Simulating intracellular calcium dynamics

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Calcium concentration in the myocytes is crucial in cardiac excitation-contraction coupling (the conversion of an electrical stimulus to a mechanical contraction of the heart cells). This part of the intricate process can be studied by simulating the calcium dynamics. In this project we consider a model that combines stochastic transitions among different states of channels and deterministic calcium dynamics and perform some computer simulations employing MATLAB. Then, we examine whether the model makes correspondence with empirical results taken from the Cellular Physiology Laboratory of the Cardiovascular Research Center (CSIC-ICCC).

Keywords: Calcium dynamics, cardiac myocytes, calcium

I. INTRODUCTION

The cardiac cycle is controlled by the heart's electrical system. When the cell membrane polarization is changed by the arrival of an electrical signal, a flux of Ca^{2+} ions goes into the cell. As a result, the Ca^{2+} concentration increases inside the cell, inducing cell contraction.

Studying this calcium dynamics can help to understand better the behavior of the heart and, as a result, the reasons why some cardiac diseases arise. For example, models similar to the one we studied are used to investigate arrhythmias, that consist in an abnormal rate of the heartbeat.

Cell contraction starts due to an increase in the concentration of intracellular calcium. Under basal conditions, most of the calcium inside the cell is located in the sarcoplasmatic reticulum (SR). In order to study how calcium moves in and out of the cell, and around the various areas of the myocyte, we focus on small spatial domains, known as calcium release units (CRU), whose structure is repeated along the cell. Each of them is a submicron scale junction where a few membrane voltage gated Ca channels (LCCs) are in close proximity with a cluster of 50 to 100 Ryanodine Receptor (RyR) channels. The former are situated across the cell membrane and connect the interior and the exterior of the cell. The latter are situated across the SR membrane and are sensitive to calcium. The opening probability of each of these channels is greater when the Ca concentration just outside the SR is high.

When an electrical pulse (action potential) arrives to a myocyte, it produces the LCC channels to open and let calcium ions cross the membrane and enter the cell from the extracellular medium. As a consequence, the local Ca concentration between the membrane and the SR increases enough to induce opening of RyRs. This way, part of the calcium that was confined inside the SR is released. Consequently, a spark is produced, i.e., a sudden rise in the local concentration of calcium.

This induces cell contraction because, when the free intracellular calcium concentration increases, calcium binds to the myofilament protein Troponin C and activates it. This activation starts the contraction process.

A cell is composed of about 10^4 of these junctions [2], and calcium can diffuse among them. When a local calcium release is produced it can induce new sparks in neighboring domains that can propagate across the cell and create a calcium wave.

There are also some other mechanisms that must be taken into account when studying calcium dynamics. After excitation of the muscular cells, relaxation must occur, so calcium concentration must decrease in order to let calcium dissociate from troponin. There are at least four well known pathways for transporting calcium out the cytosol [3], in our simulations we will consider Na-Ca exchangers and the SERCA pump.

Na-Ca exchangers are counter-transporter membrane proteins responsible for pumping calcium back to the extracellular medium. SERCA pumps calcium from the cytosol into the SR and tends to compensate the calcium released during a spark.

In this project we simulate the calcium dynamics inside the cell. By doing so, we are able to study the causes of:

- Spontaneous Calcium Release in a microdomain, that is, sparks produced when no electrical signal is sent to the membrane.
- During periodic simulation, gradual increase of the intracellular calcium concentration when the period of the pulse decreases.
- Spatial propagation of sparks, by making an extension of our simulation to a two dimensional network of microdomains.

Finally, we test the validity of our model and our simulation by contrasting its predictions with experimental data from the Cellular Physiology Laboratory of the Cardiovascular Research Center (CSIC-ICCC).

II. MODEL OF A CALCIUM RELEASE UNIT

We relayed on a model developed by Álvarez et. al. [1] for ventricular rabbit myocytes. The first part of our work is the simulation of one of the microdomains previously described. We will refer to it as a calcium release unit (CRU).

We considered five connected compartments, each with a different calcium concentration that is actualized at each step of the simulation (see figure 1): dyadic (c_d), junctional SR (c_{jSR}), subsarcolemma (c_s), cytosol (c_i) and sarcoplasmic reticulum (c_{SR}). We took the extracellular concentration to be constant.

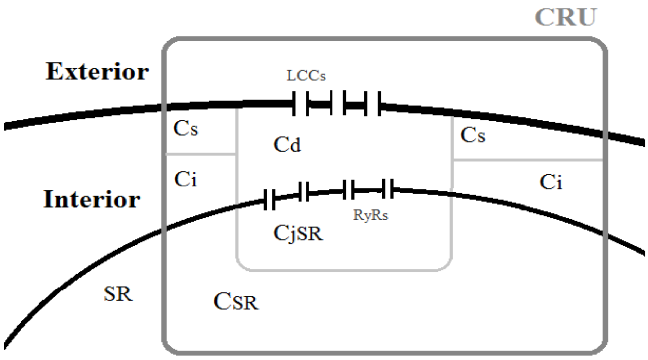

FIGURE 1. Scheme of the compartments of a CRU.

We gradually developed each of the mechanisms that take part on calcium dynamics and control the fluxes among the compartments. The first step was modeling the stochastic transitions among states of discrete channels. The flux of calcium across the membranes increases with the number of channels in the open state. We used the same transition rate schemes as in [1].

We considered a five state model for LCC channels, with two closed, two inactivated and one open state (see figure 2.a). The rate of each transition multiplied by the time step length determines the probability of transition during a time step. While the simulation is taking place, the transition rates change, depending on the potential of the membrane and on the concentration of dyadic calcium.

We will not specify here the expressions of all rates, but we highlight the major dependences. Any of the rates that directly control the transitions to the open state depend on the voltage. However, we said that the LCCs were voltage gated. This is because voltage increase the rates of transitions to states adjacent to the open state, resulting on a higher number of channels in these states and, consequently, in the open state. Some rates also depend on c_d. A rise on c_d increases the rates of transition to the inactivated state I_1.

For modeling stochastic dynamics of RyR channels we considered four possible states: two inactivated, one closed and one open (see figure 2.b). Now, any rate depends on the voltage, but the probability of opening highly depends on c_d. This fact leads to an increase on the number of open RyR channels when c_d slightly rises.

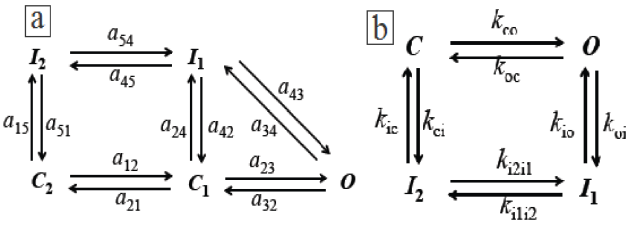

FIGURE 2. a) Five state model for an LCC channel. b) Four state model for a RyR channel.

In order to test the accuracy of the stochastic implementation of channel dynamics we made an statistical study repeating some simulations a high number of times and comparing the average behavior with the deterministic predictions. The agreement of both approaches was quite high, so we conclude that the implementation of the channels was right and we continued developing the model.

The next step was allowing the variation of the concentrations in each of the compartments of a CRU by introducing currents among them. The dynamics of all fluxes was deterministic (a part from the dependence on the number of open channels of the inward current from the exterior and the release current from the jSR towards the dyadic space).

At every time step, we updated these concentrations taking into account diffusive currents among compartments, currents due to dynamic attachment to Troponin C and other buffers and currents through channels and pumps that connect the extracellular medium, the cytosol and the sarcoplasmatic reticulum.

In order to update the concentrations, we expressed their time derivatives in terms of these currents. We also took into account the volume relations of adjacent compartments. At each time step we updated the concentrations considering a first order approximation of these derivatives.

For updating c_d and c_s, since the involved time constants were high compared to the time step length, we considered a rapid equilibrium approximation, i.e., the assumption that the time derivative of these concentrations is zero.

After developing the model for a single CRU, we connected several CRUs distributed in a two dimensional array. We fixed the spatial coordinates of each of them and incorporated diffusion among adjacent CRUs. We used a five-point laplacian for implementing it. The compartments for which this kind of diffusion was considered were the subsarcolema, the cytosol and the sarcoplasmatic reticulum. We imposed periodic boundary conditions so that we could simulate a surface surrounded by other CRUs of a cell.

Initially, we fixed the constants (transition rates, diffusion coefficients, flux multiplication constants, number of channels...) with the values specified in [1]. Even so, throughout the study we modified the values of several constants in order to test and study the response on the behavior of the system to some changes.

We discretized time taking different time steps so that they were lower than any other time constant in the problem we were solving.

III. RESULTS AND COMPARISON WITH EXPERIMENTAL DATA

Visualization of sparks

Before considering the spatial coupling among CRUs, we studied the emergence of sparks in a single CRU. The first result we obtained by doing a simulation was the visualization of the spark. We excited the membrane, that initially was maintained at -80 mV, with a single rectangular pulse signal of 20 mV and we payed attention to the time evolution of dyadic calcium. We can see the emergence of an spark in figure 3.

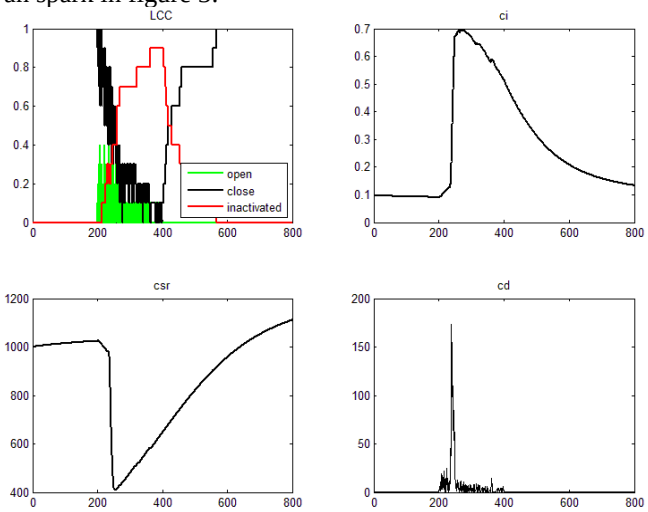

FIGURE 3. Time evolution in a CRU during an excitation. A rectangular pulse was applied between time 200 ms and 400 ms. In c_d we can observe a spark.

We visited Leif Hove-Madsen, researcher of the ICCC, and asked him for experimental data, in order to test the accuracy of our simulations. An empirical result from a human auricular myocyte, obtained by fluorescence confocal techniques, is given in figure 4. Even if the model we used is quite simple and differs in more than one point with his data sources, we can see a good agreement between the two pictures.

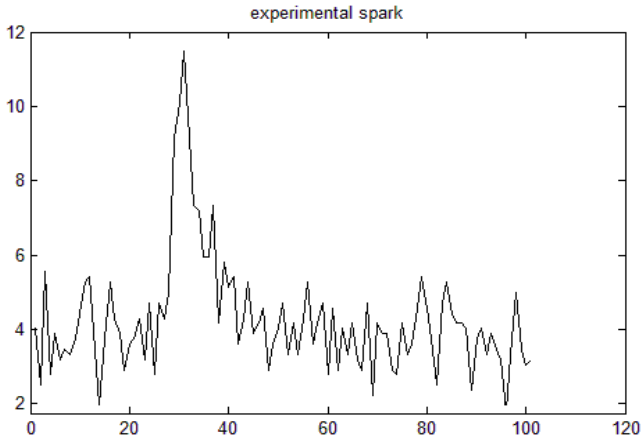

FIGURE 4. Calcium concentration in a human ventricular myocyte. Experimental. We observe a spark.

Moreover, we made this simulation several times and we observed two important things. Firstly, sometimes spontaneous sparks occurred when there was no potential applied, what we will further study. And secondly, we observed two sparks in a single pulse, which is possible, but the second spark is always much smaller than the first because many of the RyRs are calcium-inactivated.

Periodic series of pulses

Then, we focused our attention on the behavior of the system in a CRU under a periodic series of rectangular pulses. We excited the membrane with several pulses. During each of the pulses, we observed a behavior of c_d similar to the one we commented on the previous subsection. In each pulse, at least one spark was produced. Moreover, some sparks were seen while no potential was being applied.

Even if we didn't see any difference on c_d, c_s, c_{SR} and c_{jSR} among different pulses of the series, the calcium cytosolic concentration showed a different behavior. As shown in figure 5, c_i increases with time, something that is also observed in nature.

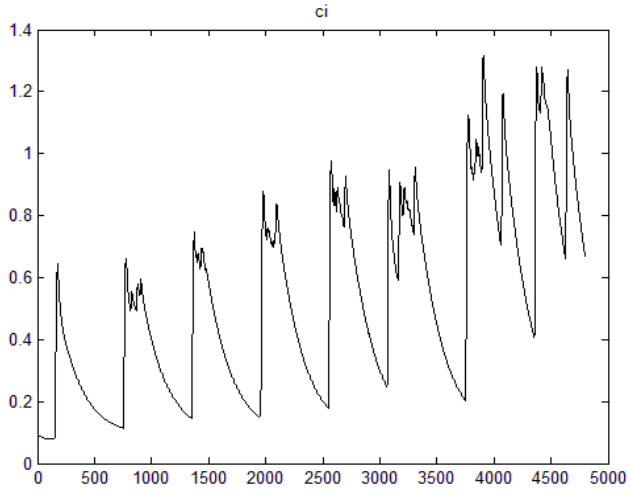

FIGURE 5. Time evolution of the c_i during 8 pulses. Maxima of the concentration increase in time.

We wondered whether c_i would reach a limit after a long series of pulses or not. We carried out some simulations in order to investigate this and we saw that, effectively, the increase of c_i accomplished an stabilization.

We also saw that the stabilization point depended on the period. For longer periods, both the stabilization time and the equilibrium reached were lower. As an example, we show figure 6 with a period of 600 ms and a pulse duration of 150 ms.

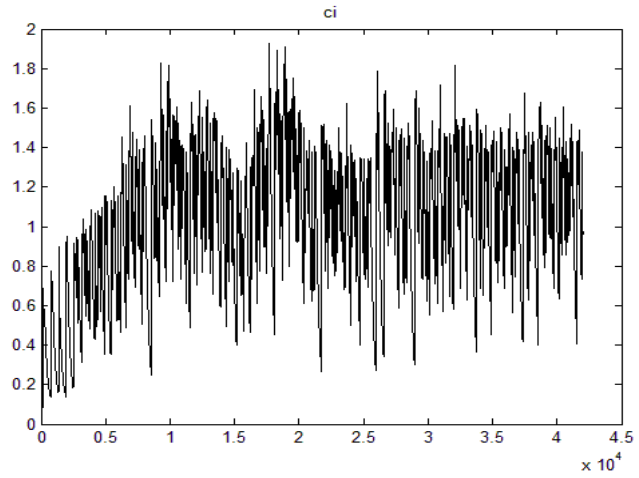

FIGURE 6. Time evolution of the cytosolic calcium concentration during 70 pulses. We observe that the calcium concentration stabilizes.

Propagation of calcium waves across an array of CRUs

Then, we focused our attention on the spatial diffusion among adjacent CRUs. When we applied the same potential to every CRU in the array, we saw a similar behavior in all of them. We checked that, even if the overall appearance of calcium dynamics was almost the same in different CRUs, sparks emerged at different moments.

In order to see if there existed any influence of a calcium release in one CRU on adjacent units, we decided to apply a potential only to some of them. In order to see the results on a more intuitive way, we created videos where the concentration of c_d in each CRU was showed simultaneously.

We did a simulation with an array of 26x26 CRUs, applying a potential pulse to a square of 3x3 CRUs in the middle. We saw that calcium propagated along the array.

Also, we did a simulation with an array of 50x50, not applying potential at all. What we expected was that an spontaneous spark was produced in a single CRU somewhere, and this spark was propagated along all the array. This was what we observed. In figure 7 we show some frame of the videos generated with the simulation.

These waves, produced by spontaneous sparks, are extremely important in the heart health, since they are produced locally but extend making global effects, they can produce serious disturbances in the cardiac functioning. The same results were obtained empirically as we checked in ICCC.

IV. DISCUSSION

Spontaneous sparks, one of the causes of several cardiac arrhythmias were predicted by our simulations. Therefore, simulations made using this model can be an initial point to investigate some pathological behaviors.

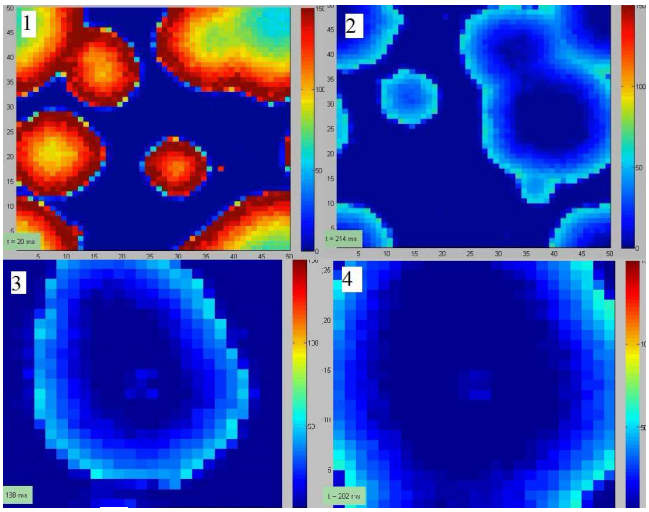

FIGURE 7. In 1 and 2 we see spontaneous waves. Whereas in 3 and 4 we observe the propagation of a wave produced in the nine central CRUs.

However, a local spark by itself is not a very important event, because it does not have a detectable effect on a macroscale. Real problems arise from abnormal contractions on a piece of tissue. So, not only it is important to study the origination of an spark in a microdomain of the cell, but also the expansion of this behavior to the whole cell, what gives rise to contraction. In our simulations we have also seen that spontaneous sparks can produce spontaneous travelling waves that propagate along the cell. The simple model we used didn't consider the contraction of the cell due to this calcium rise, but it is very useful as tool for investigating its causes. The waves we have seen produce spontaneous contractions of parts of the heart. A more detailed study in this field can lead us to understand the origin of arrhythmias and other cardiac diseases, as in [4], spontaneous release of Ca2+ in a myocyte constitutes a potential arrhythmogenic process in the heart.

Other conclusion we reached by doing some simulations was the dependence of the stabilization point of the intracellular concentration of calcium with the rate of beating of heart. As this rate increases, more calcium is accumulated inside the cell. This result could be useful when investigating the consequences of some diseases as tachycardia.

By developing this project we understood better the usefulness of simulating, as well as some simulation tools and models.

ACKNOWLEDGEMENTS

We thank Enric Álvarez Lacalle and Blas Echebarría Domínguez for their help.

We also thank Leif Hove-Madsen, leader of the Cellular Physiology Laboratory of the Cardiovascular Research Center (CSIC-ICCC) and the Sant Pau Hospital, for showing us his investigation on the topic.

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