflow of calcium into the cytoplasm. This whole process constitutes the fast subsystem of the mechanism. What causes the bursting phenomenon then? According to the major theoretical paradigm concerning oscillations, a slow negative feedback is necessary. This constitutes the slow subsystem of

the mechanism. In fact, it is hypothesized that $[Ca_i^{2+}]$ increases slowly [10] during the active phase. This perpetual increase of $[Ca_i^{2+}]$ activates the calcium-activated potassium ion channels. When the conductance $g_{K_{Ca}}$ through these channels meets a respective threshold then the plasma membrane undergoes repolarization what inactivates the voltage-gated calcium ion channels. During this silent phase, the extrusion of calcium out of the cytoplasm through calcium pumps (PMCA) [2] and subsequent decrease of $[Ca_i^{2+}]$ inhibits the calcium-activated potassium $K_{Ca}$ ion channels. This scenario ends up in a hyperpolarized plasma membrane that gradually starts depolarizing with the activation of the voltage gated potassium ion channels until it reaches the threshold at which the voltage-gated calcium ion channels are activated. So, it gives rise to a new burst of the cross membrane potential and the cyclus starts again. Mathematically, this mechanism is translated into the following dynamical equations

$$\begin{aligned} Cm\frac{dV}{dt} &= -I_{Ca} - I_K - I_L - I_{K(Ca)}, \\ \frac{dw}{dt} &= \phi\frac{w_\infty - w}{\tau}, \\ \frac{d[Ca_i^{2+}]}{dt} &= f_i(-\alpha I_{Ca} - \nu_{LMP}[Ca_i^{2+}]), \end{aligned} \tag{1}$$

where $f_i$, under the hypothesis of excess of buffer(or lower affinity), stands for the fraction of free cytosolic calcium. the parameter $\alpha$ is just a scaling factor. For ohmic-currents one has that

$$\begin{aligned} I_{Ca} &= g_{Ca}m_\infty(V)(V - V_{Ca}), \\ I_K &= g_K w(V)(V - V_K), \\ I_L &= g_L(V - V_L), \end{aligned} \tag{2}$$

and that

$$I_{K(Ca)} = g_{K(Ca)}\frac{[Ca_i^{2+}]}{K_{K(Ca)} + [Ca_i^{2+}]}(V - V_K), \tag{3}$$

where the index $L$ stands for a leakage current, which according to [3] is to embody the $Na^+$ leakage into the model. It is consistent with the mental model proposed above, seeing that the sodium-potassium pumps have an important role in this scenario. It is important to notice that the fraction of voltage-gated calcium channels is a fast variable which is consistent with empirical data. As we see in $(1)_3$, the dynamics of the cytosolic concentration is fully determined by the voltage-gated calcium

[2] In their mental model, calcium is assumed to be mainly extruded into the extracellular environment by the $Ca^{2+}$-ATPase calcium pumps.

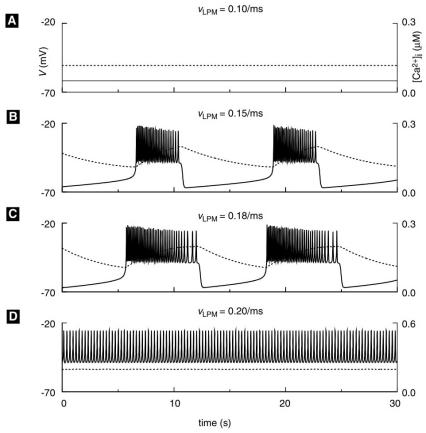


**Figure 2** Results of the simulations of the minimal model taken from [3]. In dashed-lines, the predicted sawtooth pattern of the $[Ca_i^{2+}]$-oscillations deviates from the square-wave pattern observed in the data. Moreover, the simulations predict a period of approximately 12 seconds for the spiking which utterly deviates from the one provided by the data($\approx 100s$).

ion channels and the $Ca^{2+}$-ATPase (PMCA) calcium pumps with $\nu_{LMP}[Ca_i^{2+}]$ a linearized representation thereof. Therefore, the dynamics of this model reduces to a Morris-Lecar plasma membrane oscillator augmented by an equation describing the evolution of the "slow" variable "$[Ca_i^{2+}]$".

Regarding *empirical consistency*, there are few issues. Firstly, although their identification has been proven [9], which strengthens the molecular basis of this model, calcium-activated potassium ion channels are barely open under physiological conditions [8]. Secondly, experiments revealed that blocking the $K_{Ca}$-related pathway has no relevant impact on the bursting process. These facts are really harmful for the model seeing that these channels are assumed to mediate the bursting of the cross membrane potential during the active phase [11]. Lastly, they do not consider the evidence-based relevant effects of the Endoplasmic Reticulum (ER) onto the dynamics of the cytosolic calcium concentration[3].

As for *predictive precision*, we see in the simulation of the model displayed in figure 2 that it utterly fails to reproduce the close to square-wave pattern of $[Ca_i^{2+}]$ observed in the experimental data in figure 3. This observed pattern means that the actual $[Ca_i^{2+}]$-dynamics is faster [12] than what is predicted in their simulations. Therefore, the slow variable cannot be the cytosolic calcium concentration $[Ca_i^{2+}]$ what overthrown the main hypothesis of their mental mechanism. Moreover, there is also a visible inconsistency as regards the predicted period of the spiking and the one provided by the data. However, despite the relevant drawbacks, we see the in-phase bursting and oscillation pattern in their simulations which points out that a slow variable is sufficient to yield the bursting pattern. This was *conceptual insightful* because it provided normative insights.

[3]We did not find any paper before their publication about the significant effects of mitochondrial $Na^+/Ca^{2+}$ exchanger on the dynamics of $[Ca_i^{2+}]$ so as to refer to it as another issue.

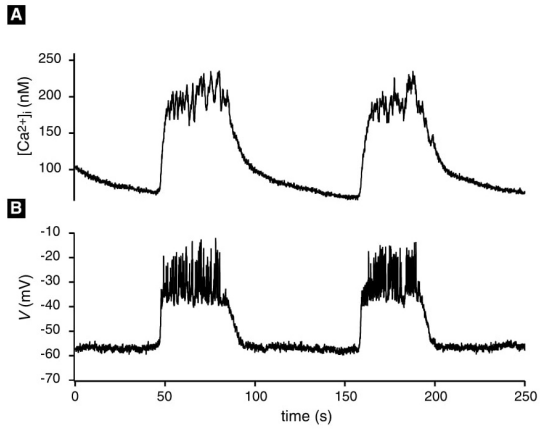


**Figure 3** Recording of electrical activity of pancreatic $\beta$-cells taken from [1]. We clearly see the in-phase bursting of the cross membrane potential(V) and $[Ca_i^{+2}]$ oscillations. Moreover, we see that the $[Ca_i^{2+}]$- oscillations are close to a square-wave pattern. Regarding this data, we presume that this was measured under normal physiological conditions. The source is neither clear about nor about the sort of $\beta$-cells used in the experiments(rodents or humans?).

## Chay's $\beta$-model

In 1997, grounded on the normative insight established by the *minimal model*[4], Chay [2] proposed a mechanism[5] to explain the effects of extracellular calcium on the cytosolic and luminal calcium oscillations in pancreatic $\beta$-cells figure 4 . The most important feature of this mechanism is the inclusion of the effects of the Endoplasmic Reticulum and SGs (insulin containing granules) on the dynamics of $[Ca_i^{2+}]$. As displayed in figure 5, this reads as follows. As in the minimal model, cellular respiration increases $[ATP_i]$ which inactivates the ATP-sensitive $K^+$ channels. $Na^+/K^+$ pumps causes the cross membrane potential to depolarise. This activates a lower-threshold inward current $I_{fast}$ which, in turn, activates the high threshold inward voltage-activated calcium ion channel $I_{Ca}$. The latter one increases $[Ca_i^{2+}]$ , upon a threshold, this activates the outward calcium-sensitive potassium ion channels $I_{K(Ca)}$. Comparing to the minimal model's mechanism, the difference here is that the voltage gated $[Ca_i^{2+}]$ ion channels deactivate themselves when $[Ca_i^{2+}]$ is high enough.

How does repolarization occur? During this active phase, $[Ca_{ER}^{2+}]$ increases slowly by storing calcium. This results in the inactivation of an inward voltage-independent cationic nonselective channel $I_{NS}$, which activates if $[Ca_{ER}^{2+}]$ becomes low. Insofar as $[Ca_i^{2+}]$ increases gradually because of the uptake of cytosolic calcium by the insulin containing granules (SGs) and by SERCA pumps in the Endoplasmic Reticulum, one has that the lower-threshold inward current $I_{fast}$ decreases. Now, the intuition of this mechanism is that repolarization occurs when $I_{K(Ca)}$ exceeds a threshold for the inward net contribution $I_{fast} + I_{NS}$ upon self deactivation of the voltage-gated

[4]We refer to the inclusion of an adequate slow subsystem:"a slow variable".
[5]There is no reason for us to name this model as the $\beta$-model. However, the lack of a clear logical connectivity among the related contributions of the author has led us to categorize the models in this way.

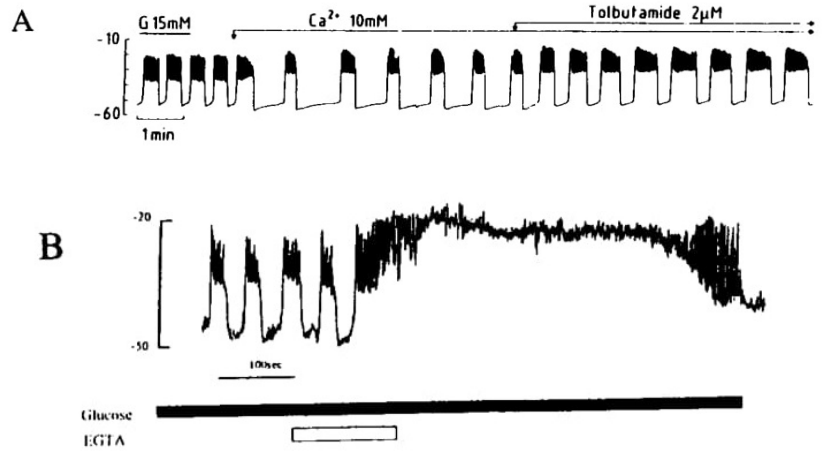


**Figure 4** (A) This figure has been taken from [2]. Here, we see the results of an experiment in which $[GLU] = 15mM$ was held constant while $[Ca_o^{2+}]$ was raised from 2.5 to 10 mM. We see that it shortens the spiking period, delays depolarization and lowers the repolarization potential. (B)When $[Ca_o^{2+}]$ is depleted, we see the repetitive spiking.

calcium ion channels. How does depolarization occur? During the silent phase, the net concentration of cytosolic calcium drops down significantly due to ER-uptake and SGs-uptake/extrusion. So ER starts releasing calcium slowly to prevent calcium from dropping abruptly. Low levels of $[Ca_{ER}^{2+}]$, during repolarization activates a voltage-independent cationic nonselective channel $I_{NS}$. This activates $I_{fast}$ and the cycle starts again.

What is the slow subsystem of this mechanism? What causes the in-phase burst of the membrane potential and the oscillations of $[Ca_i^{2+}]$? During the active phase, $[Ca_{ER}^{2+}]$(and SGs) uptakes calcium slowly which is rapidly "compensated" by $I_{fast}$, upon a threshold, it sudden activates a delayed-rectifying $K^+$ current $I_{K(DR)}$. This scenario characterizes the oscillations of the in-phase burst of the cross membrane potential and $[Ca_i^{2+}]$ oscillations. Thus, the bursting process is driven by the slow dynamics of $[Ca_{ER}^{2+}]$. Although $[Ca_i^{2+}]$ is a fast variable, it is modulated by a slow variable $[Ca_{ER}^{2+}]$ and the oscillations are mediated by the fast positive ($I_{fast}$) and fast negative feedback ($I_{K(DR)}$) driven by the slow evolution of the luminal calcium concentration.

What causes the pattern recorded in experiment when $[Ca_o^{2+}]$ is depleted? If $[Ca_o^{2+}]$ is depleted then we can imagine a scenario wherein a perpetual sequestration by ER and SGs will cause $[Ca_i^{2+}]$ to low to a "minimum" value. Why? Because this alternating uptake and release of the ER has negative feedback of the sequestration by SGs which stores calcium and extrudes it through exocytosis. Moreover, the voltage-gated calcium ion channels, if activated, have no inflow of calcium from the external medium into the intracellular compartment. So it culminates in a low $[Ca_{ER}^{2+}]$ which activates the voltage-independent cationic nonselective channels $I_{NS}$. So the intuition is that $I(N)$ will be somehow "optimal" and will oscillate seeing that $[Ca_i^{2+}]$ and $[Ca_{ER}^{2+}]$ are functioning at the minimum "level". This characterize the repetitive spikes observed upon depletion of $[Ca_o^{2+}]$. What causes the pattern

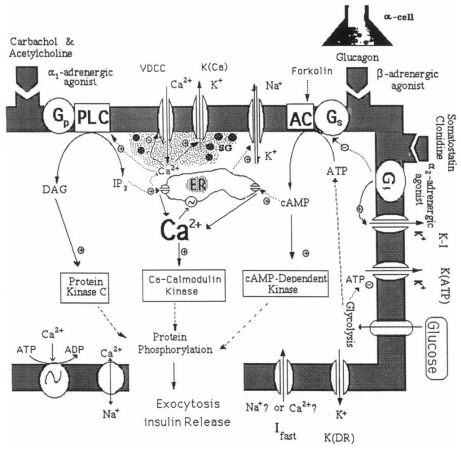


**Figure 5** This figure has been taken from [2]. Unlike the minimal model, the Chay $\beta$-model has three pathways related to insulin secretion.

recorded in experiment when $[Ca_o^{2+}]$ is increased? If $[Ca_o^{2+}]$ is increased then It is intuitive that the repolarization potential will end up being lower and take longer seeing that $[Ca_i^{2+}]$ will be high due to a high inward current $I_{Ca}$ and $I_{NS}$ will be low due to a high $[Ca_{ER}^{2+}]$. But, if it is true that the repolarization potencial is lower then one expects that the low-threshold inward current $I_{fast}$ will be optimal. Now, during the depolarization one has that the potentiated $I_{fast}$ activates the $I_{Ca}$-channels what leads to the rising of the plateau potential. The higher is the plateau, the higher is $I_{Ca}$ and this increases the cytosolic calcium concentration what maximizes $I_{K(Ca)}$ shortening the plateau length. Mathematically, this mechanism is translated into the following dynamical equations

$$\begin{aligned}
Cm\frac{dV}{dt} &= -I_{Ca} - I_{K(DR)} - I_{K(ATP)} - I_{K(Ca)} - I_{NS} - I_{Na^+} - I_{fast},\\
\frac{d[Ca_i^{2+}]}{dt} &= -\tilde{\phi} I_{Ca} - k_{Ca}[Ca_i^{2+}] + k_{rel}([Ca_{ER}^{2+}] - [Ca_i^{2+}]) - k_{pump}[Ca_i^{2+}],\\
\frac{d[Ca_{ER}^{2+}]}{dt} &= -k_{rel}([Ca_{ER}^{2+}] - [Ca_i^{2+}]) + k_{pump}[Ca_i^{2+}],
\end{aligned} \tag{4}$$

where $\tilde{\phi}$ is a surface ratio, $k_{Ca}$ is the sequestration rate of cytosolic calcium by the insulin containing secretory granules SGs, $k_{rel}$ is the release ratio of calcium from the ER and $k_{pump}$ is the calcium pumps in the ER.

As for *empirical consistency*, there are few issues. Firstly, as far as we could understand. to overcome the problem that the calcium-activated ion channels would be solely responsible for ending the active phase what would contradict some molecular based fact [9, 8], the author assumes the existence of a voltage-gated calcium ion channel that inactivates itself if $[Ca_i^{2+}]$ is sufficiently high to start repolarization. We think that this assumption is plausible, though we are not able to give any physiological insight. However, up to date, we have not found any paper in

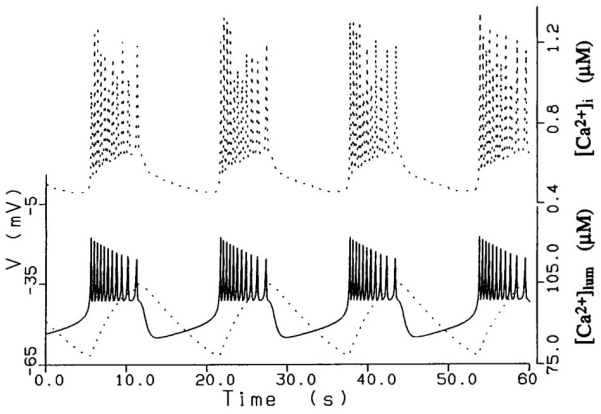


**Figure 6** In the figure, taken from [2], we see that the low dynamics of luminal calcium is sufficient to resemble the close to square wave pattern of cytosolic calcium oscillations recorded in the experiments. Luminal calcium builds slowly during the active phase when it stores cytosolic calcium and decreases gradually by releasing calcium into the cytoplasm. This slow dynamics is sufficient to reproduce the pattern. However, the period of the simulation deviates too much from the observations and also from the values of the cytosolic concentration in the experiment.

which an experiment confirms this hypothesis. Secondly, we also refer to the lack of molecular basis as regards the currents $I_{NS}$ and $I_{fast}$. They are assumed to play the role that makes it work. The author argues their existence and we find the argument convincing but an experiment should be provided(I hope I am not missing anything).

Regarding *predictive precision*, although they were able to "reproduce" the close to square-wave pattern of the $[Ca_i^{2+}]$-dynamics, if we compare figure 3 with 6 then we see that the "down to minute dynamics" requirement is not met. With respect to the role of $[Ca_o^{2+}]$ depletion or raise in the bursting process, we can say that the simulations are not precise at all. If we compare the top trace in figure 7 with 4(B) then we see no reproduction of the repetitive spiking in the experiments. As for the increase of $[Ca_o^{2+}]$, we have that the simulations resemble the data but we can point out the same inconsistencies said before.

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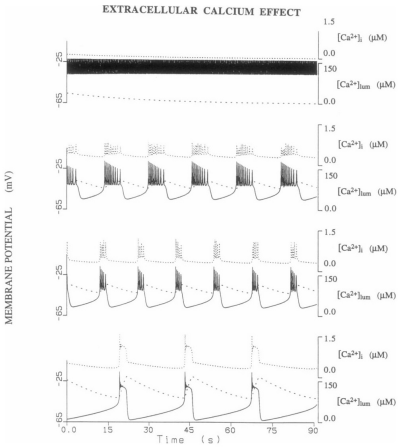


**Figure 7** This figure has been taken from [2]. From the top trace to the bottom: $[Ca_i^{2+}]$ varies from 0, 2.5, 5.0, and 7.5 mM. We see that the pattern of the experiments deviates from what is observed. The top trace line does not reproduce the repetitive spiking observed in 4(B) neither do the other simulations reproduce accurately what has been recorded in the experiment.

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