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Computational methods to study Ca^{2+} -triggered secretion at the cellular level

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ABSTRACT

Secretion is a widespread and fundamental physiological process that is tightly controlled by intracellular Ca^{2+} . Here, we present computational methods to study the functional dynamics of cellular secretion. We propose two computational schemes in order to reproduce the Ca^{2+} dynamics and exocytosis associated to different spatiotemporal scales: a microscopic scheme, suitable for fast spatiotemporal processes and a whole-cell scheme, suitable for slower (larger) scales. Some results showing the dynamic behaviour of two specific cell types are included in order to show the interest of a computational approach to gain insight about the molecular aspects of secretion, as well as to make theoretical predictions.

Keywords: calcium dynamics, stochastic methods, deterministic methods.

1 INTRODUCTION

Calcium is mainly concentrated in teeth and bones in higher organisms as mammals, and it is moved through the body as a divalent ion (Ca^{2+}). This ion is the most ubiquitous cell signaler that nature uses to trigger and control a lot of vital processes such as muscular contraction, genetic transcription, cell proliferation and differentiation, as well as regulated secretion of hormones and neurotransmitters [58]. Ca^{2+} homeostasis is carefully kept inside the cells to maintain fixed amounts of Ca^{2+} in the intracellular and extracellular liquids. For example, in resting conditions, the ratio between extracellular and intracellular calcium concentrations is of four orders of magnitude. This difference guarantees an endless source for cells, and at the same time, it makes cells highly sensitive to very small changes, even of few ions, in the internal calcium ion concentration [15]. The huge gradient of Ca^{2+} between the extra- and intracellular medium is kept thanks to a cell membrane that acts as a barrier and to specific Ca^{2+} mechanisms which constantly extrude the excess. However, there are Ca^{2+} channels that allow Ca^{2+} to enter into the cell in a selective manner. These channels are proteins located across the cell membrane and are normally closed (i.e., not allowing Ca^{2+} to go from the outside to the inside), but they can open in response to a specific stimulus (voltage, ligand or emptying of intracellular stores) [15]. Since the calcium ion is a charged particle, when some of them cross the cell membrane they can be detected as an ionic (electric) current using two electrodes, one in the cell inside and one in the external side.

In 1968, the term stimulus-secretion coupling was coined to describe the vital role of Ca^{2+} as the coupling agent between the stimulation and the secretion process, based on observations in neuroendocrine cells [12]. The physiological process starts when

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an adequate stimulation opens some Ca^{2+} channels, producing a calcium current, and consequently increases the intracellular calcium level. The presence of incoming Ca^{2+} is decoded to trigger secretion through its binding to specific Ca^{2+} sensors located in the cell membrane and/or in the secretory vesicles [4]. To always keep the vital calcium gradient, the extrusion mechanisms will work constantly to take out all the extra Ca^{2+} which is inside the cell. However, some ions will have time to act as signals activating intracellular responses. Thus, the temporal dynamics of the intracellular calcium concentration ($[Ca^{2+}]_i$) is strongly related to the corresponding dynamics of the Ca^{2+}-triggered secretion, also named exocytosis [58].

The optimal functioning of the nervous system strongly depends on neurotransmision, that is, the whole process of passing a signal from one neuron (presynapse or presynaptic) to another (postsynaptic) by releasing a substance (neurotransmitter) into the synapse, i.e., the junction between them. The efficiency of neurotransmitter release depends in turn on the number of active zones (releasing areas) located in the presynaptic neuron, as well as on the speed of vesicle fusion. Vesicles are small bags inside the cell that contain the releasing substance. The secretion process finishes when the vesicle fuses its membrane with the cellular one, opening a pore through which its contents are released to the cell exterior. As all secretory processes, neurotransmitter releasing depends on vesicles, but it is also influenced by the organization of the exocytotic machinery, including Ca^{2+} channels and other relevant proteins; they all shape the spatiotemporal dynamics of the stimulus-secretion coupling [3]. Presynaptic terminals of the central nervous system are characterized by their high efficiency because the secretory response appears just a few milliseconds after a single action potential stimulates the presynaptic neuron. Fast secretion is possible thanks to effective geometrical and physiological factors such as the spatial coupling of vesicles and Ca^{2+} channels, and the availability of a rapid pool of vesicles. Indeed, Ca^{2+} channels are known to play a key role in the control of neurotransmitter release, in particular, those that open in response to cell depolarization (voltage-gated channels) [5]. Vesicles are also very important and they must have a fast response, so there needs to be enough of them and they have to be ready to be fused in order to achieve an efficient stimulus-secretion coupling [33]. Despite the great relevance of neurotransmitter release in the adequate functioning of the nervous system in mammals, the study of the stimulus-secretion coupling has enormous experimental restrictions since fast, microscopic, and not invasive systems are needed to simultaneously record a stimulus and its corresponding response. Theoretical modeling and simulation are very useful tools for complementing experimental studies in such a way that their combination allows a more thorough interpretation of results, as we discuss in [1].

Secretion is not only observed in the central nervous system, where neurotransmitters such as glutamate or acetylcholine, are the released substances [45]. Neuroendocrine cells are peripheral cells that secrete hormones needed to start vital process like glucose regulation, fat metabolism, stress control, etc. In this case, secretion is also triggered by Ca^{2+}, in a manner similar to neurotransmitter release. However, main differences are present: one, the time delay in-between stimulus and release is much longer; two, the sources for $[Ca^{2+}]_i$ elevations are multiple; and three, the associated $[Ca^{2+}]_i$ dynamics spans different time scales [35]. Again, theoretical modeling and simulations may not only help experimental studies to unveil how $[Ca^{2+}]_i$ is being increased and how it is decoded to trigger secretion, but also point out the main factors affecting the secretory response. Moreover, since dynamic variables are hard to be recorded in experiments, modeling could serve for testing some experimental protocols to predict cellular responses to stimulation. Also, experiments can be suggested by the model to test the possible influence of a mechanism, or the benefits of a drug therapy.

The need for resorting to computational approaches is further amplified when analyzing secretion in cells that display Ca^{2+} oscillations after stimulation by an hormone or a neurotransmitter. To quote only a few examples, secretion occurs in response to an oscillating level of Ca^{2+} in pancreatic beta [25] and alpha cells [20], parotid acinar cells [48], pancreatic acinar cells [51] or pituitary gonadotrophs [52]. In most cases, these Ca^{2+} oscillations occur on a time scale longer than the secretion process. Periods indeed range from seconds to several minutes. These oscillations are brought about by a rise in the concentration of inositol 1,4,5-trisphosphate ($InsP3$), which in turn provokes the release of Ca^{2+} from the endoplasmic reticulum (ER). The Ca^{2+} releasing activity of the $InsP3$ receptor is stimulated by cytosolic Ca^{2+} at low concentrations of this messenger and inhibited by this same compound at higher concentrations. In combination with an active pumping of Ca^{2+} back into the ER, this biphasic dependency can give rise to sustained Ca^{2+} oscillations. In complement to the numerous experimental approaches, the detailed molecular mechanism responsible for Ca^{2+} oscillations has been much investigated by computational modeling. However, the relationship between relatively slow

Ca^{2+} oscillations and fast secretory processes is still poorly understood, except for some specific cases as, for example, saliva secretion [37]. In particular, the question arises as to whether secretion can be sensitive to slow Ca^{2+} changes, as reported for some experimental studies [20, 27].

In this work, we discuss theoretical approaches to study the dynamics of Ca^{2+}-triggered secretion. We focus on two computational schemes aimed at simulating this process, either microscopically or at the cellular level. The first approach is required if the time and length scales are so small that stochastic effects need to be considered. This is the case for glutamate secretion by a presynaptic terminal, such as the calyx of Held, which occurs in a few milliseconds. In contrast, in pancreatic α-cells where secretion occurs on time-scales of the order of minutes, a deterministic approach taking into account the large variability of Ca^{2+} dynamics among the cells is appropriate. Some results showing the dynamic behaviour of both cell types are included. In particular, we show how these models can be used to gain insight into the functional dynamics of Ca^{2+}-triggered secretion.

2 METHODS

Our starting point is that Ca^{2+}-triggered cellular secretion follows similar steps in different cellular types [9], that is: An external stimuli opens some Ca^{2+} channels that leads to an intracellular Ca^{2+} increase which initiates Ca^{2+} regulation, including buffered diffusion and extrusion; meanwhile, secondary processes such as exocytosis are triggered. Secretion is activated since the exocytotic machinery, mainly formed by proteins in the vesicle and in the cellular membrane, is sensitive to calcium levels. Then, vesicles are fused, so they release their contents to the extracellular medium. On this basis, a modeling study of exocytosis in different cell types should include these same steps but taking into account the spatiotemporal differences of each cell.

Whole-cell and compartmental approximations of the Ca^{2+} dynamics related to secretion [16, 29, 57] are useful to study the slow components of secretion, that last for minutes or hours [28]. However, these approximations are not accurate for synapses where Ca^{2+} influx and secretion occurs very fast (in a few milliseconds) since channels and vesicles are usually clustered (in a few nanometers) [3]. Then, active-zone models are more accurate to study the Ca^{2+} influx leading to neurotransmitter secretion. Some active-zone models include spherical terminals [30], and cubic volumes [31].

2.1 Fast Ca^{2+} and secretion dynamics

Our approach is based on a small-sized spatial domain representing the active zone, where the main cell features involved in fast Ca^{2+}-triggered secretion (Ca^{2+} channels, vesicles, and buffers) are located. Since the simulation times are below 10 milliseconds and an active zone of the calyx of Held is about 100 nm of radius [44], we use a fully microscopic model with high spatial and temporal resolution. A value of 10 nm for the spatial resolution is adequate for this microscopic study. In contrast to previous active-zone models [16, 30, 57], all the processes are computed stochastically in our scheme: the entry of Ca^{2+} ions through calcium channels, the three-dimensional diffusion of Ca^{2+} and mobile buffers, and the kinetic reactions of Ca^{2+} ions with buffers and vesicles (vesicles are treated as buffers in our approach). Then, it is a Monte Carlo scheme that allows the simulation of local Ca^{2+} values near vesicles. For the defined spatial resolution, it can be expected that only few Ca^{2+} ions enter or move, and only few can be bound to a buffer or a vesicle too, so the stochastic effects become very important. Therefore, it is much more accurate to keep track of each particle (ion or molecule) as we do, than to use average concentrations to describe the fast dynamics of Ca^{2+} and secretion in an active zone of a synaptic terminal. Actually, this aspect was already pointed out in [31].

In our approach, an active zone is modeled as a conical o cylindrical domain. The domain base represents a cell membrane patch, and we assume that the flux of ions and buffer molecules through the lateral sides of the domain is zero. An orthogonal three-dimensional grid maps the domain of simulation with a distance between grid points chosen by the user, defined as the spatial discretization (Δx). Each point of the grid is associated with a cubic compartment of volume $(\Delta x)^3$. Therefore, grid points or cubic compartments are equivalent concepts. Calcium ions and buffer molecules may move from one compartment to another due to diffusion, which is modeled as a random walk process. The time scale for the random walk is calculated from the spatial discretization and the fastest diffusion coefficient of the system, following the equation $\Delta t = (\Delta x)^2/4D$, where Δt is the time step and D is the diffusion coefficient of the fastest particle in the medium (in this case, Ca^{2+} ions). The random walk algorithm allow mobile particles to be moved every time step in which each particle will have 50 % chance to remain in its initial position, and 25 % chance to move either in the positive or negative direction, for each spatial dimension. In the first slice of the domain, which represents the cell submembrane region, Ca^{2+} channels and vesicles are distributed. Different distribu-

tions can be chosen: cluster, correlated cluster or random. The scheme was solved with Monte Carlo methods implemented in Fortran, based on those described in [18].

The following algorithm was used for simulations of fast Ca^{2+}-triggered secretion in the calyx of Held:

Main input data:

- Channel distribution (random/cluster/correlated-cluster)
- Initial Ca^{2+} and buffer concentrations
- Depolarization pulse (voltage, duration)
- Simulation time

Presimulation stage:

- Transform the macroscopic input variables into microscopic quantities
- Map the simulation domain into a 3-D orthogonal grid
- Distribute calcium channels over the upper layer of the domain
- Distribute vesicles over the upper layer of the domain
- Distribute uniformly the initial number of calcium ions and buffers over the whole domain

Simulation stage: At each time step, Δt, calculate the following values:

- Number of incoming calcium ions
- New position of calcium ions and mobile buffers due to diffusion
- Number of free and bound calcium ions due to kinetic reactions of the buffers and the vesicle calcium sensor, as well as the number of fused vesicles

Output data: Number and location of Ca^{2+} ions and fused vesicles.

It is important to note that, in the algorithm, the Ca^{2+} influx comes from calcium channels which are located in the cell membrane. To model these channels, we use discrete Markov models as described in [21]. In the particular case of neurotransmitter secretion, Ca^{2+} influx is mainly managed by P-type voltage-gated Ca^{2+} channels [59], which is the model included for our simulations. Due to the modularity of the algorithm, alternative Ca^{2+}-channel models could be used for the simulations in order to study other cellular conditions, such as the one we explored in [54]. The AZ scheme does not consider main calcium-extrusion mechanisms found in presynaptic terminals, such as the Na^{+} - Ca^{2+} exchanger and the Ca^{2+} pump, since the former is not colocalized with release sites, and the latter has a slow extrusion rate that is out of the simulation timescale [26]. Other phenomena not considered are the effect of diffusion barriers, such as release granules or mitochondria, which were studied by A. Gil and collaborators in [47].

Calcium ions trigger exocytosis thanks to the existence of a calcium-binding protein (*calcium sensor*) in the cytoplasm that activates the secretory machinery. It is known that 3 to 5 calcium ions could be bound to these proteins to activate them [49]. Calcium binding to the calcium sensor leads to vesicle fusion and finally to release, and these process could be modeled as a biochemical reaction consisting of several steps [9]. Secretion has been well described considering five binding sites for synapses [8], and three binding sites for neuroendocrine cells [24, 55]. On this basis, we use a secretion model that includes the last Ca^{2+}-triggering steps to study the glutamate secretion in the calyx of Held, considering five binding sites (Fig. 1). In a similar manner, we use a secretion model including the possible states and transitions for granules in α-cells; in this case, we consider three binding sites to describe the Ca^{2+}-triggering steps (Fig. 2).

2.2 Slow Ca^{2+} and secretion dynamics

There are several cell types exhibiting exocytosis in mammals besides neurons. They include neuroendocrine and endocrine cells, which release hormones that serve to many physiological functions. In these cell types, Ca^{2+} elevations do not occur as fast as in neurons, but they occur more slowly (lasting milliseconds to seconds) or they even appear as oscillations (lasting seconds to minutes or hours). Thus, the time scale of the simulations, which have to cover at least one oscillatory period, are orders of magnitude larger than those characterizing neuronal processes. On the other hand, the intracellular Ca^{2+} elevations involve the opening of plasma-membrane Ca^{2+} channels but also the release of intracellular stores. Thus, the simulation scheme must consider the whole cell and cannot focus only on active zones, as it is the case for neurons. Our approach for slow Ca^{2+}-triggered secretion has been to use a deterministic computational scheme for long timescales. Moreover, in order to incorporate realistic Ca^{2+} changes taking into account the very large cell to cell variability, the inputs to the simulation algorithm are time series of Ca^{2+} evolutions obtained experimentally, at different levels of

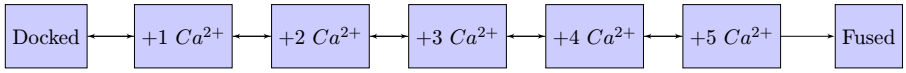

Figure 1 – Secretion model used for the calyx of Held.

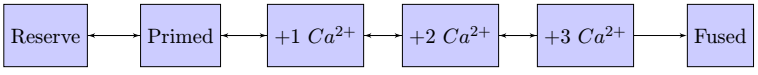

Figure 2 – Secretion model used for α-cells.

stimulation. Then, the algorithm describes the transitions between the six possible states of the secretory granules: granules serving as "reserve" and located in the cytoplasm, primed granules, granules bound to 1, 2 or 3 Ca^{2+} ions and fused granules (see Fig. 2). Transitions between the different states are described by the law of mass action, except for the transition between "reserve" and "primed" that is sensitive to cytoplasmic Ca^{2+} and external glucose. Ca^{2+}-binding to primed granules is sensitive to the level of Ca^{2+} just beneath the plasma membrane. However, only average values of cellular Ca^{2+} are provided by the experiments. Then, to compute the cytoplasmic and sub-membrane Ca^{2+} concentrations, we use the shell model described in [22]; this model allows the estimation of intracellular Ca^{2+} in concentric layers of specific thickness for a spherical cell, considering buffered diffusion and the Ca^{2+} pump. Taking into account the average radius of an α-cell (5.3μm) and the average size of the nucleus in this cell type (60% of the whole cell volume), we could estimate that the Ca^{2+} concentration up to 100 nm from the cell membrane is 1.28 larger than in the rest of the cytoplasm. From this calculation, we were able to reconstruct the time series of membrane and cytoplasmic Ca^{2+} concentration from the experimental data of average cell Ca^{2+} (Fig. 3).

The following algorithm was used for simulations of slow Ca^{2+}-triggered secretion in pancreatic α-cells:

Main input data:

- Cell radius
- Shell thickness
- Experimental time series of Ca^{2+} oscillations
- Simulation time

Presimulation stage:

- Uniformly distribute the concentration of Ca^{2+} and endogenous buffer in each shell

Simulation stage: At each time step, Δt, calculate the following values:

- Cytoplasmic and membrane Ca^{2+} concentration
- Number of vesicles in each state

Output data: Ca^{2+} concentrations, and number of fused vesicles.

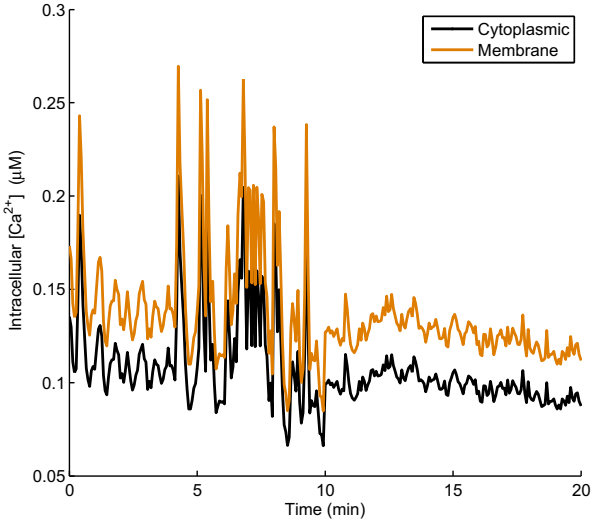

Figure 3 – Simulation of the cytoplasmic and membrane Ca^{2+} concentrations in an α-cell. These concentrations are used by the secretion model (Fig. 2).

3 RESULTS

3.1 Rapid Ca^{2+} and secretion dynamics: the case of the calyx of Held

We have analyzed the Ca^{2+} and secretion dynamics observed in the calyx of Held, which is a large synapse widely used to study neurotransmitter release [46], in response to the protocols we have described in [17]. The calyx of Held synapse is particularly interesting because it is located in the trajectory of the mammalian auditory central nervous system and it has been related

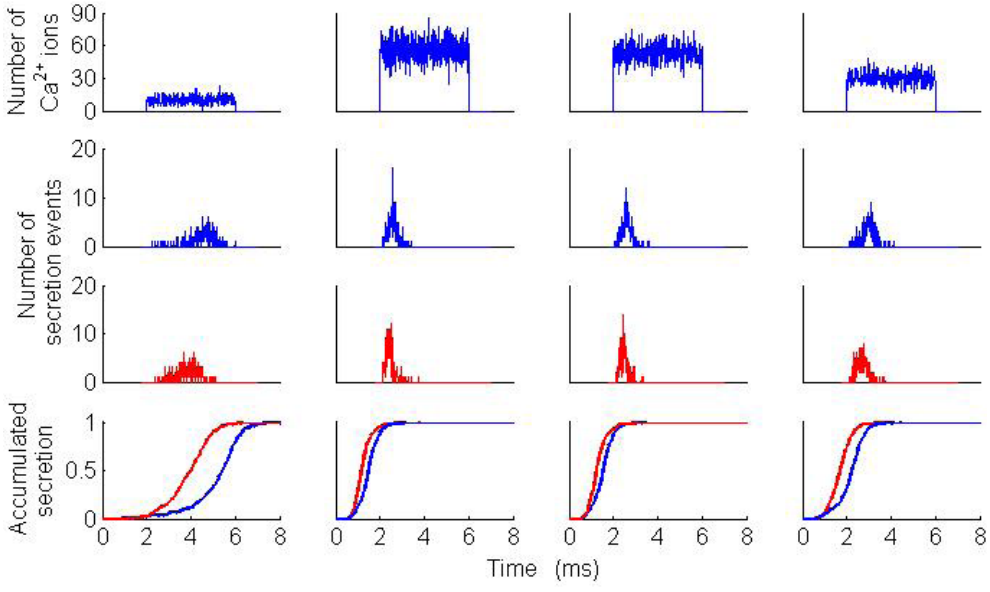

Figure 4 – Ca^{2+} and release dynamics in a short spatiotemporal scale referred to glutamate secretion in the calyx of Held. Ca^{2+} and secretion events are simulated in response to four different stimulation strengths: one weak, two strong, and one intermediate (first to fourth columns), respectively. Simulations show that Ca^{2+} influx (first row) can induce different secretory responses (second and third rows) depending on the presence of different Ca^{2+} buffers (BAPTA buffer in blue, EGTA buffer in red). Accumulated secretion is shown in the fourth row to emphasize the temporal delay induced by the BAPTA buffer (blue trace). Notice that the simulation timescale is in the order of milliseconds, and that these are plots of discrete events occuring in an active zone of 0.1 μm of radius.

to high-frequency sound source localization [50]. Neurotransmitter secretion has been accurately simulated with our model (Fig. 4): the release triggered by Ca^{2+} due to a brief stimulation, as well as the release delays due to the presence of mobile buffers, such as EGTA or BAPTA, are as well reproduced. This figure also shows that with microscopic models it is possible to take into account the inherent noise associated with the stochastic nature of voltage-gated calcium channels, buffered diffusion and Ca^{2+} binding [19, 32]. Moreover, the calyx of Held, as other synapses, illustrate that macroscopic descriptions obtained with differential methods in homogeneous geometries are not always valid or accurate: as shown in our simulations, calcium concentrations at the low regime (low influx) are so small that, for the spatial resolutions considered, just few calcium ions enter into the active zone [17]. Then, particle-based methods, such as those based on Monte Carlo schemes, are more accurate and useful. These features have, however, a price to pay: the computational cost of solving the AZ model is larger than the corresponding solution of deterministic schemes. For example, the CPU time spent for the simulation of a calcium time course (and secretion dynamics) lasting 5 milliseconds for an AZ model with typical parameters given in [17], is about 3 minutes in a Intel Core 2 Duo of 2.26 GHz (2GB of RAM).

Another interesting feature of our scheme is the possibility of studying effects related to non-regular geometrical configurations of calcium channels and/or secretory vesicles: as can be seen in Figure 5, very different scenarios arise in the local calcium concentration maps when uniform random or clustered distributions of calcium channels are considered in the cell membrane. Geometrical effects related to secretion in presynaptic terminals are interesting since we have found that when Ca^{2+} channels are non-uniformly distributed, i.e. when they are grouped in a cluster, and the stimulus induces low Ca^{2+} influx in the active zone, there is a reduction of the apparent value of the kinetic cooperativity for secretion [17]. This microscopic organization, disposed by nature, is related to the developing changes suffered during maturation of the calyx of Held and of the whole auditory pathway in mammals [56].

3.2 Slow Ca^{2+} and secretion dynamics: the case of α-cells

Using the deterministic computational scheme, we have studied the relationship between Ca^{2+} oscillations and glucagon secretion in pancreatic α-cells exposed to low glucose levels. Pancreas has at least three types of exocytotic cells named α, β and δ grouped in areas called Islets of Langerhans. Among

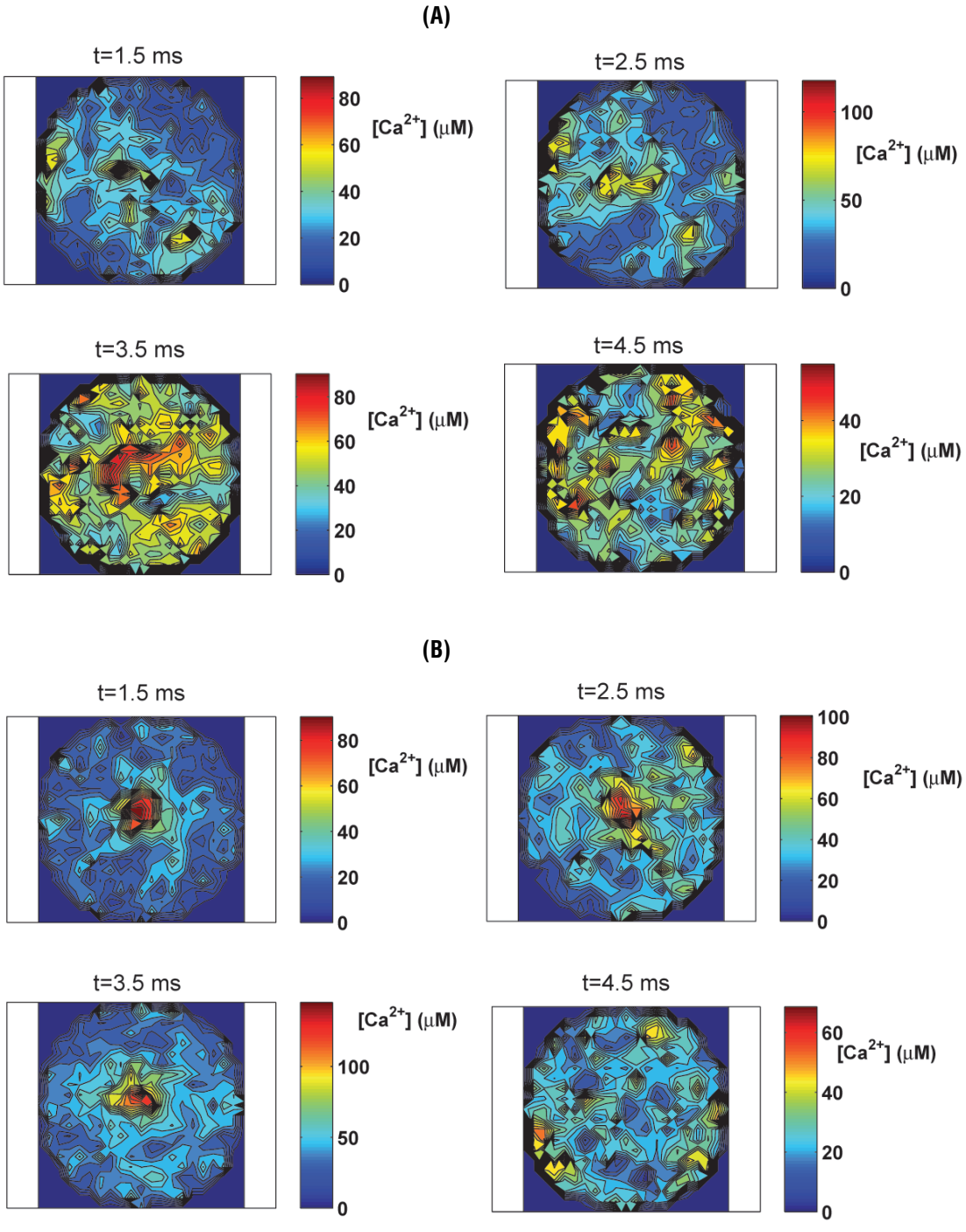

Figure 5 – Local calcium maps at four different times obtained for the calyx of Held model, considering a strong stimuli lasting 4 ms. (A) Five Ca^{2+} channels are randomly distributed in the active zone. (B) Five Ca^{2+} channels are clustered in the center of the active zone. Notice differences in the spatiotemporal Ca^{2+} distribution and concentration.

them, α and β cells are considered highly relevant because they release glucagon and insulin hormones, respectively, both involved in glucose regulation [39]. Glucagon is released by α-cells through a Ca^{2+}-dependent process when cells are exposed to low-glucose (below normal) levels [39], and during these periods, about 30% of total islet α-cells exhibit Ca^{2+} oscillations [41]. These oscillations are asynchronous and highly irregular [6, 7, 38, 53], in opposition to what happens in β-cells that all

undergo synchronized oscillations upon stimulation with high-glucose (above normal) levels [38].

Experimentally, glucagon secretion is induced by keeping a low glucose level for several minutes. Commonly, glucagon is measured as the accumulated quantity per islet per hour [53]. It has been observed that the secretory response takes some minutes to become stable [23]. Then, relevant conditions for a simulation are to take into account the behaviour of α-cells coming from the same islet, and to analyze it in a time scale in the order of minutes. However, the rates of Ca^{2+} binding and unbinding are 0.5 per micromolar of Ca^{2+} per second and 4 per second, respectively [1]. These values imply that if the intracellular Ca^{2+} concentration reaches one micromolar, the binding rate would be 0.5 per second. Then, the faster reaction (an unbinding reaction) may occur in one quarter of second; i.e., a time step below 250 milliseconds will be needed to run an accurate simulation of the kinetic scheme of secretion. Notice that this temporal resolution means that it will take 14,400 time steps to simulate one hour of secretion; this computing time should be added to the time needed to compute the associated Ca^{2+} dynamics. Moreover, the molecular mechanisms responsible for the highly irregular Ca^{2+} oscillations in pancreatic α-cells remains largely unknown. They would include the plasma-membrane Ca^{2+} channels, the sodium-calcium exchanger, the endoplasmic reticulum as well as the buffered diffusion of Ca^{2+}, but the way these phenomena interact with each other to create irregular small-amplitude, long-period Ca^{2+} oscillations is still unknown. Therefore, we decided to directly feed the simulation code with real Ca^{2+} data obtained from experiments (data provided by the group of Dr. Ivan Quesada from CIBERDEM – Insto. Bioingeniería, Elche, Spain).

In Figure 6 there are some examples of real α-cell Ca^{2+} oscillations that appear in response to controlled stimulation (low and high glucose levels). Notice the temporal scale associated to this cellular response which is in the order of minutes. These Ca^{2+} increases have been clearly associated to glucagon secretion [40], although there is no clear idea about the pathways followed by Ca^{2+} to activate exocytosis. Moreover, there are few experimental data about Ca^{2+} dynamics recorded simultaneously to exocytosis, and still fewer about secretion dynamics on an extended time interval.

Starting from the assumption of a standard secretory scheme (see Fig. 2 and Methods section), we have identified the most plausible way that α-cells could follow to manage secretion [20]. One important theoretical result is the prediction of the primary role played by the activation of the priming process by extracellular glucose, which has also been observed experimentally [2]. It appears indeed that by decreasing glucose, the cell both activates membrane secretory processes by increasing Ca^{2+}, but inhibits at the same time the refilling of granules at the membrane. One plausible explanation for this paradoxical observation is that it allows the cell to respond rapidly after a decrease in blood glucose, while keeping enough reserve granules to be able to provide enough glucagon during extended hypoglycemic periods. We have also found that, in contrast to observations in other cell types, there is no frequency encoding phenomenon in α-cells secretion, as there is clearly no correlation between the frequency of Ca^{2+} oscillations and the rate of secretion (Fig. 7). However, for a same average Ca^{2+} concentration, oscillating cells secrete more than non-oscillating ones [20]. Indeed, the majority of α-cells display higher secretion at low (0.5 mM) than at high (11 mM) glucose, with low glucose corresponding to the presence of Ca^{2+} oscillations.

The reason for this behavior is related to the strongly non-linear relationship between Ca^{2+} concentration and secretion (Fig. 8). This non-linearity arises because three Ca^{2+} ions need to bind to granules to trigger vesicle fusion and glucagon release. Because of this non-linearity, in the course of oscillations, the ratio between secretion levels reached at the top of the peak and at basal Ca^{2+} much exceeds the ratio between Ca^{2+} levels. This potency of Ca^{2+} oscillations to optimize physiological responses has been reported for other Ca^{2+}-induced responses, such as gene expression [11] or enzyme activation [14]. In contrast to frequency-encoded phenomena such as Ca^{2+}-calmodulin kinase II activation [13], this sensitization of secretion by oscillations can be achieved with irregular oscillations. Also, it does not require Ca^{2+} oscillations to be synchronized among the individual cells. Synchronized Ca^{2+} oscillations would lead to a pulsatile secretion of hormone, as it is the case for insulin secretion by pancreatic β-cells [25].

4 PERSPECTIVES

The development of different computational methods to study the Ca^{2+} dynamics leading to cellular secretion in different cell types can be considered a powerful approach to study subcellular mechanisms that underly the physiological functions observed in humans and other animals. Cellular models allow researchers to link macroscopic behaviour with those biological elements that may be studied using a model prediction [34].

In the present work we have discussed the implementation and applicability of two computational schemes intended to

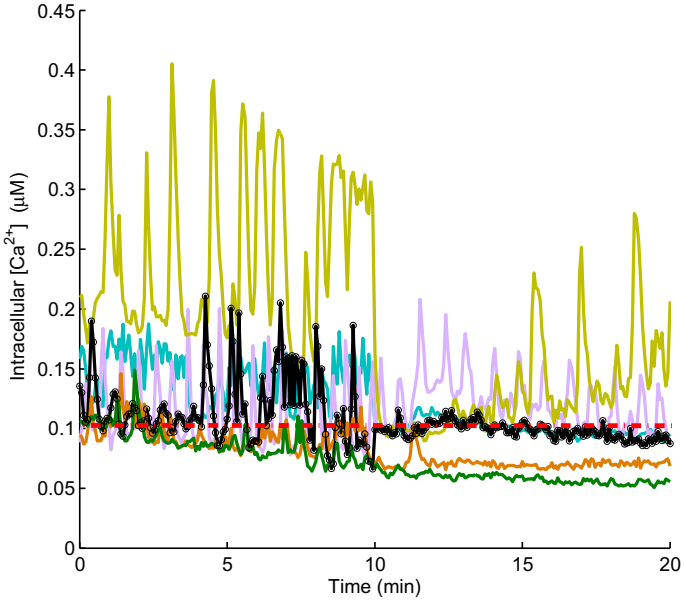

Figure 6 – Some spontaneous α-cell Ca^{2+} oscillations recorded when pancreatic islets are exposed to low glucose for 10 minutes, and then to high glucose for another 10 minutes. Remarked (in black) is the oscillation corresponding to the cell which secretes more than the others at low glucose (see black diamond in Fig. 8). The mean Ca^{2+} level of this cell is also indicated (red dashed line) to emphasize its oscillatory dynamics that crosses this level several times inducing more secretion, as discussed in the Results section. (Data recorded by the research group of Dr. Iván Quesada, Inst. Bioingeniería – CIBERDEM, Universidad Miguel Hernández, Spain.)

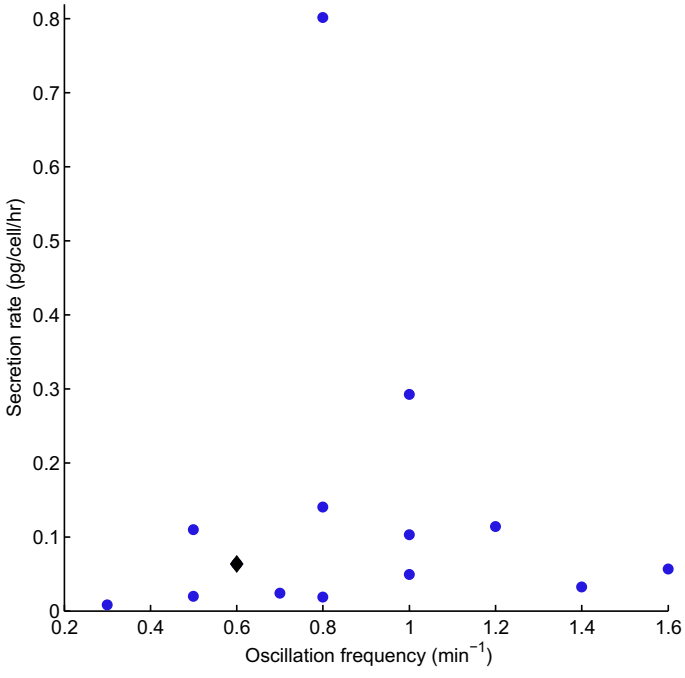

Figure 7 – Predicted individual secretion as a function of the frequency of individual α-cell oscillations taken from experimental data (shown in Fig. 6). The frequency – corresponding to a time average for a single cell – is defined as the number of times Ca^{2+} exceeds the mean value of the whole time series. Notice that there is no clear relationship between these variables. Remarked (black diamond) is the value for the cell which has the highest secretion rate at low glucose.

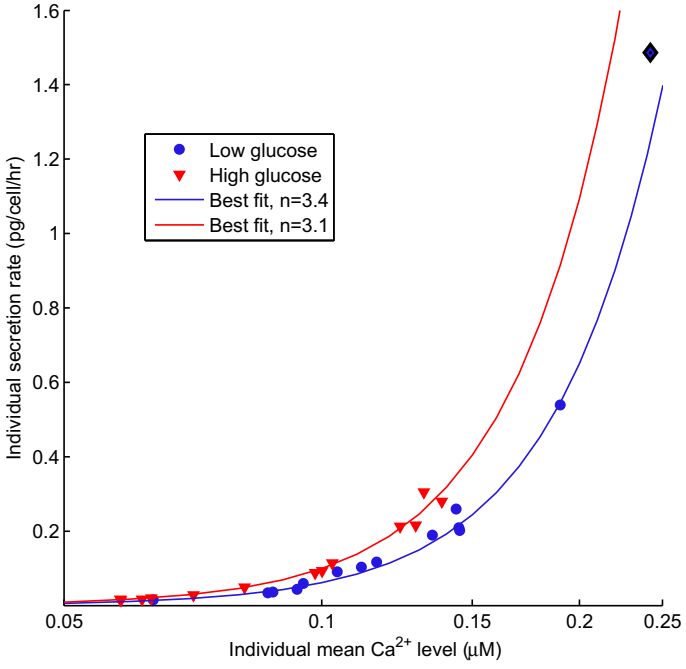

Figure 8 – Predicted individual secretion as a function of the mean Ca^{2+} level. The secretion rate predicted in simulations for each cell is plotted against the individual mean Ca^{2+} level. There is a clear third-order relationship between these variables. Remarked (black diamond) is the value for the cell which secretes much more than the rest of the group at low glucose.

study the dynamic behaviour of Ca^{2+} increases inside a cell, and its involvement in cellular secretion. Our mathematical models are based on original or published experimental data on Ca^{2+} and take into account the main sub-cellular mechanisms related to Ca^{2+} dynamics and secretion. The implementation was done in simple but adequate geometries to study local or average Ca^{2+} distribution in time and space. The application of the schemes has been reported for neurotransmitter and hormone secretion which are two useful cases to compare the reliability of using computational approaches to study the same process occurring in different time scales. These scales are related to the underlying spatial cell features associated with the Ca^{2+} signal, as well as with the kinetics of secretion.

In cells, when a Ca^{2+} channel opens in response to stimulation, a Ca^{2+} microdomain is formed around the channel. Inside this microdomain elevations of intracellular Ca^{2+} are steeper than in the cytosol, and these increments change very fast in synapses but not so fast in neuroendocrine cells [43]. In synaptic terminals a nanodomain is formed because of the small distance (tens of nanometers) between channel clusters and vesicles, whereas in neuroendocrine and endocrine cells the distance between these subcellular mechanisms is greater (hundreds of nanometers) leading to macrodomains. Nano- and microdomains have gained importance since they seem to be responsible for the fast component of secretion evoked by action potentials [36]. Therefore, in order to study Ca^{2+}-triggered secretion in both cell types, and even in endocrine cells, models and computational implementations must be able to deal with the actual spatial organization and the characteristic response times. For fast Ca^{2+} and secretion dynamics, geometries that allow non-uniform location of channels and vesicles are needed as well as an implementation with spatial resolutions around nanometers and temporal resolutions around microseconds [3]. In contrast, slow dynamics may be studied with more regular geometries that model the whole cell features. These geometries assume uniform distributions of channels and vesicles, and this is adequate since slow Ca^{2+} and secretion dynamics are less sensitive to local Ca^{2+} increases, but more sensitive to the spatiotemporal Ca^{2+} dynamics involving other Ca^{2+} controlling mechanisms [28]. Indeed, slow Ca^{2+} elevations associated to secretion are commonly induced by substances such as glucose or hormones, and these cellular processes seem to be modulated in long timescales [14, 40]. Then, models for slow Ca^{2+} and secretion dynamics could be implemented with a spatial resolution

around microns and a higher time step, which open the possibility to simulate longer periods in a reasonable computational time.

For the cell types studied in the present work, simulations were produced following experimental protocols. Each cell type was specific, with own values for size and characteristic times associated to the Ca^{2+} dynamics. Therefore, our basic modeling frameworks were suited to fit these specifications. Up to now, we have shared some common subcellular models, and we have used stochastic or deterministic solutions considering real cellular behaviour and features. However, we consider as a future goal to combine stochastic and deterministic approaches in a dynamic way [42] to improve our understanding of the real functional dynamics of biological processes.

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