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Modeling buffered Ca^{2+} diffusion in a single human β-cell: the role of endogenous Ca^{2+} buffers and Ca^{2+} extrusion mechanisms

G.J. Félix-Martínez[1] and J.R. Godínez-Fernández[1]

[1] Department of Electrical Engineering, Universidad Autónoma Metropolitana, D.F., México

Abstract— **In this paper, a model of the electrical activity of the human β-cell was used in conjunction with a reaction-diffusion model of Ca^{2+} in order to analyze the spatiotemporal distribution of Ca^{2+} in the intracellular space. The model includes both Ca^{2+} influx and extrusion mechanisms, as well as a two endogenous buffer systems with different kinetic characteristics. The effect of buffering and clearence of Ca^{2+} on the time course of the Ca^{2+} signal driven by spiking electrical activity was addressed.**

Keywords— **β-cell, calcium, diffusion, action potential, electrical activity**

I. Introduction

Insulin, the only hormone directly responsible for lowering the blood glucose levels, is secreted in response to an increase of the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) following glucose stimulation[1]. In β-cells, Ca^{2+} transients are mediated by the electrical activity produced by the interplay of several cellular mechanisms, including ionic channels, Ca^{2+} handling mechanisms and metabolism. It is widely accepted that after being transported into the cell, glucose stimulates metabolism (i.e. ATP synthesis) producing an increase of the cytosolic ATP concentration, which inhibits the activity of the ATP-dependent K^+ channels . The resulting depolarization of the membrane potential (Vm) triggers the onset of the electrical activity allowing the influx of Ca^{2+} through the VDCCs and the increase of $[Ca^{2+}]_c$ that finally triggers insulin secretion. Several cellular mechanisms contribute to the generation of the electrical activity of β-cells (for a review see Ref. [2]). In human β-cells, action potential (AP) firing (i.e. *spiking*) is the most common electrical behavior, although bursts of APs are also occasionally observed[2].

Ca^{2+} signals in the intracellular space are shaped by several cellular mechanisms (e.g. pumps and exchangers). In addition, endogenous Ca^{2+} buffers (Ca^{2+}-binding proteins) buffer Ca^{2+} to low levels, preventing an abnormal increase of $[Ca^{2+}]_c$. It has been shown that the secretory sites co-localize with the VDCCs[3], where Ca^{2+} concentration reaches much higher levels than the rest of the cytosol, forming the so called Ca^{2+} microdomains[4]. Given that the increase of cytosolic Ca^{2+} generated by the influx of Ca^{2+} through the VDCCs triggers both mobilization and exocytosis of the insulin granules, it is important to determine how the different mechanisms involved in Ca^{2+}-handling contribute to the shape of the $[Ca^{2+}]_c$ transients.

Mathematical modeling has been extremely useful to the understanding of the physiology of the β-cell, although the spatial aspects have been always neglected, assuming a uniform distribution of Ca^{2+} throughout the cytosol. In contrast, in this work we have used a Ca^{2+} reaction-diffusion model in conjunction with a model of the electrical activity of the human β-cell to simulate the diffusion of Ca^{2+} through the intracellular space in response to changes in Vm that resemble the electrical behavior of the actual cell during glucose stimulation.

II. Material and Methods

A. Model of the electrical activity of the human β-cell

The time course of Vm was simulated using the model of the human β-cell of Pedersen[5] without modifications. The model is based mainly on the electrophysiological data from Braun et al.[6] and includes K^+, Na^+ and Ca^{2+} currents. Of special interest for this work are the Ca^{2+} currents, given that they are responsible for the influx of Ca^{2+} that produces the Ca^{2+} transients throughout the intracellular space. Three voltage-dependent Ca^{2+} currents (L-, T-, and P/Q type) were included. Thus, the total Ca^{2+} current is given by $I_{Ca} = I_L + I_T + I_{PQ}$ (see Ref. [5] for the details of the model).

B. Buffered diffusion

This model includes three species: $[Ca^{2+}]_c$, a fixed and a diffusable endogenous buffers (B_i), and the Ca^{2+}-fixed buffer complexes (CaB_i). It was assumed that the single human β-cell has a spherical shape, which is a reasonable approximation when the cell is isolated (Braun, 2013, personal communication). Neglecting the tangential components of diffusion and assuming that the VDCCs are homogeneously dis-

tributed over the cell membrane, the diffusion equation can be reduced to a one-dimensional equation:

$$\frac{\partial[\mathrm{Ca}^{2+}]}{\partial t} = \frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2 D_{\mathrm{Ca}}\frac{\partial[\mathrm{Ca}^{2+}]}{\partial r}\right) - \sum_i R_i \quad (1)$$

$$\frac{\partial[\mathrm{B}_i]}{\partial t} = \frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2 D_{\mathrm{B}_i}\frac{\partial[\mathrm{B}_i]}{\partial r}\right) - R_i \quad (2)$$

$$\frac{\partial[\mathrm{CaB}_i]}{\partial t} = \frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2 D_{\mathrm{B}_i}\frac{\partial[\mathrm{CaB}_i]}{\partial r}\right) + R_i \quad (3)$$

where r is the radius of the cell, D_X is the diffusion coefficient for the corresponding species and R_i is the term of interaction between Ca^{2+} and i denotes the type of endogenous buffer (fixed f and movie m). Ca^{2+} buffering follows a first order reaction, thus:

$$R_i = k_f[\mathrm{Ca}^{2+}]\cdot[\mathrm{B}_i] - k_r[\mathrm{CaB}_i] \quad (4)$$

where k_f and k_r are the forward and backward binding rates for both the fixed and movie buffers, respectively.

C. Ca^{2+} influx and extrusion

Ca^{2+} influx and extrusion were imposed only to the boundary representing the cell membrane. The influx of Ca^{2+} is mediated by the total Ca^{2+} current (I_{Ca}, see above). The plasma membrane ATPase (PMCA) and the Na^+/Ca^{2+} exchanger are responsible for the extrusion of Ca^{2+} from the cytosol (I_{PMCA} and I_{NCX} respectively). Both extrusion mechanisms were simulated as in the model of Meyer-Hermann[7]:

$$I_{PMCA} = \rho_{PMCA} i_{PMCA}\frac{[\mathrm{Ca}^{2+}]^2_{MD}}{[\mathrm{Ca}^{2+}]^2_{MD} + K^2_{PMCA}} \quad (5)$$

$$I_{NCX} = \rho_{NCX} i_{NCX}\frac{[\mathrm{Ca}^{2+}]_{MD}}{[\mathrm{Ca}^{2+}]_{MD} + K_{NCX}} \quad (6)$$

where ρ_{NCX} and ρ_{PMCA} are the densities of the NCX and PMCA respectively and i_{PMCA} and i_{NCX} are the maximal currents. In order to maintain a fixed $[Ca^{2+}]_c$ at rest, a steady leakage of Ca^{2+} given by Eq. 5 with $[Ca^{2+}]_{MD} = [Ca^{2+}]_0$ was included (J_{Leak}). All the Ca^{2+} fluxes were calculated as $J_X = I_X/(2FA)$, where X indicate the corresponding mechanism (ICa, PMCA, NCX and Leak), and A is the surface area of the cell. Given that Braun et al.[6] reported an average capacitance of 9.9pF, assuming an equivalence between capacitance and surface area of 10 fF/μm^2[8], a radius of a surface area of 990 μ^2 was calculated, corresponding to a radius of 8.8μm. The total Ca^{2+} flux through the cell membrane is thus:

$$J_{Ca} = J_{ICa} - J_{PMCA} - J_{NCX} + J_{Leak} \quad (7)$$

The parameters used for the Ca^{2+} buffering systems are shown in Table 1. The parameters and expressions for the Ca^{2+} extrusion mechanisms (PMCA and NCX) can be consulted in Ref. [7] and were used without modification.

Table 1: Parameters for the model of buffered diffusion of Ca^{2+}. Obtained from Ref. [9]

Parameter	Value
D_{Ca}	220 $\mu m^2/s$
D_{B_f}	0 $\mu m^2/s$
D_{B_m}	15 $\mu m^2/s$
k_f	$5\times10^8 M^-1 s^-1$
k_d	10 μM
$[B_f]$	0.5 mM
$[B_b]$	100 μM

D. Computational Aspects

Simulations were performed in COMSOL Multiphysiscs 4.4, using the finite element method. The relative and absolute tolerance were 1E-7 and 1E-8 respectively. A maximum time step of 0.1 ms was used in all cases.

III. Results

A. Effects of endogenous buffers on the time course of the Ca^{2+} transients during spiking electrical activity

Figure 1A (*top*) shows the simulated electrical activity (Vm) consisting in action potential (APs) firing with a frequency of 5 APs/s. Changes in Vm promotes the influx of Ca^{2+} through the VDCCs, resulting in an increase of $[Ca^{2+}]_c$. In Fig. 1A (*bottom*) the concentration of Ca^{2+} in the microdomain (at a distance of 10 nm from the membrane, $[Ca^{2+}]_{MD}$) is shown both in the presence and absence of endogenous buffers. When endogenous buffers are not considered, it can be seen that the amplitude of the Ca^{2+} spikes remained approximately constant, while the baseline of $[Ca^{2+}]_{MD}$ transiently increased upon a steady level of $1\mu M$, where peak $[Ca^{2+}]_{MD}$ reached $> 5\mu M$. In contrast, when endogenous buffers were included in the model, both peak $[Ca^{2+}]_{MD}$ and the rise of the baseline were inhibited considerably (compare also Fig. 1 D1 *vs.* D2 and D3).

It can be observed that the time course of the free fixed endogenous buffer ($[B_1]$) closely follows the behavior of

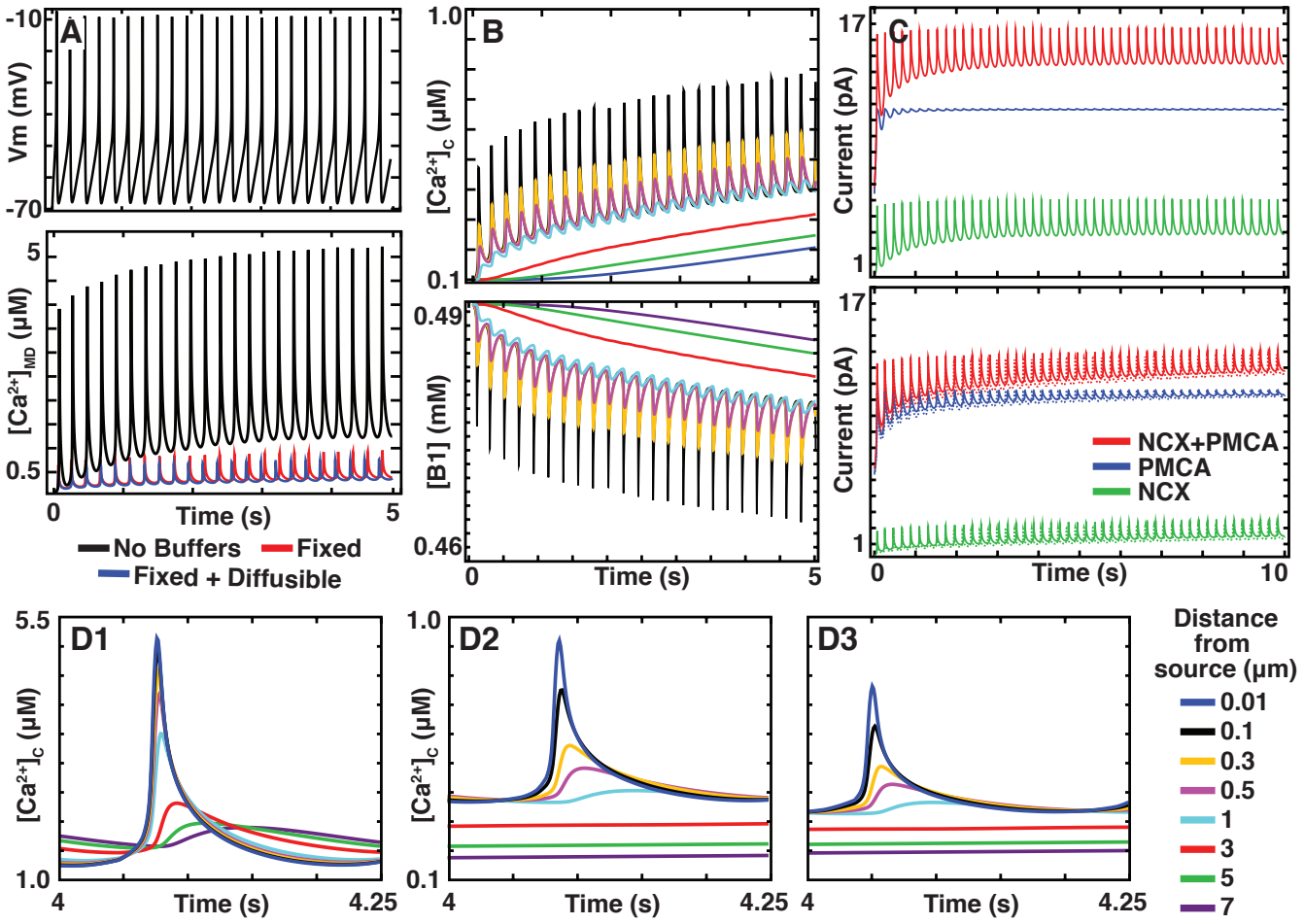

Fig. 1: **A.** Spiking electrical activity (*top*) and Ca^{2+} concentration at the microdomain (*bottom*). **B.** Ca^{2+} concentration at different depths of the intracellular space (*top*) and the corresponding free fixed endogenous buffer concentrations. Colors indicate different depths (see legend in **D**). **C.** Current due to the Ca^{2+} extrusion mechanisms in absence (*top*) and presence (*bottom*) of the fixed endogenous buffer. **D.** A single Ca^{2+} transient is shown at different depths of the intracellular space in absence (**D1**) of Ca^{2+} buffers; when a fixed endogenous buffer is included (**D2**) and when both a fixed and movie endogenous buffers are considered (**D3**).

$[Ca^{2+}]_c$ (compare Fig. 1B *top* and *bottom*). It is important to mention that as $[B_1]$ decreases, the complex $[CaB_1]$ is formed in such a way that the total concentration of B_1 is conserved (not shown). A single $[Ca^{2+}]_c$ transient is shown in Figs. 1 D for the three cases simulated: absence of buffers, presence of a single fixed endogenous buffer and presence of both a fixed and a diffusible buffers. In the absence of buffers (Fig. 1 D1) the Ca^{2+} transient was observed even at 7 μm of depth. In contrast, when endogenous buffers were added, the Ca^{2+} transient was only observed at depths $\leq 1\mu m$ (Figs. 1 D2 and D3), although $[Ca^{2+}]_c$ remained increasing slowly at all depths (see Fig. 1 B *top*).

B. The effect of Ca^{2+} extrusion mechanisms on $[Ca^{2+}]_{MD}$

According to our simulations, Ca^{2+} extrusion mechanisms play an important role limiting the rise of $[Ca^{2+}]_c$ (Fig. 2). In Fig. 1C it can be seen that in the absence of buffers, the activity of the PMCA was practically at its maximum level ($I_{PMCA} \sim 10$ pA) given its higher sensitivity to Ca^{2+}. On the other hand, the NCX contributed in a lesser extent to the clearing of Ca^{2+} from the cytosol, as its sensitivity to Ca^{2+} is ten fold lower than that of the PMCA. Figure 2 clearly shows the effects of both the PMCA and NCX on $[Ca^{2+}]_{MD}$ when a single fixed endogenous buffer is included The presence of the Ca^{2+} extrusion mechanisms slows the accumulation of Ca^{2+} in the submembrane space. Similar results were ob-

tained when the diffusible buffer was included (not shown).

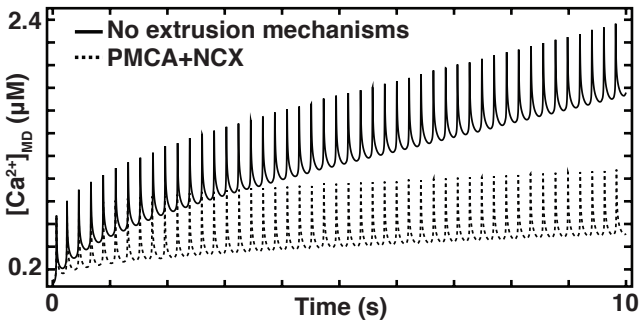

Fig. 2: Effects of Ca^{2+} extrusion mechanisms on $[Ca^{2+}]_{MD}$

IV. Discussion

In this work we have shown that endogenous buffers, in conjunction with the PMCA and NCX, limit to a great extent the increase of $[Ca^{2+}]_c$ during repetitive firing of APs. In β-cells, it is known that insulin granules are released to the extracellular space in response to a increase in $[Ca^{2+}]_{MD}$. Moreover, insulin granules in the cytosol are also mobilized to the cell membrane by a Ca^{2+} signal. By means of computational simulations we have estimated that action potential firing, resembling the most common electrical behavior of the human β-cell, produces a Ca^{2+} transient observable even at a distance of $\sim 1\mu m$ from the Ca^{2+} channels in the presence of endogenous buffers. To our knowledge, experimental measurements of $[Ca^{2+}]_c$ in human β-cells are lacking, although some studies of $[Ca^{2+}]_c$ in rodent β-cells have been performed. For instance, Theler et al.[10] reported a submembrane Ca^{2+} of less than $1\mu M$. In addition, other authors have reported slightly higher concentrations in the range of 1.8 to 2.3 μM[3]. Our simulations agree with these experimental reports.

Future development of the model must include a secretory component in order to be able to evaluate how Ca^{2+} oscillations regulates the mobilization of insulin granules in the intracellular space.

V. Conclusions

Computational models are very useful tools as a complement to the experimental work. In this paper we have shown how by combining a model of the electrical activity of the human β-cell with a model of the buffered diffusion of Ca^{2+} it is possible to estimate the cytosolic Ca^{2+} levels achieved during spiking electrical activity.

VI. References

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Author: Gerardo J. Félix Martínez
Institute: Universidad Autńoma Metropolitana
Street: San Rafael Atlixco No 186, Iztapalapa, Vicentina, 09340
City: México D.F.
Country: México
Email: gjfelix2005@gmail.com