Mathematical Biosciences 270 (2015) 66–80

Contents lists available at ScienceDirect

Mathematical Biosciences

journal homepage: www.elsevier.com/locate/mbs

Modeling Ca^{2+} currents and buffered diffusion of Ca^{2+} in human β-cells during voltage clamp experiments

Gerardo J. Félix-Martínez*, J. Rafael Godínez-Fernández

Department of Electrical Engineering, Universidad Autónoma Metropolitana Iztapalapa, México, D.F., Mexico

ARTICLE INFO

Article history:
Received 9 October 2014
Revised 3 September 2015
Accepted 28 September 2015
Available online 20 October 2015

Keywords:
β-cell
Electrical activity
Ca^{2+}
Microdomains
Diffusion
Voltage clamp

ABSTRACT

Macroscopic Ca^{2+} currents of the human β-cells were characterized using the Hodgkin–Huxley formalism. Expressions describing the Ca^{2+}-dependent inactivation process of the L-type Ca^{2+} channels in terms of the concentration of Ca^{2+} were obtained. By coupling the modeled Ca^{2+} currents to a three-dimensional model of buffered diffusion of Ca^{2+}, we simulated the Ca^{2+} transients formed in the immediate vicinity of the cell membrane during voltage clamp experiments performed in high buffering conditions. Our modeling approach allowed us to consider the distribution of the Ca^{2+} sources over the cell membrane. The effect of exogenous (EGTA) and endogenous Ca^{2+} buffers on the temporal course of the Ca^{2+} transients was evaluated. We show that despite the high Ca^{2+} buffering capacity, nanodomains are formed in the submembrane space, where a peak Ca^{2+} concentration between ~76 and 143 μM was estimated from our simulations. In addition, the contribution of each Ca^{2+} current to the formation of the Ca^{2+} nanodomains was also addressed. Here we provide a general framework to incorporate the spatial aspects to the models of the pancreatic β-cell, such as a more detailed and realistic description of Ca^{2+} dynamics in response to electrical activity in physiological conditions can be provided by future models.

1. Introduction

In the pancreatic β-cell, insulin is released in response to an increase in the intracellular Ca^{2+} concentration [1,2] ($[Ca^{2+}]_i$), mediated by the entry of Ca^{2+} through specific voltage-dependent Ca^{2+} channels (VDCCs), which are activated by membrane depolarization caused by the closure of the ATP-dependent K^+ channels (K_{ATP}) due to an increased metabolic activity (i.e. ATP production) after glucose stimulation. In general, the latter mechanism is responsible for insulin secretion both in rodent and human β-cells; however, several differences between species at different levels have been found recently. For instance, it has been shown that the fraction and distribution of the different hormone-secreting cell types in the islets of Langerhans differ between rodents and humans [3,4]. Similarly, differences in the glucose threshold for insulin secretion [5], the kinetics of exocytosis [6], the ionic channels expressed and their role in electrical activity have been reported [7–11].

Insulin secretion shows a biphasic response to glucose stimulation consisting of a first fast transient phase, followed by a second sustained phase with a slower rate of secretion [12]. It has been proposed that this biphasic behavior is due to the existence of distinct pools of insulin granules [12,13] that are distinguished both by the proximity to the cell membrane and their sensitivity to Ca^2 [14,15]. A minor fraction of the insulin granules forms the readily releasable pool (RRP), located in the immediate vicinity of the VDCCs, where $[Ca^{2+}]_i$ reaches much higher levels than the rest of the cytosol and the so called Ca^{2+} nano and microdomains are formed [16–18]. It has been hypothesized that the release of the RRP granules accounts for the first phase of insulin secretion [13,19], ending once the RRP pool has been depleted. According to this proposal, the second phase of secretion is sustained by the mobilization of insulin granules from a reserve pool to the plasma membrane, where they can be released to the extracellular space [13]. Recently, direct evidence supporting this proposal was obtained by means of imaging techniques [20,21]. A detailed description of the mechanisms involved in insulin granule exocytosis can be found in recent reviews [22,23].

The main signal for the release of the RRP granules is the increase of $[Ca^{2+}]_i$, thus the formation of Ca^{2+} nano and microdomains near the mouth of the VDCCs is extremely important for an adequate secretory response in β-cells. It has been shown that in rodent β-cells, the secretory sites and the VDCCs are colocalized and that both the entry of Ca^{2+} and the secretion of insulin are limited to a certain region of the plasma membrane [17,24–26].

In contrast to mice β-cells, in which Ca^{2+} influx is mediated mainly by the L- and R-type Ca^{2+} channels, the ionic channels responsible for the entry of calcium to the cytosol in human β-cells are the

* Corresponding author. Tel.: +525558044600, Ext. 6437.
E-mail address: gjfelix2005@gmail.com (G.J. Félix-Martínez).

http://dx.doi.org/10.1016/j.mbs.2015.09.009
0025-5564/

T-, L-, and P/Q-type Ca^{2+} channels [27] (for recent reviews regarding the electrophysiology of β-cells see Refs. [11,28–30]). Braun et al. [8] described in detail the electrophysiological characteristics of the ionic currents expressed in the human β-cell and their relationship with insulin secretion [6,8]. However, simultaneous measurements of Ca^{2+} in the intracellular space were not performed. In light of the above limitations we have built a computational model to simulate the spatiotemporal distribution of Ca^{2+} produced by the Ca^{2+} currents under voltage clamp conditions by using detailed models of the Ca^{2+} currents, which were derived directly from electrophysiological data. To our knowledge, studies addressing the dynamics of $[Ca^{2+}]_i$ in human β-cells are lacking. Since the Ca^{2+} signal plays a key role in glucose-stimulated insulin secretion, it is important to study how Ca^{2+} is distributed throughout the intracellular space following entry through the VDCCs.

Computational models have been widely used to simulate the spatiotemporal distribution of Ca^{2+} in the intracellular space in different types of cells [31–36]. In these models, both exogenous and endogenous Ca^{2+} buffers have been considered, since they play an important role in determining the distribution of $[Ca^{2+}]_i$. Several methods have been adopted in these studies to solve the resulting reaction–diffusion problem, being among them the finite differences method [31–34], the Monte Carlo method [35] and recently the finite element method [36]. Owing to the characteristics and limitations of each method, different simplifications have been made in these works. For example, most of the models based on finite differences schemes [31–33] assume that Ca^{2+} channels are uniformly distributed over the cell membrane. As a consequence, important geometrical aspects, like the non-homogeneous distribution of the Ca^{2+} channels, the tangential components of diffusion and the curvature of the cell are often neglected. On the other hand, the model of Gil et al. [35], which is based on the Monte Carlo method, is capable of considering the distribution and number of channels, though in a simplified three-dimensional geometry. In this work we have built a three-dimensional model of an isolated human β-cell using the finite element method with two main objectives: (1) to develop a model of the macroscopic Ca^{2+} currents accounting for the dependence of the activation and inactivation processes on both membrane potential and Ca^{2+} concentration in the nanodomain; (2) to simulate the spatiotemporal distribution of Ca^{2+} in the intracellular space under voltage clamp conditions considering the morphological characteristics of a typical human β-cell.

2. Methods

2.1. Conceptual model

In order to simulate the spatiotemporal distribution of Ca^{2+} in the intracellular space due to the entry of Ca^{2+} through the VDCCs in voltage clamp conditions, a model of each of the macroscopic Ca^{2+} currents found in the human β-cell (T-, L-, and P/Q-type) was developed using the Hodgkin–Huxley formalism. A model of Ca^{2+} buffered diffusion in a three-dimensional cell was simultaneously used to estimate the concentration of Ca^{2+} at different depths from the Ca^{2+} sources, using the flux of Ca^{2+} generated by the simulated currents as the input signal. Since L-type Ca^{2+} channels of the human β-cells are inactivated by Ca^{2+} itself [8], the concentration of Ca^{2+} at the nanodomain ($[Ca^{2+}]_{ND}$), estimated from the reaction–diffusion model, was used to couple the model of buffered diffusion with the model of the Ca^{2+} currents. In addition, $[Ca^{2+}]_{ND}$ dynamically regulates the activity of the Ca^{2+} extrusion mechanism and the value of the reversal potential. This approach allows us to evaluate the effect of the localization of the ionic channels over the cell membrane (Fig. 1B) on the spatiotemporal distribution of Ca^{2+} in the intracellular space. A schematic diagram of the conceptual model is shown in Fig. 1A.

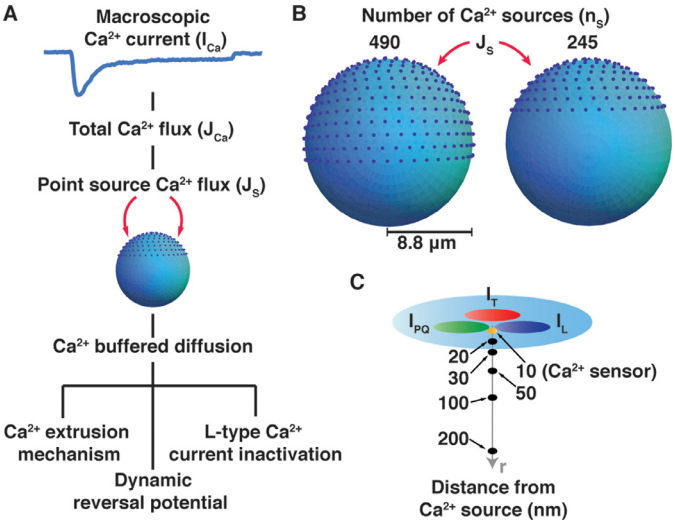

Fig. 1. A. Conceptual model. From the simulated macroscopic currents, a corresponding total Ca^{2+} flux (J_{Ca}) was calculated and distributed among 245 or 490 Ca^{2+} point sources (J_S). The problem of buffered diffusion of Ca^{2+} considering both exogenous (EGTA) and endogenous (END) buffers was solved using the finite element method. The concentration of Ca^{2+} at the nanodomain, estimated at 10 nm from the point sources ($[Ca^{2+}]_{ND}$), is used to determine the level of inactivation of the L-type Ca^{2+} channels, to calculate the value of the dynamic reversal potential and to regulate the activity of the Ca^{2+} extrusion mechanism. **B.** Geometry of the simulated β-cell. Two cases with 245 and 490 Ca^{2+} sources distributed among ~30% and 57% of the total sphere surface area were considered. **C.** Diagram of a Ca^{2+} point source. It was assumed that the point sources include the three types of Ca^{2+} channels (L, T and P/Q). Ca^{2+}, EGTA and END concentrations were measured at points located 10, 20, 30, 50, 100 and 200 nm from the Ca^{2+} sources.

2.2. Model of buffered diffusion of Ca^{2+}

Different buffering conditions were simulated. Initially, simulations with a single exogenous buffer (EGTA) were performed, thus considering three species in the model: free Ca^{2+} ($[Ca^{2+}]_i$), unbound EGTA ([EGTA]) and bound EGTA ([EGTA•Ca^{2+}]). Then, we evaluated the effect of adding a non-diffusible endogenous buffer (END) on the distribution of Ca^{2+}, thus adding two more species to the model: unbound END ([END]) and bound END ([END • Ca^{2+}]). In both cases the buffered diffusion of Ca^{2+} was simulated using the standard reaction–diffusion equations:

$$\begin{aligned}\frac{\partial [Ca^{2+}]_i}{\partial t} &= D_{Ca}\nabla^2[Ca^{2+}]_i - \sum_i R_i \\ \frac{\partial [B_i]}{\partial t} &= D_{B_i}\nabla^2[B_i] - R_i \\ \frac{\partial [B_i \bullet Ca^{2+}]}{\partial t} &= D_{B_i}\nabla^2[B_i \bullet Ca^{2+}] + R_i \end{aligned} \quad (1)$$

where D_X is the diffusion coefficient for each species, ∇ is the three-dimensional partial differential operator, B_i represents the type of Ca^{2+} buffer (END or EGTA) and R_i is the reaction term given by a first order kinetic scheme:

$$[Ca^{2+}] + [B_i] \underset{k_-}{\overset{k_+}{\rightleftarrows}} [B_i \bullet Ca^{2+}].$$

which, according to the mass-action law, can be written as:

$$R_i = k_+[Ca^{2+}]_i \cdot [B_i] - k_-[B_i \bullet Ca^{2+}]. \quad (2)$$

The parameters k_+ and k_- are the forward and backward binding rates respectively, and are given in Table 1.

2.2.1. Geometry

In this model, the human β-cell geometry is assumed to be spherical, which is a reasonable approximation when it is studied in isolation (Braun 2013, personal communication). A three-dimensional