

# Effect of RyR2 Refractoriness and Hypercalcemia on Calcium Overload, Spontaneous Release, and Calcium Alternans

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## Abstract

*In this work we use a computer model of a ventricular rabbit myocyte to study several factors that affect sarcoplasmic reticulum (SR) calcium load, such as hypercalcemia, SR calcium buffering, or ryanodine receptor (RyR2) dynamics. General conditions for the appearance of calcium spontaneous release in situations with calcium overload are obtained. Furthermore, we study the appearance of alternans under these conditions. The presence of calcium alternans may be due to either a steep calcium load release relation, resulting in oscillations in SR Ca content, or to a slow recovery from refractoriness of the RyR2. We find that a change in calsequestrin buffering dynamics or extracellular calcium concentration greatly affects the appearance of the former type of alternans, but not of the latter.*

## 1. Introduction

Dysfunctions in calcium dynamics at the cardiomyocyte level have been associated, under certain conditions, with cardiac arrhythmias. Instabilities in the calcium transient may give rise to the appearance of calcium alternans, or to the generation and propagation of intracellular calcium waves [1, 2].

Calcium alternans have been linked to the appearance and break-up of spiral waves, giving rise to tachycardia and ventricular fibrillation. Typically, alternans appears as the pacing rate is increased due the inability of the cell to produce the same release of calcium at every beat. This may be due to a slow refilling of the sarcoplasmic reticulum (SR) or to a long refractoriness time of the RyR2. Changes in SR calcium load due to hypercalcemia or altered buffering dynamics are expected to produce a larger effect in the former than in the latter scenario.

Alternatively, a variety of cardiac arrhythmias are initiated by a focal excitation whose origin is a large release of calcium from the sarcoplasmic reticulum (SR) which

is not timed with the external signaling provided by the depolarization of the tissue [3]. Although the factors involved in the timing of these excitation have not been fully elucidated [4], it is known that these excitations provide a proarrhythmic substrate through the generation of either delayed (DAD) or early afterdepolarizations (EAD), that may give rise to ectopic beats and lead to reentry and conduction blocks. It is generally believed that sudden cardiac death is indeed induced by a focal excitation that, if occurring during the DI window where the tissue is able to conduct, can form a new depolarization wave which may break and generate a reentry [3, 5–7]. Similarly, a large synchronous spontaneous release of calcium during afterdepolarization may also generate ectopic beats due to the increased activity of the sodium-calcium exchanger. In both situations, calcium overload and Ca cycling are critical to the generation of these arrhythmias.

In this paper we investigate under which conditions global high levels of calcium load can be present in the cell due to changes in extracellular calcium or in calsequestrin dynamics and, more importantly, the effects of these high loads in the arrhythmic substrate associated with anomalies of calcium release.

## 2. Model and approach

We use the rabbit ventricular whole-myocyte model described by Shannon et al. [8], and study the effect of changes in the extracellular calcium concentration and the parameters related with RyR2 cycling and calsequestrin dynamics. Regarding the RyR2 which considers transitions among four states, one open, one closed, and two inactivated, we use the nomenclature shown in Figure 1 where activation and inactivation rates are given by the functions  $\tilde{k}_a = k_a/k_{CSR}$ , and  $\tilde{k}_i = k_i k_{CSR}$  and  $k_{CSR}$  is a factor that depends on SR calcium concentration [8].

We should recall here that the myocyte model by Shannon et al. [8] using the original parameters  $k_a = 10 \text{ mM}^{-2} \text{ ms}^{-1}$ , and  $k_i = 0.5 \text{ mM}^{-1} \text{ ms}^{-1}$  does not give rise to calcium alternans neither at normal pacing rates (3 Hz) nor

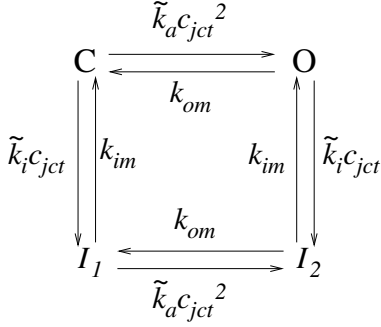


Figure 1. Dynamics of the RyR2

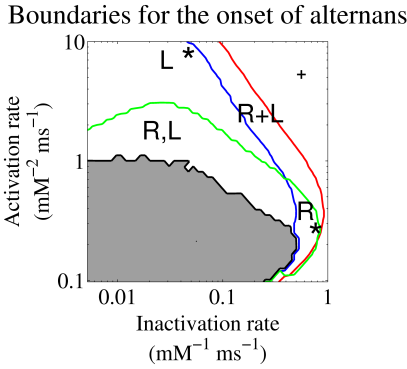


Figure 2. Graph indicating the different calcium alternans mechanisms for different pairs of activation and inactivation rates, at a pacing rate of 3Hz (see text for more details). The stars correspond to sets of activation and inactivation values for which alternans is due either to fluctuations in SR calcium loading ( $k_a = 7 \text{ mM}^{-2} \text{ ms}^{-1}$ ,  $k_i = 0.05 \text{ mM}^{-1} \text{ ms}^{-1}$ ) or to a slow recovery from inactivation of the RyR2 ( $k_a = 0.25 \text{ mM}^{-2} \text{ ms}^{-1}$ ,  $k_i = 0.75 \text{ mM}^{-1} \text{ ms}^{-1}$ ). We use these values as examples to study the effect of a change in the dynamics of calsequestrin. Similarly, the cross for the study of an increase of extracellular calcium.

when the pacing interval is shortened further. Given that calcium alternans appears roughly at 5Hz in rabbit, as observed in isolated rabbit cardiomyocytes [9] the standard activation and inactivation rates given in the original Shannon paper should not be considered the baseline for this work. We consider as standard values for the activation and inactivation rates those that generate transient alternans at high frequency (5 Hz). As a general rule, activation and inactivation rates must be reduced by 50% with respect to the values in [8] to generate alternans. We also considered that the RyR2 recovery from inactivation benchmark time is  $t_r = 750 \text{ ms}$ , which has been shown to be consistent with experiments on the restitution of the calcium transient [10, 11].

In Figure 2 we show the regions presenting alternans as a function of the activation and inactivation rates of the

RyR2. We have used the same numerical protocol as in [12] to analyze the mechanism behind the onset of alternans in each case; let it be RyR2 refractoriness ( $R$ ) or slow SR calcium loading refilling ( $L$ ). For that, the myocyte was stimulated at a constant pacing rate until steady state was reached. Then, we proceeded to eliminate alternations in SR Ca load or in the level of recovered RyR2s, by dynamically accelerating the dynamics of either the RyR2 recovery or the SR Ca uptake. For some activation-inactivation rates, both alternations are necessary to sustain the alternans (what we call  $R + L$  alternans). Finally if both activation and inactivation are very low then both the alternation of SR Ca load and of the level of recovered RyR2 can sustain on their own the presence of alternans (we call them  $R, L$  alternans).

A summary of these result is presented in Figure 2 where different alternans mechanism at 3Hz are indicated as different areas in a 2D plot, with the inactivation rate as horizontal axis and the activation rate as vertical axis. The upper right corner of high activation and inactivation rates is the area where calcium alternans is not present. The lower left grey area is also an area with no simple alternation but more complex periodicity, sometimes similar to chaotic regime. On the upper left, indicated as  $L$ , between the blue and green line, an area where alternans are due alternations in the SR Ca load. In the lower right corner, indicated as  $R$ , and also delimited by the blue and green lines, an area where alternans are due to recovery from inactivation. Finally, areas  $R + L$  and  $R, L$  are also indicated, showing respectively where both mechanism are necessary, and where each one can sustain alternans on its own. These regions are related to the nonlinear responses studied in [13].

### 3. Calsequestrin level and dynamics can eliminate SR Ca induced alternans

Due to the different regions present in Figure 2, one would expect that a change in SR calcium loading would have a differential effect on alternans depending on its underlying mechanism. We have checked this point modifying the dynamics of SR calcium buffering to calsequestrin (CSQN), that is known to have an effect on the occurrence of alternans [14]. The basic reaction is given in the model by

$$\frac{d[Ca \cdot CSQN]}{dt} = k_{on}[Ca]_{SR}(B_{max} - [Ca \cdot CSQN]) - k_{off}[Ca \cdot CSQN] \quad (1)$$

The two key features that we have changed are the binding rate to calsequestrin  $k_{on}$  and the maximum amount of calsequestrin buffering available in the cell  $B_{max}$ . The original values in the Shannon model are  $k_{on} = 100$

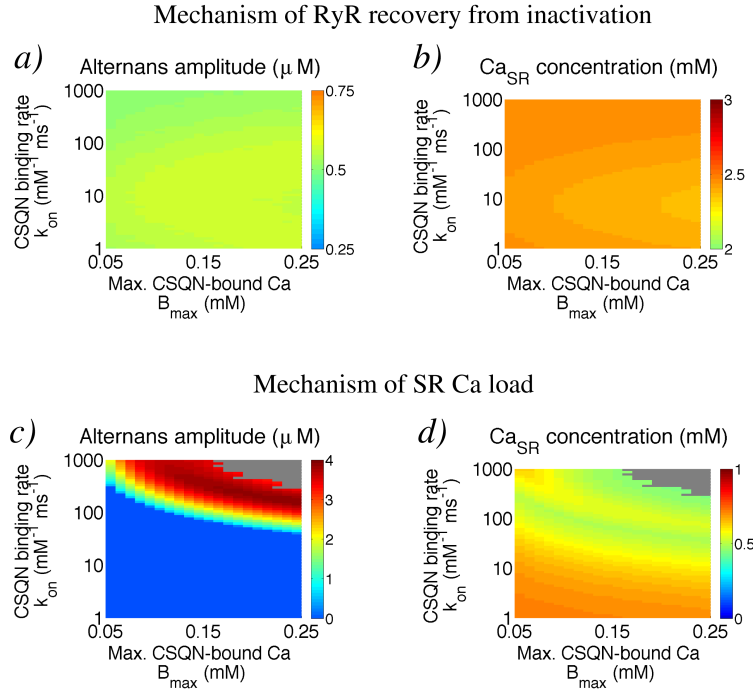


Figure 3. Top panels, color-code level of cytosolic calcium alternation (left) and pre-systolic SR Ca load as a function of calsequestrin level ( $B_{max}$ ) and binding rate ( $k_{on}$ ) show the persistence of alternans due to recovery from inactivation. Bottom panel with the corresponding graphs when SR Ca load alternation is the main mechanism behind calcium alternans. In this latter scenario, graphs show a strong effect of calsequestrin on pre-systolic SR Ca load (right) and the elimination of alternans and the return to normal calcium cycling for a wide range of calsequestrin dynamics scenarios (left)

$\text{mM}^{-1} \text{ms}^{-1}$  and  $B_{max} = 0.14 \text{ mM}$ . The activation rate is varied from 1 to  $1000 \text{ mM}^{-1} \text{ms}^{-1}$  and maximum concentration of free CSQN from 0.05 to 0.25 mM.

Given that we can not visualize a four-dimensional plot changing activation and inactivation rates of the RyR2 together with the level of calsequestrin buffering and rate of binding, we have picked activation-inactivation pairs which set us in a particular alternation scenario. In Figure 2, we show two points (marked as stars), one in the  $L$  area and another in the  $R$  which will act as characteristic examples. The first one presents alternation due to SR Ca load fluctuations and the second due to recovery from inactivation.

We consider first a set of activation and inactivation rates ( $k_a = 0.25 \text{ mM}^{-2} \text{ms}^{-1}$ ,  $k_i = 0.75 \text{ mM}^{-1} \text{ms}^{-1}$ ) where alternation is due to recovery from inactivation (see Figure 2). For these parameters the SR is strongly loaded and its concentration depends very slightly on the dynamics of buffering with calsequestrin (Figure 3b). As expected, this does not have any appreciable effect on cytosolic alternans, that persist under strong changes on buffering dynamics (Figure 3a).

A very different situation occurs in the case where alternans is due to fluctuations in SR calcium loading ( $k_a = 7 \text{ mM}^{-2} \text{ms}^{-1}$ ,  $k_i = 0.05 \text{ mM}^{-1} \text{ms}^{-1}$  in Figure 2). In

this situation, the calcium concentration in the SR is much lower and depends strongly on buffering dynamics (Figure 3d). As expected, the amplitude of alternans varies accordingly, finding big regions in the  $(k_{on}, B_{max})$  parameter space for which it completely disappears (Figure 3c).

#### 4. Effects of hypercalcemia

Another situation that affects calcium homeostasis is hypercalcemia, i.e., high levels of extracellular calcium. In this situation the calcium concentration in the SR is increased and this has an important effect on the size of the alternans regions shown in Figure 2 [15].

Even for values of activation and inactivation of the RyR2 where no alternans are present, high values of the external calcium concentration can induce spontaneous calcium oscillations (Figure 4a,b). In the paced case, the competition between the period of the spontaneous oscillations and the external stimulus results in frequent EAD or DAD and very irregular dynamics (Figure 4c,d).

#### 5. Discussion and conclusions

We have used a numerical model of a rabbit ventricular myocyte, as well as a numerical protocol developed

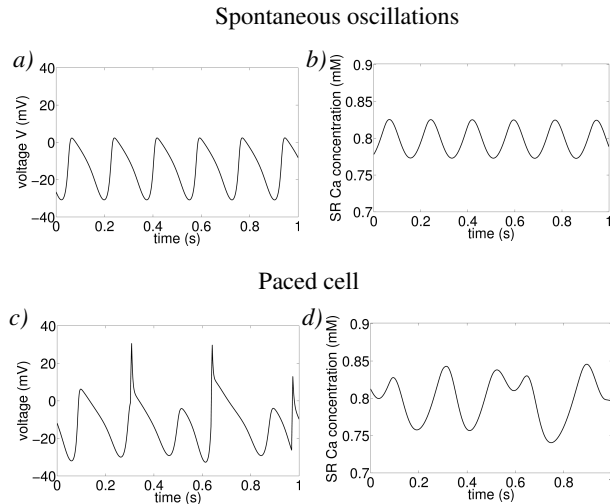


Figure 4. Transmembrane voltage (left) and SR calcium (right) dynamics at high values of the external calcium concentration ( $[Ca]_o = 10$  mM) for (top panels) no external stimulation, showing spontaneous oscillations and (bottom panels) a periodic stimulation of 3Hz, resulting in very irregular dynamics. The activation and inactivation rates of the RyR2 correspond to the cross in Figure 2 ( $k_i=0.5$  mM $^{-1}$  ms $^{-1}$ ,  $k_a=5$  mM $^{-2}$  ms $^{-1}$ ).

recently, to study the effect of calsequestrin buffering dynamics on the appearance of calcium alternans. We find that this effect is negligible when alternans is due to oscillations in RyR2 dynamics but can prevent the appearance of alternans when it is due to fluctuations in SR calcium loading. This opens the possibility of experimentally studying the underlying mechanism behind calcium alternans by changing the buffering dynamics. Similarly, hypercalcemia can also produce a differential effect on calcium alternans, or induce DAD and EAD, known to be highly proarrhythmic.

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