Calcium Alternans Produced by Increased Sarcoplasmic Reticulum Refractoriness

I R Cantalapiedra, C A Lugo, A Peñaranda, B Echebarria

Universitat Politècnica de Catalunya, Barcelona, Spain

Abstract

Despite the important role of electro-mechanical alternans in cardiac arrhythmogenesis, its molecular origin is not well understood. In this paper we want to study alternans that can be associated with alternations in the cytosolic calcium transient. To this end we study particularly, how a malfunction of Ryanodine Receptor (RyR) recovery time from inactivation may induce beat-to-beat alternations in intracellular calcium concentration and how this could affect the stimulus propagation in inhomogeneous tissue, whose cells exhibit different calcium dynamics.

1.   Introduction

Cardiac arrhythmias are often associated with irregularities in intracellular calcium dynamics, which is coupled with the transmembrane voltage. A prominent example of this is the presence of calcium alternans, a beat-to-beat alternation in the level of intracellular calcium concentration [1]. Numerous experimental studies have shown that isolated cardiac cells, when paced rapidly or pharmacologically stressed, exhibit a beat-to-beat alternation in both the action potential duration (APD) and the intracellular Ca transient [2-4]. The mechanism responsible for this irregular rhythm is not well understood. Alternans at the single cell level can be caused by unstable membrane voltage (V) dynamics linked to steep APD-restitution, or unstable intracellular calcium (Ca^{2+}). The existence of a dynamical instability of Ca cycling independent of V dynamics has been clearly demonstrated by experiments [5,6], where alternans of intracellular Ca^{2+} can occur even though the membrane V is periodically clamped.

A possible explanation has been given in terms of fluctuations in the Ca^{2+} content of the Sarcoplasmic Reticulum (SR), at fast pacing rates, in which the Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase (SERCA) does not have enough time to refill completely the SR before the next excitation. Coupled with a steep SR calcium load - calcium release relationship, this has been shown to give rise to sustained alternans. However, alternans has also been observed in experiments in which the SR content remains constant in successive beats [7]. Thus, it has been hypothesized that other mechanisms, related with calcium release restitution, must underlie the changes in cytosolic calcium dynamics. We investigate calcium alternans in a situation when the SERCA pump is able to rapidly eliminate calcium from the cytoplasm and fully recover the same SR calcium level before the next voltage activation.

We show in this work that calcium alternans appears under these circumstances if the recovery from inactivation of the Ryanodine Receptors (RyR) malfunctions and becomes too slow. This agrees with post-rest potentiation experiments that show that several seconds are needed to recover full SR calcium depletion [7]. We then analyze the arrhythmogenic implications of our results, by performing simulations of action potential propagation in tissue, using a model for an atrial myocyte [8] with modified calcium dynamics. We describe in detail how the presence of alternans affects the stimulus propagation in inhomogeneous tissue in which cells with different calcium dynamics are present. Depending on the size and spatial distribution of the region with alternans, they produce local conduction block and reentry due to dispersion in action potential duration [9]. We analyze different distributions, and the minimum size that gives rise to conduction block.

2.   Methods

We used a monodomain tissue model representing a 2D sheet of human atria myocytes with isotropic diffusion, a diffusion coefficient of D=1.00 cm^2 s^{-1}, and a specific capacitance of C_m=1 µF cm^{-2}. This model was solved using an explicit finite difference scheme with a space step of dx=0.025 cm, and time step dt=0.01 ms. The 2D simulations were performed in a rectangular region of dimensions L_x=2.25 cm, L_y=3 cm. No-flux boundary conditions were imposed at each edge. The model by Nygren *et al* [8] was modified [10] in order to reproduce calcium alternans at high pacing rhythms, as has been observed in experiments [4]. The modified model [10] includes three different compartments (Fig.

1A): i) a dyadic (junctional) space with the presence of LCC and RyR channels, ii) a subsarcolemmal space where the other transmembrane currents act, and iii) the bulk cytosolic compartment where the calcium concentration transient is caused by diffusion from the subsarcolemma and luminal calcium efflux from the SR through the RyR channels, as in [11]. For the RyR dynamics we consider a markovian four state model based on the one in Ref. [12] (see Figure 1B), with the parameters used in Ref. [13]. The SR Ca release includes inactivation/adaptation and SR Ca load dependence of activation and inactivation. The model reproduces the nonlinear dependence of gain and fractional SR Ca release upon SR Ca load. At fast pacing it presents alternans, due to slow recovery from inactivation of the RyR.
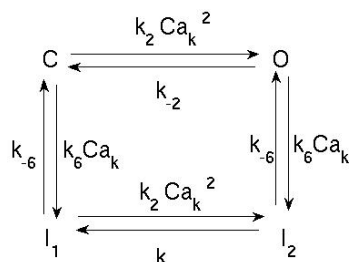
A)
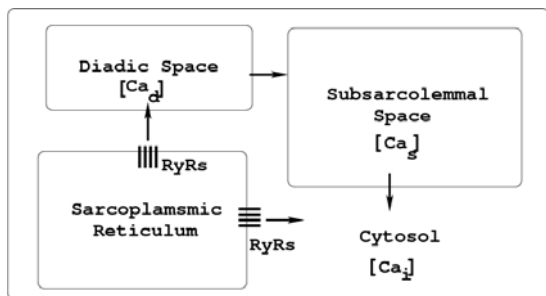
B)

Figure 1: A) Sketch of the different compartments employed by the cell model. B) Diagram of the Markov model for the state of the RyR. The states I_1, I_2, C, O, are similar to those employed and discussed in other models [12,13]. The dynamics of activation (opening), inactivation and recovery of the RyR is given by the rates k_2, k_{-2}, and k_{-6}. The recovery time is defined as the inverse of the recovery rate $t_{REC}=1/k_{-6}$. The subindex k in Ca refers to the dyadic or bulk cytosolic space.

The model is simulated first for a single cell, to fit the original intracellular calcium values given by Nygren *et al* [8]. Once the single cell model matched the results in [8], but exhibiting also alternant behaviour, we explored the effect on voltage propagation in tissue, due to the alternant-type cells "cluster" size, alongside the

conditions under which full or partial signal blocking could occur.

First, in order to investigate the conditions under which conduction block occurs, we divided the domain into three regions of two different types (see Fig. 3A). Type I region is populated with cells of regular behaviour, separated by a strip of width h perpendicular to the propagation direction of the AP waves (Fig 3A), and populated with cells exhibiting alternans (type II cells). By varying the width h of the strip we determined the critical size for which the system undergoes a transition from a full blocking regime to a stable local 2:1 block regime.

Then, in order to establish the effect of blocking in the formation of arrhythmias, different shaped areas of type II cells were also tested.

Finally, numerical ECG's were obtained using the "dipole" formula [14], ECG= $\int(D\Delta V \cdot r/r^2)dx$, where $r=r_{tissue}-r_p$, and r_p denotes the point at which the ECG is calculated.

3. Results

In Fig. 2 we show typical action potentials and calcium transients for type I and type II cells. From there it can be seen that a modification in the refractoriness of the RyR in type II cells results in electromechanical alternans.
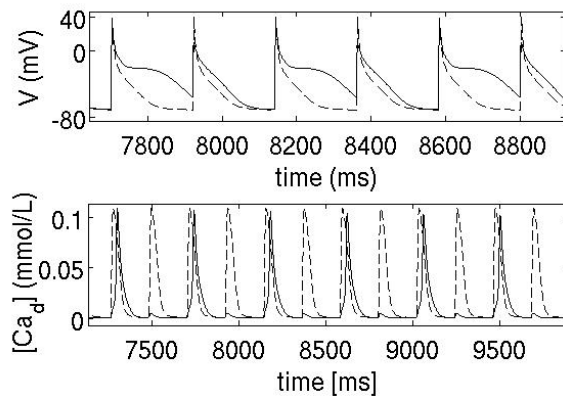

Figure 2: Single cell action potentials and calcium concentration in the dyadic space for two values of the refractoriness parameter $t_{REC}=200$ ms (type I cell) and 750 ms (type II cell), giving rise to a regular calcium dynamics (dashed line) and alternans (solid line). The stimulus period was set to $T_S= 220$ ms.

Distributing cells as shown in Fig. 3A, we were able to determine the minimum size h of the region with alternating cells that results in conduction block. In these simulations the tissue is stimulated from the upper side, at a pacing period of $T_S=220$ ms. As the AP wave

propagates through the area that presents alternans, the duration of the AP changes. In particular, after a long AP in this region, the recovery time for the following wave becomes smaller, resulting in conduction delay and eventually conduction block. When this zone is small, diffusive effects smear out the gradient in transmembrane voltage, and the waves propagate through it. As the size of zone II is increased, gradients of repolarization develop, and the waves following a long calcium transient start to be delayed, up to a point beyond which conduction block is produced (Fig. 3B). In our simulations, for h=1.5 cm there is an irregular behaviour, with intermittent conduction blocks, while for h=2.0cm there is a 2:1 blockade. We have also computed the electro cardiogram (ECG) corresponding to this situation (Fig. 3B), clearly showing the transition from normal stimulus propagation, to irregular blockade and to 2:1 block. Although not shown here, we have tested that this minimum size depends also on the alternans region geometry and its position with respect to the stimulation site [10].
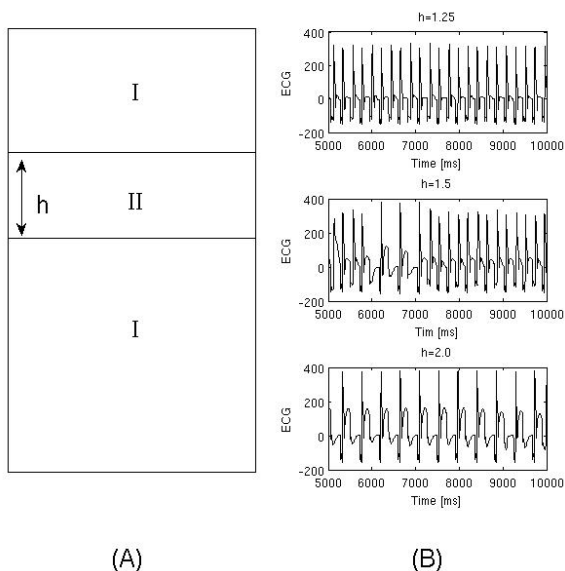

(A)   (B)

Figure 3: A) Simulation geometry, showing the different regions, with and without alternans (respectively, zones II and I). B) Virtual ECGs corresponding to simulations with a central region of width h=1.25cm, 1.5cm, and 2.0 cm. The stimulus period T_S =220 ms and recording site r_P =(1.125, 1.5, 0.025) cm.

Once we know the typical size of the region with alternans that produces conduction block, we have studied the case of a tissue paced from one corner, that presents a circular region of these characteristics (see Fig. 4). As shown, the ensuing dispersion of repolarization is enough to produce localized block and the induction of reentry. This is also evident in the corresponding ECG in Fig. 4, for a case in which reentry disappeared spontaneously.
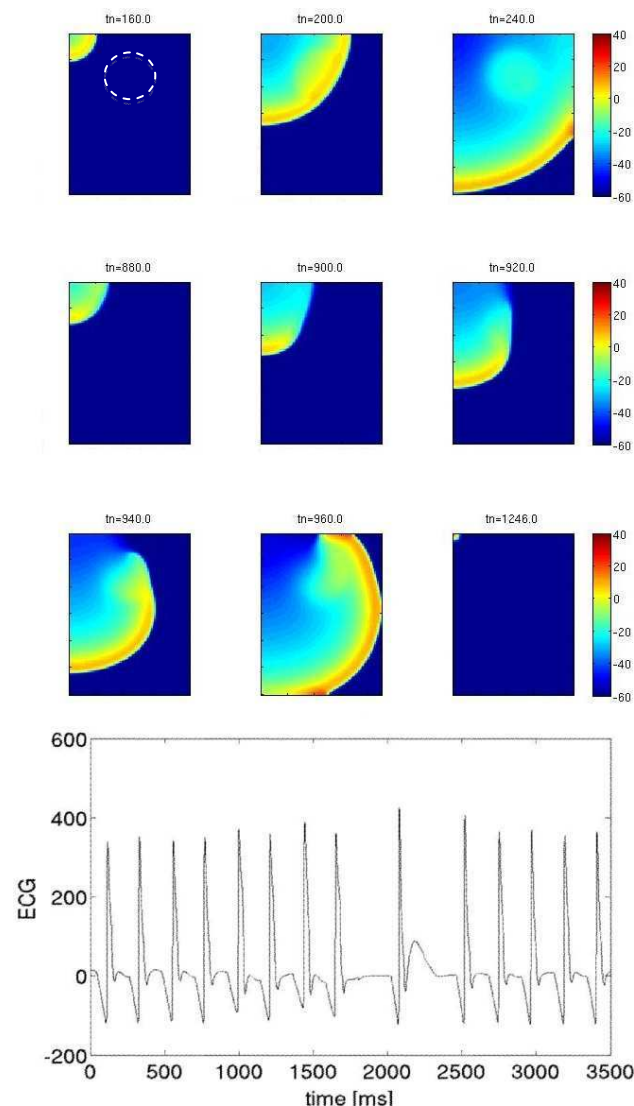

Figure 4: Wave propagation in tissue with a heterogeneous distribution of RyR refractoriness, such that alternans are develop in a circular region of side R=0.5cm (dashed line). As the waves go pass this region, they get delayed, until at some point, localized conduction block is produced. In the lower panel we show the corresponding virtual ECG.

4. Discussions and conclusions

There is increasing evidence that anomalies in the RyR dynamics can result in a proarrhythmic substrate. One of the ways this occurs is through the induction of calcium

alternans that, due to the action of the sodium-calcium exchanger, change the duration of the action potential. Once they appear, AP alternans can be concordant (with the AP at a certain beat being either long or short, but homogeneous in all tissue) or discordant, with the formation of defect lines that separate regions oscillating out of phase. It is, in fact, close to these defect lines that strong dispersion of repolarization can be created, resulting in localized block and reentry. In AP alternans driven by an instability in voltage (for instance, due to steep AP restitution), the origin of discordant alternans can be traced back to a dynamic instability related with conduction velocity restitution [15].

In this paper we have considered an alternative mechanism that can create dispersion of repolarization (with its subsequent proarrhythmic effects): a heterogeneous distribution in the dynamical characteristics that regulate the appearance of alternans in the dynamics of intracellular calcium. In particular, we have considered the proarrythmic effect of a spatial heterogeneity in the refractoriness of the RyR. We have considered first the idealized situation of an abrupt change in RyR refractoriness in a region of tissue, and confirmed that this results in a large dispersion of refractoriness in the border region, that gives rise to conduction block as its width is increased. Depending on the size of the alternans region, there maybe delayed conduction (for small sizes), localized block at every other beat (for large sizes), or a complex behaviour with blocks occurring at intermittent intervals (for intermediate sizes). Then, we have shown that this can result naturally in reentry, considering a circular region with intracellular calcium alternans in an otherwise normal tissue.

Acknowledgements

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Address for correspondence.

Inma R. Cantalapiedra.
Departament de Física Aplicada. Universitat Politècnica de Catalunya, Av. Dr. Marañon 50 (EPSEB) E-08028, Barcelona, Spain.
inma@fa.upc.edu.