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DEVELOPMENT AND APPLICATION OF ATRIAL MYOCYTE MODELS TO INVESTIGATE MECHANISMS THAT CONFER PATIENTS A HIGH RISK OF ATRIAL FIBRILLATION

Blas Echebarría Domínguez
Escola Politècnica Superior d'Edificació de Barcelona.
Universitat Politècnica de Catalunya

1. Overview of the project

Introduction

The main objective of the present project was to develop and adapt atrial human computational models to study mechanisms by which risk factors may give rise to atrial fibrillation. As a more specific goal, we planned to study the effect of a genetic variant, related to deficiency in the transcription factor PITX2, that affects calsequestrin-mediated calcium buffering, SERCA activity and RyR2 gating. The main hypothesis is that the mechanistic link of atrial fibrillation (AF) and given polymorphisms associated to it occurs through a modification of calcium homeostasis, leading to spontaneous calcium release events and triggered arrhythmias.
Thus, the aim of the project is to develop a multiscale *in silico* model of the atrial myocytes and, using this model, to test the ability of spontaneous release events to produce spontaneous arrhythmogenic (large) membrane depolarizations in human atrial myocytes and tissue. To explore the role of these mechanisms we used mathematical models of single and multicellular atrial myocytes. The single cell models allow investigation of the risk that a change in a single molecular mechanism imposes on myocyte function. In particular, we studied the results of loss of SK3 channel function, up-regulation of SERCA, calsequestrin (CSQ), mechanical stress, etc, and analyze their arrhythmogenic effects at the subcellular, cellular and tissue levels.

Goals

The general aim of this project is to identify molecular and cellular electrophysiological risk factors for atrial fibrillation that confer on patients a high risk for this arrhythmia. The specific objectives of the project are:

1. Set-up of a human atrial myocyte model (single cell and multicellular model) with detailed SR calcium handling (CSQ buffering, SERCA activity, RyR2 gating) and calcium-activated potassium channels.
2. Study the functional consequences of alterations in CSQ-mediated calcium buffering, SERCA activity and RyR2 gating.
3. Cross-talk between calcium handling and SK3-channels in the regulation of atrial electrical activity.
4. Modification of the atrial myocyte model to investigate how electro-mechanical

coupling modifies atrial electrical activity.

5. Introduction of subcellular structure in the atrial model. Study of the conditions for local calcium release events, calcium waves and membrane depolarizations.

Methodology

It is based on the development and simulation of mathematical models in the atria, ranging from the dynamics of calcium at the subcellular level to propagation of the action potential in sheets of tissue. Nowadays, there are several human atrial models available (for instance, Grandi model (Grandi et al., Circ Res, 2011) or Lugo model (Lugo et al., Am J Physiol, 2014), that consider average calcium concentrations in different compartments in the cell (cytosol, sarcoplasmic reticulum, etc). They are, thus, fast from a computational point of view, which makes them a very good tool to study functional consequences of modifications in ion currents or calcium handling regulation. However, they are deterministic models so they do not consider stochastic calcium release events, including, for instance, spontaneous calcium release giving rise to DADs or EADs. Besides, they do not account for concentration gradients within the cell and cannot describe local release events such as calcium sparks, or global events such as calcium waves. This can have an important effect when studying the homeostatic response of the cell. To overcome these deficiencies in the project we proposed to develop a detailed human calcium model that would take into account the distribution of RyR clusters in the cell and allow the study of subcellular calcium release events.

2. Results

1) Study the functional consequences of alterations in CSQ-mediated calcium buffering, SERCA activity and RyR2 gating

Using already published and validated human atrial models we ran simulations for a set of variations in these parameters, both individually and in combination. We then measured changes in systolic and diastolic cytosolic calcium, SR calcium concentrations (free and CSQ-bound), SR fractional release and action potential duration (APD). We found that a change in the maximal reuptake speed of SERCA has little effect by itself, except at fast pacing rates. An increase in the CSQ-buffer capacity increases diastolic

SR calcium concentration, as well as global calcium content, while an increase in RyR activity has the opposite effects. Besides its intrinsic interest to understand how variations in calcium handling regulatory factors may result in changes in calcium homeostasis and action potential shape and duration, we have focused on a specific case: lower expression of PITX2, which has been observed to result in reduced ICaL maximal conductance, upregulation of Casq2, Atp2a2 and increased RyR activity. Following the experimental results in Lozano-Velasco et al., Cardiovasc. Res, 2016, we took a 30% decrease in I_{CaL} maximal conductance and an upregulation of RyR activity, SERCA and CSQ. This results in lower levels of cytosolic and SR calcium concentrations, compared with WT, while the total SR concentration (free and bound to CSQ) is increased.

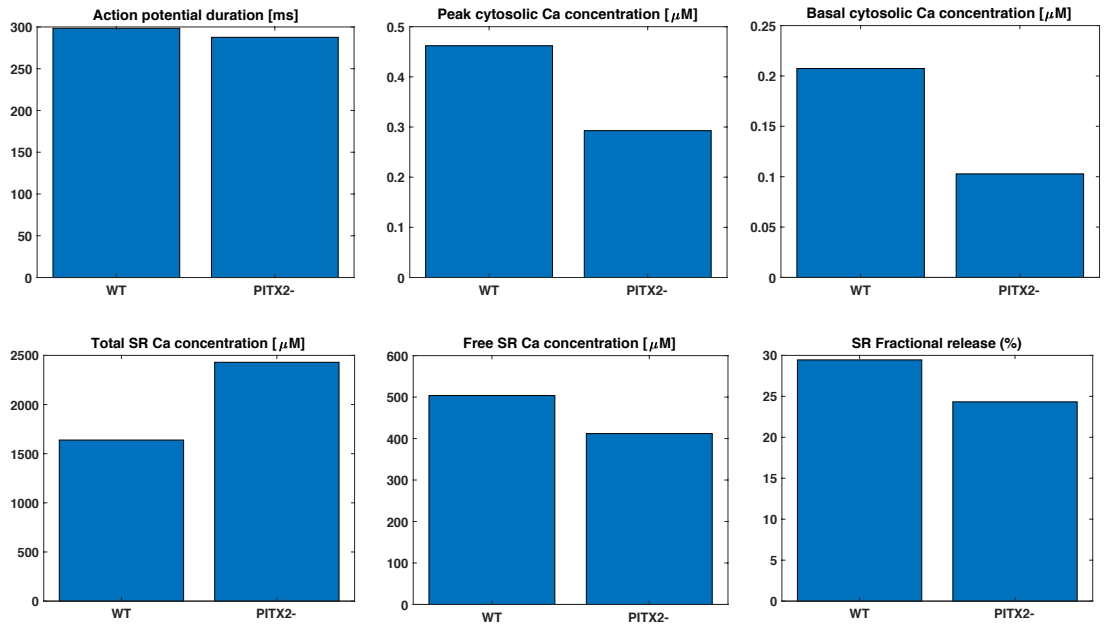

Figure: Comparison of the original Grandi model (WT) and the one modified to model PITX2 deficiency.

Then, we addressed the effect of these homeostatic changes on the appearance of calcium waves. For that, we produced simulations of the subcellular calcium model developed in this project with different levels of average calcium concentration and calsequestrin (CSQ) buffer. We observed that when the calcium load increases, the system starts to spontaneously show calcium waves. These waves persist in time with different shapes and durations, giving rise to a nearly periodic oscillation in the global calcium signal. Roughly, we observed one calcium wave per second. Waves are normally initiated at different sites each time but they appear systematically. Upon

reduction of the CSQ concentration, the oscillations appear at lower values of total average concentration, they have a higher frequency and the range of oscillations becomes broader.

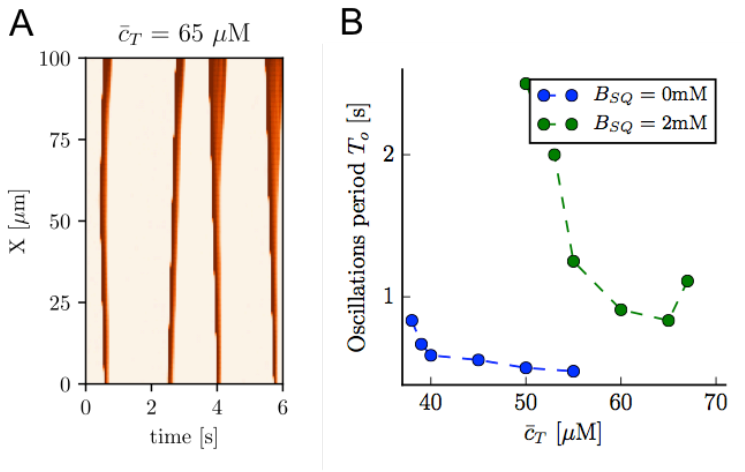

Figure: A. Line-scan showing the appearance of periodic calcium waves. B. Average period of oscillations at different values of the average calcium concentration c_T, for a concentration of CSQ of B_{SQ} = 2mM (green dots), and in the absence of CSQ (blue dots).

2) Cross-talk between calcium handling and SK3-channels in the regulation of atrial electrical activity

We performed a study of the different existing models for the SK3 channels and their effects for the form of the action potential. The current is modeled assuming an ohmic dependence on transmembrane voltage and an activation gate for the SK channels, whose steady state presents a sigmoidal dependence on intracellular calcium in the subsarcolemmal space. We have introduced this current in four different human atrial models and studied the resulting changes in APD, calcium homeostasis, etc. for different values of the parameters of the current, i.e. maximal conductance, activation Hill exponent, half activation concentration, and activation time. The calcium-activated potassium current, I_{KCa}, has the expected effect of reducing the action potential duration (APD), but this reduction is only appreciable at rates larger than 1Hz. Results obtained with the Lugo et al. model show a big dependence of the I_{KCa} current with its conductivity and the type of gate dynamics. A change in the calcium sensitivity of the channels, by changing the half activation Ca^{2+} constant, K_{KCa}, also shows two different regimes. For K_{KCa} above ~500 nM, lowering the sensitisation of the channel (increasing K_{KCa}) does not change much the value of the APD. On the other hand, decreasing it below this value produces a fast reduction of the APD. By introducing the I_{KCa} current in

other atrial models, like Nygren, Courtemanche and Grandi, we have observed that the effects of the current are qualitatively the same.

Then we studied whether the presence of a strong I_{KCa} current could avoid the pro-arrhythmic effects (EADs, DADs) of spontaneous calcium release (SCR) events. We included SCR in the simulations by allowing the opening of the ryanodine receptors at random times. We compared similar conditions with and without I_{KCa} current and observed that I_{KCa} currents did indeed reduce the effect of SCR on extradepolarizations. The presence of the I_{KCa} current prevents SCR induced afterdepolarizations in a narrow region of parameters but this seems to suffice to reduce the chance of induced depolarizations. However, it is not clear whether this protective effect stems from the current itself, which counteracts the effect of the sodium-calcium exchanger, or from the change in the resting potential, which presumably affects the refractory period.

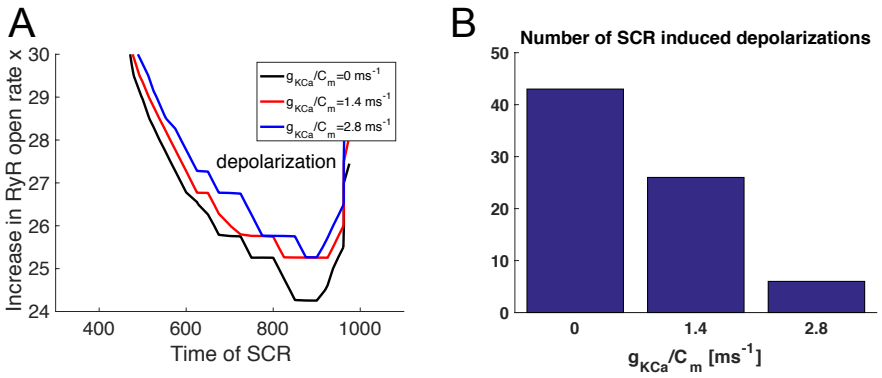

Figure: A. Instances of depolarization as a function of the timing and strength of SCR, for different values of the conductance of I_{KCa}. B. Number of depolarizations in a simulation with 1000 stimulations at a pacing period of T=1000ms, where a SCR event is produced at a randomly chosen time in each stimulation period.

3) Modification of the atrial myocyte model to investigate how electro-mechanical coupling modifies atrial electrical activity.

There is increasing evidence of the potential role of oxidative stress in the pathogenesis of AF. Excessive production of reactive oxygen species (ROS) is likely involved in the structural and electrical remodeling of the heart. Atrial stretch may increase the production of ROS, affecting the open probability of the RyR, and resulting in occurrences of spontaneous calcium release and calcium waves. We introduced this

effect by modifying the description of the RyR2, such that the transition among its different states depends on the amount of reactive oxygen species (ROS).

Furthermore, we consider that the RyR can be in oxidized states, with an increase probability of RyR opening. We performed simulations introducing this new model for RyR gating in a model of calcium cycling. We found that an increase in the level of ROS gives rise to spontaneous calcium oscillations over a wide range of parameters. Then, we introduced this description of the effect of ROS in the subcellular model developed in this project in order to study the effect of an increase in ROS production on the frequency of sparks and spontaneous calcium waves. In order to account for ROS production, diffusion and degradation, a few modifications were made to the model. ROS was assumed to be produced by NOX2 at a constant rate at the membrane. The diffusion process regarding ROS was set with the same diffusion constant as Ca^{2+} but also accounting for the degradation with a rate of $2 \cdot 10^{3} ms^{-1}$. We performed simulations with different magnitudes of ROS production. For a low, basal value of ROS production (j_{ROS} = 10^{-3}□M μm ms^{-1}) the observed number of sparks is low. For growing values of the production, a higher number of sparks is observed, which are also larger. From our results we found that the number of sparks shows a dependence on ROS concentration of the form: $N_{sparks}=a+b$ log[ROS]. Besides, when ROS production is enhanced two orders of magnitude over the low, basal value, calcium waves appear in the cell.

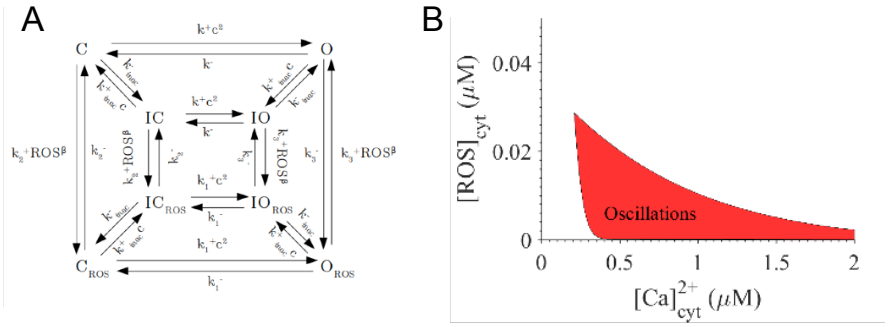

Figure: A. Eight-state model of the RyR, including oxidized states by ROS. The four central states are inactivated and the bottom four are the oxidized ones. There are two open states, O and O_{ROS}. B. Region of existence of oscillations as a function of cytosolic Ca^{2+} and ROS concentrations.

4) Introduction of subcellular structure in the atrial model. Study of the conditions for local calcium release events, calcium waves and membrane depolarizations

We developed a detailed model of the atrial cell, with a discretization at the submicron scale, that allowed us to also study the effect of changes in the RyR clusters size and

distribution during AF, according to available experimental data at this scale. For that, we proceeded in several steps, defining:

1. The internal structure of the cell. We introduced the transverse and axial tubule (TAT) network and studied the effect of variations in the amount of both t-tubules (TT) and axial tubules (AT).
2. The size and form of the RyR clusters. We have incorporated an algorithm into the model to construct heterogeneous distributions of RyR cluster sizes.
3. The RyR gating dynamics. We improved existing models of RyR gating by identifying the mechanisms of RyR inactivation.

Then, we modified the subcellular calcium model to account for pathological conditions, such as homeostatic changes or oxidative stress, explained in the previous points. We have also had access to experimental data from the group of Leif Hove-Madsen, at Institut de Investigaciones Biomedicas Barcelona, CSIC, Barcelona, regarding the subcellular distribution of CSQ levels and RyR phosphorylation in atrial myocytes from patients with AF. We used the subcellular calcium model with some modifications to include modulation of RyR opening by the CSQ level and RyR2 phosphorylation observed in the experiments. Mathematical modeling taking into account this spatial distribution of CSQ, RyR2 and phosphorylation shows a differential increase in subsarcolemmal calcium release events that favor electrogenic Na-Ca exchange and consequently a higher incidence and amplitude of transient inward currents, which translates into a higher incidence of spontaneous DADs or APs that can break normal electrical activity.

3. Relevance and potential future applications

The results of this project will have an impact on the knowledge of the underlying mechanisms of cardiac arrhythmias, and more specifically, atrial fibrillation (AF). One of the main questions about AF is how the accompanying atrial remodeling affects the recurrence of episodes of AF until it becomes permanent. The new human atrial model developed in the present project can help to predict how structural and molecular alterations at subcellular level may affect calcium homeostasis and spontaneous

electrical activity. In particular, in this project we studied the impact of several elements that are modified during remodeling (calcium-activated potassium current, reactive oxidative species, changes in phosphorylation and calsequestrin buffer concentration, etc) and how this affects the number of spontaneous calcium release events, which may result in delayed afterdepolarizations that can break normal electrical activity. Thus, the models we have developed can be considered as an *in silico* models, which can be used, in a first stage, to understand different factors that affect AF, or to test the effects of new pharmacological treatments.

4. Scientific production

Publications

Cantalapiedra IR, Alvarez-Lacalle E, Peñaranda A, Echebarria B
Minimal model for calcium alternans due to SR release refractoriness
Chaos: An Interdisciplinary Journal of Nonlinear Science 27, 093928 (2017)

Peñaranda A, Cantalapiedra IR, Alvarez-Lacalle E, Echebarria B
Effects of Small Conductance Calcium Activated Potassium Channels in Cardiac Myocytes
Computing in Cardiology 44 (2017).

Marchena M, Echebarria B
Computational model of calcium signaling in cardiac atrial cells at the submicron scale
Frontiers in physiology 9, 1760 (2018)

Peñaranda A, Cantalapiedra IR, Alvarez-Lacalle E, Echebarria B
Electrophysiological effects of small conductance Ca2+-activated K+ channels in atrial myocytes
Nonlinear Dynamics in Biological Systems, J. Carballido-Landeira, B. Escribano (eds.), SEMA SIMAI Springer Series 7 (2019)

Romero L, Alvarez-Lacalle E, Shiferaw Y
Stochastic coupled map model of subcellular calcium cycling in cardiac cells

Chaos: An Interdisciplinary Journal of Nonlinear Science 29, 023125 (2019)

Conesa D, Echebarria B, Peñaranda A, Cantalapiedra IR, Shiferaw Y, Alvarez-Lacalle E
Two-variable nullcline analysis of ionic general equilibrium predicts calcium homeostasis in ventricular myocytes (submitted)

Marchena M, Echebarria B, Shiferaw Y, Alvarez-Lacalle E
Buffering and total calcium levels determine the presence of oscillatory regimes in cardiac cells (submitted).

Marchena M, Echebarria B
Influence of t-tubular network on the characteristics of calcium transients in cardiac myocytes (submitted)

Wei J, Belke D, Zhong X, Sun B, Guo W, Yao J, Wang R, Vallmitjana A, Benitez R, Hove-Madsen L, Alvarez-Lacalle E, Echebarria B, Chen SRW
Ca^{2+}-Calmodulin Dependent Inactivation of Cardiac Ryanodine Receptor Underlies Ca^{2+} Alternans in Intact Hearts (submitted)

Besides, 10 communications have been presented in conferences and workshops.

PhD Thesis and Student training

Miquel Marchena will shortly present his PhD thesis centered in the studies of the present project. Miquel Marchena was hired with the grant granted by Fundació La Marató de TV3.

Besides, three master final projects: Miquel Marchena (2016), Nikolina Krizanec (2017), David Conesa (2019) and two degree final projects: David Conesa (2018), Miquel Bosch (2019), have been supervised, related to this project.