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Nonlinearities due to Refractoriness in SR Ca Release

A Peñaranda, E Alvarez-Lacalle, IR Cantalapiedra, B Echebarria

Departament de Física Aplicada, Universitat Politècnica de Catalunya-BarcelonaTech, Barcelona, Spain

Abstract

Calcium alternans is a pro-arrhytmic cardiac dysfunction related to beat-to-beat changes in the amplitude of intracellular calcium transient, that typically occurs at rapid pacing rates. Although oscillations in sarcoplasmic reticulum (SR) content have been related with calcium alternans, the experimental appearance of alternans without these oscillations suggests that another mechanism related with refractoriness of SR calcium release might be key, at least, under certain conditions. We investigate how RyR2 refractoriness modulates calcium handling on a beat-to-beat basis using a numerical rabbit cardiomyocyte model. We find that a slow recovery from inactivation of the RyR2 might be crucial. On one hand, a steep relation between sarcoplasmic reticulum (SR) load and calcium release makes regular calcium cycling unstable at high SR calcium load and/or fast pacing rates, in agreement with previous explanation when RyR2 inactivation is not important. On the other hand, we show that calcium release can also depend strongly on the number of RyR2 ready to open if an important number of RyR2s inactivate after the release. This gives rise to a steep nonlinear relation between the calcium release and the level of recovered RyR2, so that a small change in the later produces big changes in calcium release. A conclusion of this result is that RyR2 refractoriness can be the main nonlinearity behind alternans even when alternation in SR-Ca load is present.

1. Introduction

Cardiac alternans is a disturbance in the normal rhythm of the heart characterized by oscillations in the action potential duration (APD). Clinically it can be recognized as a beat-to-beat alternation in the magnitude of the electrocardiographic T-wave and it has been identified as a risk factor for cardiac arrhythmias. Earliest explanations of this irregular rhythm attributed it to an instability of the cell membrane potential when the APD restitution curve (dependence of the APD with the time elapsed between the end of the previous action potential and the beginning of the current one) presented a steep slope. This results in a nonlinear feedback that amplifies small beat-to-beat differences in APD. The persistence of alternans in pacing protocols with periodic action potentials waveforms [1] suggested an alternative mechanism, i.e., that this rhythm could be produced by beat-to-beat changes in the intracellular calcium response. The originating mechanism must be related to a steep nonlinear relation between calcium release at a given beat and the state of certain variable. In this regard, several experiments focused their attention in the calcium content of the sarcoplasmic reticulum (SR) [2]. The prevailing explanation is based on a steep relation between calcium release and SR load that makes regular calcium cycling unstable at fast pacing rates [3,4]. However, cytosolic calcium alternans has also been found without concurrent appreciable changes in SR calcium content [5]. This suggests that other variables, such as SR-Ca release refractoriness, can play a role in cytosolic calcium alternans [6,7]. Refractoriness could be due to a slow refilling of the SR, i.e., a slow SERCA pump, or to a slow recovery of the ryanodine receptor (RyR2). In fact, recent experiments in atrial myocyes by Shkryl *et al* [8] confirmed the existence of calcium alternans without SR content oscillations and related its appearance with refractoriness of calcium release through RyR2.

In this work, we use a numerical ventricular myocyte model to study the SR calcium fractional release (defined as the percentage of the calcium content of the SR that is released in a stimulation) as a function of the SR calcium load and the number of recovered RyR2s. We show that, although there is often a strong nonlinear dependence on SR-Ca load, under certain conditions it can also depend on the number of RyR2s ready to open, i.e., on the RyR2s recovered from inactivation. This gives rise to a steep nonlinear relation, so that the quantity of calcium released is very sensitive to small changes in the number of recovered RyR2s. Then, alternans appears as an instability when the recovery time increases. This leads to an alternation mechanism that does not depend mainly on SR-Ca load but on refractoriness of the SR-Ca release related with the lack of recovery from inactivation. Depending on the type of dysfunctions or pathologies one or the other mechanism may have prevalence as precursor

of alternans.

2. Materials and methods

In our study we used a rabbit ventricular myocyte based on the model described by Shannon *et al.* [9]. The original model does not reproduce neither calcium alternans nor the post-rest potentiation described by Picht *et al.* [5], so we made some changes in the parameters in order to account for these experimental results [10]. In this sense, the same formal equations as in the model by Shannon et al. were used, but differences in the values of some parameters related with the RyR2 kinetics were introduced. A schematic representation of dynamics of gating of RyR2 channels is shown in Figure 1.

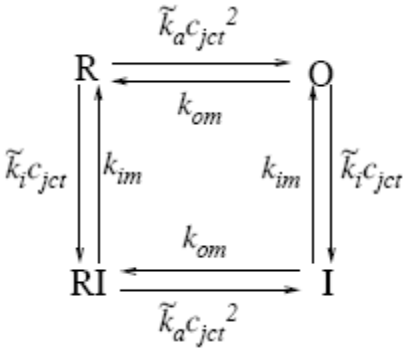

Figure 1. Representation of RyR2 gating dynamics**.** The four markovian states of RyR2 are O (open), I and RI (inactivated) and R (recovered). Respective rates for transition between states are represented. The recovery time is defined a $1/k_{im}$.

In order to obtain the variation of calcium release with the SR load and/or RyR2 recovered, the following protocol was used: we paced the cell until it reached steady state at different values of activation and inactivation of the RyR2. Then, for the variation as a function of SR-Ca load, during one beat we dynamically moved the SR-Ca load to a different pre-systolic value by changing the SERCA strength. Upon returning SERCA to normal behavior, we measured the fractional release in the next beat. Repeating this scheme for different pre-systolic values we obtained the fractional release curve as a function of SR-Ca load under a given working condition. Given our interest on the influence of RyR2 refractoriness in the generation of calcium alternans we also computed the fractional release as a function of the level of recovered RyR2 receptors. This curve was now obtained maintaining SERCA unchanged but with the recovery dynamics of the RyR2 accelerated during one beat after the steady behavior had been achieved.

Thus, we compute the fractional release as a function of either SR calcium load or number of recovered RyR2s, dynamically changing the starting value of these variables. Notice that in principle we could dynamically change both variables at the same time. However, we prefer to present both curves independently for clarity. It should be clear, though, that the fractional release curve as a function of SR-Ca load is computed with a given value of recovered RyR2, which is given by the steady state obtained with the specific activation and inactivation rates. Similarly, the fractional release curves as a function of RyR2 are obtained with the steady-state value of the SR-Ca load. When alternans are present, there are two possible working conditions, depending on whether we take the high or low calcium transient. Therefore, we must compute and compare two fractional release curves for each variable in the same manner as before, for the two alternating working conditions.

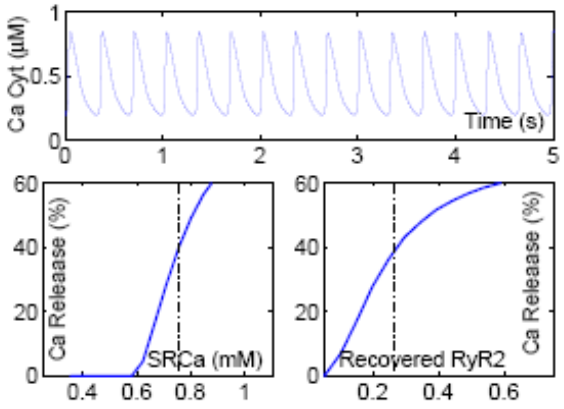

Figure 2. Fractional release for non-alternating calcium dynamics. Top) Intracellular calcium for the model by Shannon *et al.* with activation and inactivation reduced by 30 % of the values in the original model. No alternation is present. Bottom) Fractional release computed using a dynamic change of the variables as a function of SR-Ca load (left) and fraction of recovered RyR2s (right). At a given beat, the values of SR Ca load or RyR2 are modified so as to change its pre-systolic values, as explained in the methods section. The vertical lines indicate the pre-systolic values for this under non-altered conditions

3. Results

The original ventricular myocyte model by Shannon *et al* does not give rise to alternans at any pacing rate. To obtain the alternans observed experimentally it is necessary to change activation, inactivation rates and/or recovery from inactivation. In Figure 2 we show the calcium transient for modified parameters that do not produce calcium alternans at a frequency of 3Hz. We also present the fractional release, as a function of the SR calcium content and the percentage of recovered RyR2s, where we indicate with a vertical line the steady-state

working condition at pre-systolic values. Although the behavior is nonlinear in the case of SR-Ca load since release only starts for loads above 0.6 mM, the steady-state value occurs at a region where the steepness of the slope is not high enough to produce alternans. In the same manner, this curve as a function of RyR2 recovery does not present any abrupt onset of release but increases gradually up to the working condition at roughly 26% level of recovery.

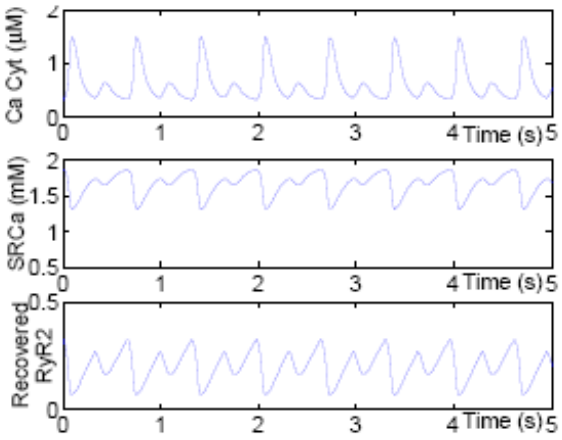

Figure 3. Small activation or inactivation leads to calcium alternans in the model by Shannon et al. Both SR-Ca load and the fraction of recovered RyR2 oscillated from beat to beat. For the case presented here, activation has been reduced by 95% and recovery time set to 750 ms

Upon reduction of activation and inactivation of the RyR2 alternans in the calcium transient appears at the same pacing frequency (see Figure 3). During alternans both the level of SR calcium loading and the fraction of recovered RyR2s oscillate. To get insight into which alternation is more relevant for the generation of alternans, we compute the corresponding nonlinearities of the fractional release as a function of SR-Ca loading and recovered RyR2.

We find two main behaviors for the different responses. In one, shown in Figure 4, there are steeper relations with both variables than in the non-alternating case, but with a stronger nonlinearity in the dependence on SR Ca load. The best way of noticing this is to look at the spread of the red and blue curves in the figure, corresponding to the fractional release curves after a small and a large calcium transient, respectively. On the left panel the two curves are very similar, meaning that release does not depend strongly on the change in the level of recovered RyR2s at the small and large calcium transients. Thus, this variable can hardly be of relevance for the onset of alternans in this case. In fact, on the right panel we see that the two curves split, so for the two values of SR Ca load during alternans we obtain completely different release curves as a function of the recovery of the RyR2, stressing the importance of SR Ca load for the appearance of alternans in this case.

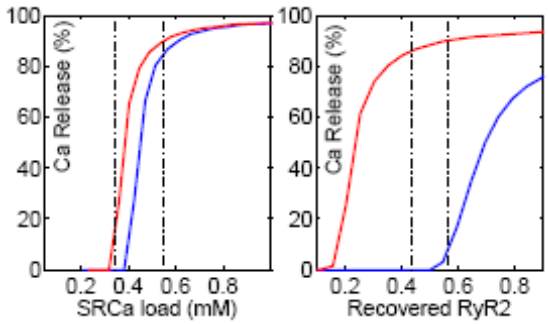

Figure 4. Highly nonlinear fractional release as a function of SRCa load (left) for very low inactivation. Blue and red line represent the two curves obtained depending on whether the fractional release is computed after a beat where the previous release was low in red (high in blue), and therefore the number of recovered RyR2 in the beat is high (low). Notice that the curves of the SRCa are very similar indicating lack of sensitive to the number of recovered RyR2. On the left, the fractional release for RyR2 is completely dependent on the level of SRCa load given the difference between the two curves. The vertical lines indicate the two pre-systolic values for each variable so that the relevant non-linear interval is between those two lines. For this case activation was reduced 30%, while inactivation was 95%, from the original nominal values.

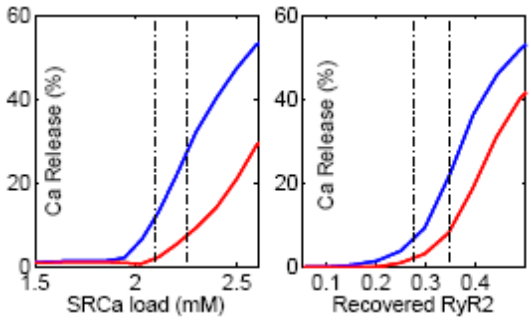

Figure 5. Highly nonlinear fractional release as a function of SRCa load (left) and as a function of recovered RyR2 (right) for low activation with significant inactivation. Blue and red lines represent the two curves obtained depending on whether the fractional release is computed after a beat where the previous release was low in red (high in blue).Both curves present a nonlinear response of the same order indicating that recovery from inactivation can contribute and be an important mechanism behind some type of alternans. The vertical lines indicate the two pre-systolic values for each variable so that the relevant non-linear interval is between those two lines. For this case activation was reduced 95% while inactivation was increase 50% from the original values of the model.

The dominance of the SR-Ca load nonlinearity is not always present. We have found a second situation (see Figure 5), typical for significant calcium inactivation of the RyR2. Here, both curves are steeper than in the non-alternating case and, more importantly, the fractional release onsets in a highly nonlinear manner once a finite number of RyR2s are recovered (around 20%). Actually, although both oscillations in SR Ca load and in the fraction of recovered RyR2s have a strong effect in fractional release, it can be observed that the split of the curves is larger in the SR-Ca load case (left panel) than in the level of recovered RyR2 (right panel). This figure seems to suggest that the relative importance of each of these variables for the onset of alternans is tilted towards the RyR2 recovery. It is clear, however, that both nonlinear mechanisms contribute at least on equal footing to sustain the alternans rhythm.

4. Discussions and conclusions

A possible explanation for the origin of calcium alternans relies on a steep relation between SR calcium content and calcium release. If reloading is slow (due to a slow SERCA, for instance) then, at fast pacing rates, after a big release, the SR-Ca concentration would not have time to regain the previous value, resulting in a small release. This feedback mechanism would give rise to beat-to-beat oscillations in the calcium transient, i.e., calcium alternans. However, experiments that do not show fluctuations in SR-Ca loading during alternans have stressed the importance of other possible mechanisms, such as recovery from inactivation of the RyRs after release. For this to work, a strong relation between Ca release and the number of recovered RyR2s must exist, coupled with a slow recovery of the RyR2s from inactivation, so, at fast pacing rates, they do not have enough time to reach the same value at every beat.

By analyzing the fractional release as both a function of SR-Ca load and RyR2 recovery we observe that the established case of strong dependence of calcium release as a function of SR Ca load is present when inactivation of the RyR2 is low. While for this situation the SR Ca load is clearly the underlying mechanism for alternans, we have found parameters for which recovery of the RyR2 from inactivation has a clear relevance in the origin of alternans. We indeed find a strongly nonlinear relation between calcium release and the level of recovered RyR2 in conditions where the inactivation of the RyR2 is large and the activation is low enough to load strongly the SR.

Acknowledgements

This work has been financially supported by Spanish Ministry of Science and Innovation under projects FIS2008-06335-C02-01 and FIS2009-13360-C03-03, and by CNIC under project CNIC-12. AP also acknowledges financial support from EPSEB (UPC).

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Address for correspondence.

Name. Angelina Peñaranda
Full postal address. Applied Physics Dept. (EPSEB) Dr. Marañon 44-50, 08028 Barcelona
E-mail address (optional). angelina@fa.upc.edu