CALCIUM TRIGGERED ARRHYTHMIAS AND THE GOLDILOCKS' PRINCIPLE

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ABSTRACT

Beyond the importance of calcium cycling on cardiac contractile function, calcium can also affect membrane currents and lead to arrhythmias.

In this revision we will discuss how the balance between release and reuptake of calcium from and to the sarcoplasmic reticulum affects the probability of arrhythmias.

A mathematical human cardiomyocyte model that can simulate increasing RyR2 open probabilities (Po) along with high or low SERCA2A mediated reuptake velocities showed that arrhythmias occur in an intermediate physiological zone for both RyR2 Po and reuptake velocity. These results allow to make a parallelism with the "goldilocks and the tree bears" story in which she is looking to drink a cup of porridge with an intermediate optimal temperature.

Along the text we explain how the Goldilocks principle is fulfilled in different well-known experimental situations.

Keywords: Heart, Arrhythmias, Calcium, Sarcoplasmic Reticulum, RyR2, PLN

RESUMEN

Más allá del papel que el ciclado de calcio cumple en la función contráctil del corazón, el manejo de calcio en los cardiomiocitos es capaz de afectar corrientes de membrana y, de este modo, las alteraciones en el manejo de calcio son capaces de generar arritmias cardíacas. En esta revisión discutiremos los efectos que el balance entre liberación y recaptura de calcio desde y hacia el retículo sarcoplasmático (RS) tiene sobre la probabilidad de gatillar arritmias cardíacas. Mediante un modelo matemático de miocito cardiaco humano, que simula probabilidades de apertura (Po) crecientes de los receptores de rianodina (RyR2) en asociación con aumentos y disminuciones de la velocidad de secuestro de Ca^{2+} por la Ca-ATPasa del RS (SERCA2a), se determinó que las arritmias ocurren con más probabilidad en una zona intermedia (zona óptima para las arritmias) tanto de Po como de la velocidad de secuestro de Ca^{2+}, en tanto que las zonas con mayor Po o mayor o menor velocidad de secuestro de Ca^{2+} están libres de arritmias. Estos resultados permiten establecer un paralelismo con la historia de "Ricitos de oro y los tres osos" y su búsqueda de una taza de avena con temperatura intermedia como aquella óptima para ser bebida.

A lo largo del texto explicaremos cómo este principio se cumple en situaciones experimentales conocidas.

Palabras Clave: Corazón, Arritmias, Calcio, Retículo Sarcoplasmático, RyR2, PLN

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Introduction

Mechanical dysfunction and arrhythmias are a leading cause of morbidity and mortality worldwide [1,2], and it is now well established that a large fraction of ventricular arrhythmias is initiated at the cellular level by focal triggered mechanisms such as abnormal spontaneous Ca^{2+} discharges (Ca^{2+} sparks) from the sarcoplasmic reticulum (SR). This excessive Ca^{2+} release during diastole propagates as regenerative Ca^{2+} waves that travel through the cytosol and activate inward membrane currents, mainly the electrogenic Na^{+}/Ca^{2+} exchanger (NCX) working in the forward mode [3-5].

Abnormal spontaneous Ca^{2+} discharges from the SR occur under conditions in which SR Ca^{2+} load exceeds a threshold that is largely determined by the state of the ryanodine receptors (RyR2). It is known, for instance, that RyR2 point mutations render the channel more prone to spontaneous SR Ca^{2+} release during adrenergic stimulation. Patients with this inherited anomaly exhibit catecholaminergic polymorphic ventricular tachycardia, a known cause of sudden cardiac death [6]. We and others have described that RyR2 phosphorylation by Ca^{2+}–calmodulin-dependent protein kinase II (CaMKII) at the Ser2814 site is associated with SR Ca^{2+} leak and arrhythmogenesis in cardiac pathologies of different etiologies [7-12]. These results define the crucial role of altered RyR2 activity on triggered arrhythmias.

In contrast, the effect of increasing SR Ca^{2+} uptake on cardiac triggered events is not clear and there is concern on whether the increase in SR Ca^{2+} uptake, which has been shown to be a useful therapy to revert depressed cardiac contractility in human and experimental heart failure [13], is protective against Ca^{2+} triggered arrhythmias or exacerbates them. Indeed, either the increase or decrease of SR Ca^{2+} uptake has led to contradictory results [14-21].

We believe that a possible explanation to these conflicting results may rest, at least in part, in the opposite effects inherent to the augmented cytosolic SR Ca^{2+} uptake (Figure 1) i.e., increasing the rate of SR Ca^{2+} uptake would reduce cytosolic Ca^{2+} overload and the risk of cardiac arrhythmias, but would necessarily increase SR Ca^{2+} content, favoring RyR2 Ca^{2+} sensitization, improving diastolic SR Ca^{2+} leak and the risk of Ca^{2+} waves. This situation might be exacerbated if the increase in SR Ca^{2+} uptake coexists with an increase in the open probability of the RyR2, as that produced by CaMKII-dependent phosphorylation of the Ser2814 site [7, 12, 22].

Of note, SR Ca^{2+} uptake and release are highly regulated processes. The activity of SERCA2a and RyR2 open probability (Po) are dependent on a complex regulation that includes SR Ca^{2+} load and different proteins interacting with either SERCA2a, RyR2 or both (See for review [23-25]. Among these regulatory proteins we will mention phospholamban (PLN) not only because of its relevant action on SERCA2a activity but also because some of the experiments to be described here use genetic modified mice with PLN ablation (PLNKO mice, (26)). Under dephosphorylated conditions, PLN tonically inhibits SERCA2a. PLN phosphorylation or ablation, relieves this inhibition increasing SERCA2a activity and SR Ca^{2+} load [26-28].

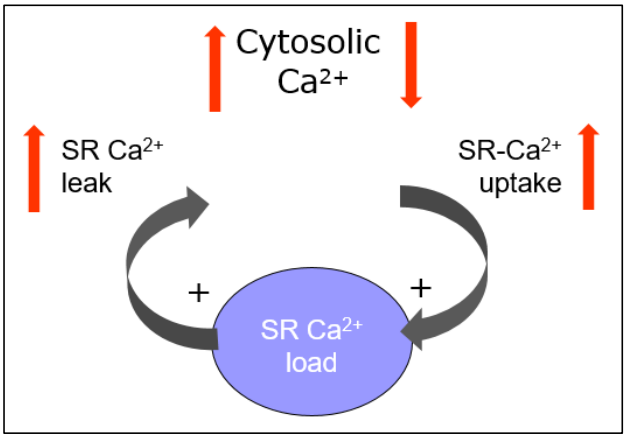

Figure 1. Opposite effects of increasing SR Ca^{2+} uptake on cytosolic Ca^{2+}. The increase in SR Ca^{2+} reuptake diminishes cytosolic Ca^{2+} and increases SR Ca^{2+} leak.

Triggered arrhythmias and the relationship between SR Ca^{2+} uptake and leak

By using genetic altered mice and the myocyte mathematical model of Negroni- Lascano [22, 29-31], we previously showed that triggered arrhythmias are highly dependent on the relationship between SR Ca^{2+} uptake, given by the activity of SERCA2a and SR Ca^{2+} leak, given by the Po of RyR2. In these experiments, we were able to define an arrhythmogenic zone (arrhythmogenic island) and a non-arrhythmogenic area (in blue), surrounding "the island", constituted by different combinations of RyR2 Po (conductance in the model) and SR Ca^{2+} uptake.

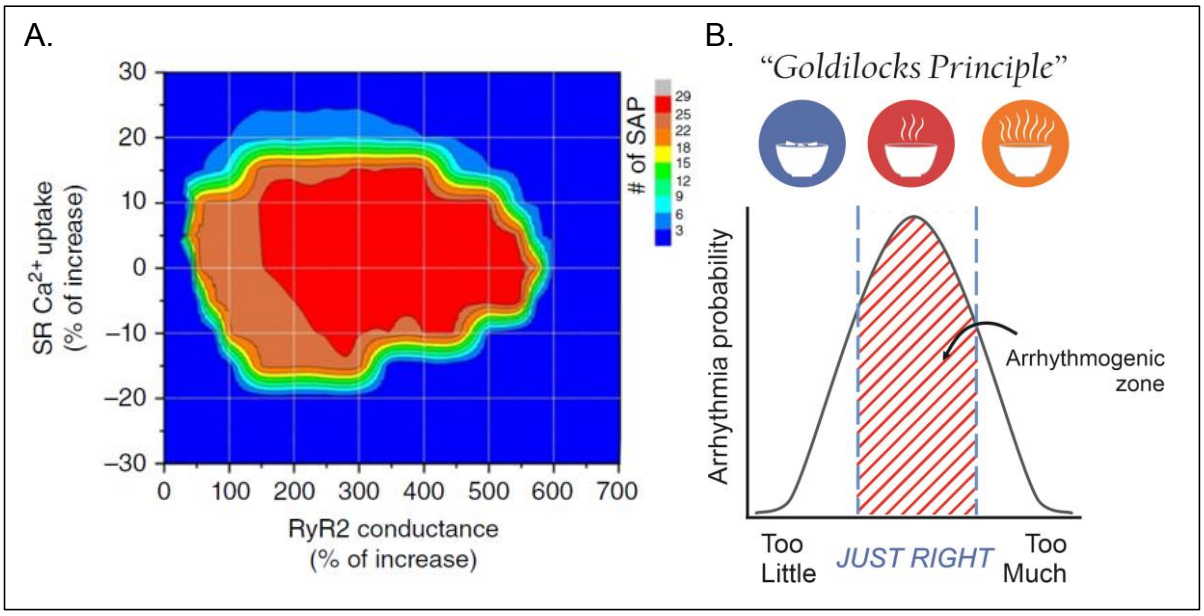

Figure 2. A. The interplay between SR Ca^{2+} uptake and leak define the occurrence of arrhythmias. **B.** The Goldilocks principle. Too much uptake (with respect to the leak) or too much leak with respect to the uptake), decreases the probability of arrhythmias.

As it is shown in the figure, very low or very high SR Ca^{2+} uptake or leak (blue zone), is unable to evoke triggered arrhythmias. Instead, maximal arrhythmia probability occurs in the middle zone of the relationship, which reminds the classic Goldilocks principle by analogy to the children's story "The Three Bears" by Robert Southey [32]. In this tale, a young girl named Goldilocks tastes three different bowls of porridge and finds she prefers porridge that is neither too hot nor too cold, but has just the right temperature. In our case, the red zone of the island constitutes the area with the just right combination of SR Ca^{2+} uptake and leak that produces arrhythmias.

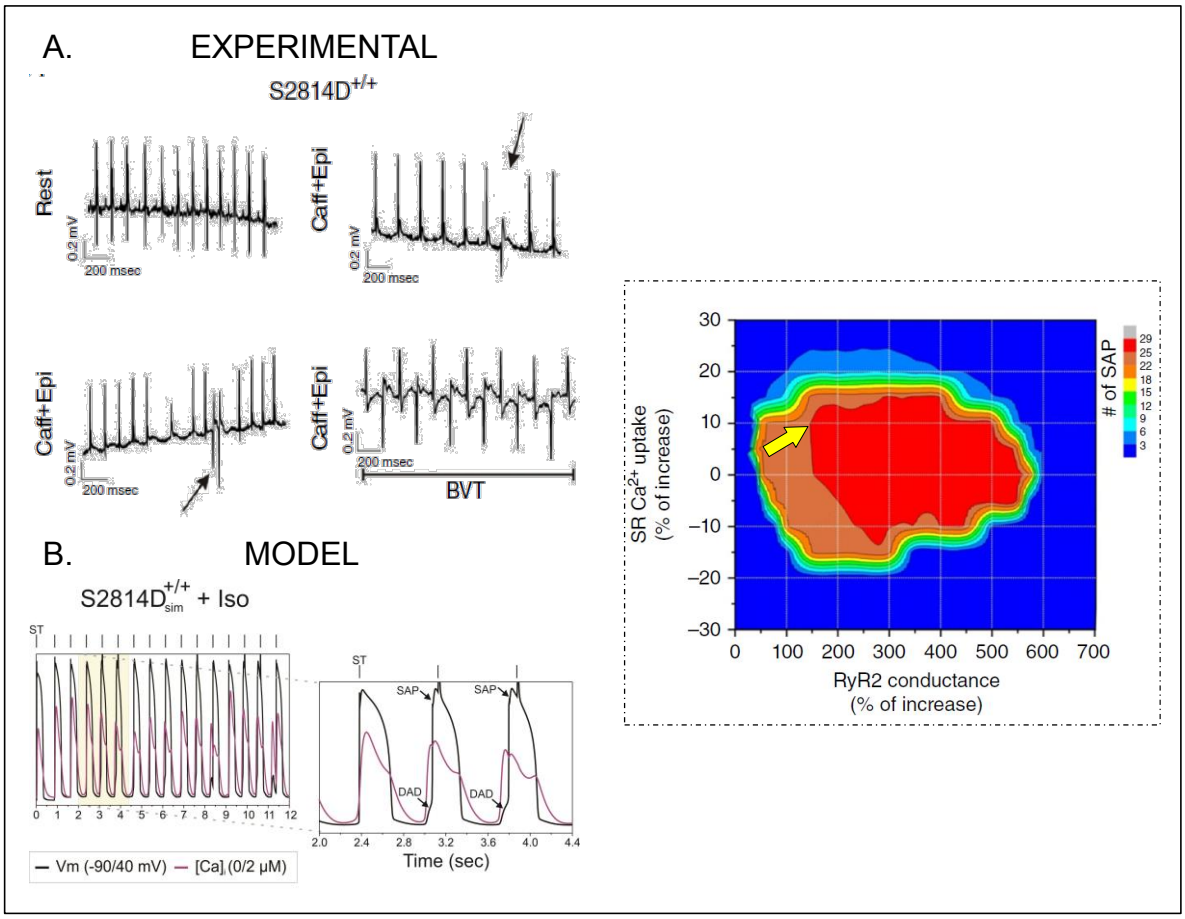

Figure 3. A. Representative ECG tracings in conscious S2814D+/+ knock-in mice at rest and after I.P. injection of caffeine/Adrenaline (Caff/Adr). Arrows in S2814D+/+ tracings indicate premature ventricular complexes (PVCs). Bidirectional ventricular tachycardia (BVT) was observed in the majority of S2814D+/+ mice after Caff/Adr challenge. **B.** The model reproduces the experimental results. Under conditions of stress (Isoproterenol (Iso) stimulation), there is an increase of spontaneous action potentials (SAP) originated from delayed afterdepolarizations (DADs). ST: Stimulus. Inset: The yellow arrow indicates the possible position in the graph of S2814D hearts under stress conditions. Experimental.

Figure 3A depicts the results obtained in a genetic modified mouse with an increased Po produced by constitutive pseudo-phosphorylation of RyR2 by CaMKII without SR Ca^{2+} uptake modifications (Ser2814D mice, [33]). Under resting conditions, there are no detectable arrhythmias. However, under stress conditions (Norepinephrine plus caffeine infusion), there is an increase in cardiac arrhythmias. **Figure 3B** shows that the mathematical model simulating the conditions of S2814D hearts, reproduces the experimental behavior of these hearts under stress conditions [22].

The behavior of Ser2814D hearts would follow the yellow arrow in the inset of Figure 3. Under control conditions the SR Ca^{2+} load is low, because of the increased SR Ca^{2+} leak of these myocytes without any increase in SR Ca^{2+} uptake. When submitted to stress (Adrenaline plus caffeine or Isoproterenol) there is an increase in SR Ca^{2+} uptake, SR Ca^{2+} load and SR Ca^{2+} leak that approximates S2814D myocytes to the red zone of the graph (high arrhythmia probability).

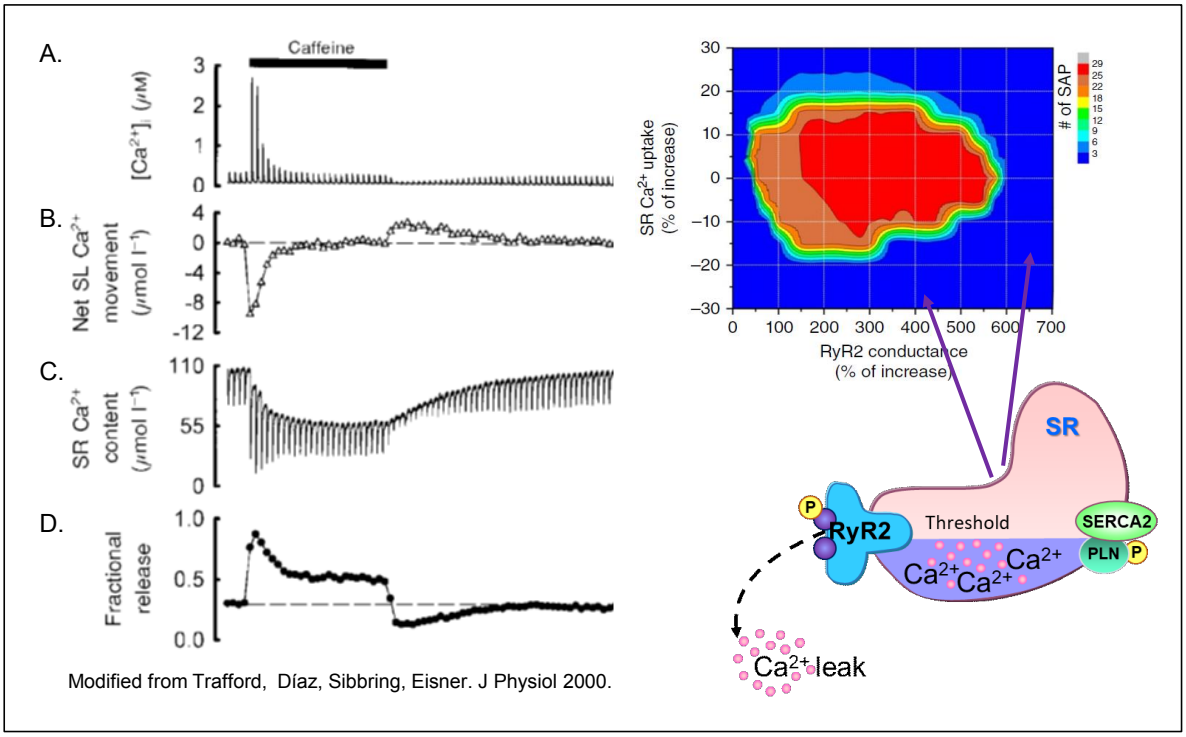

Figure 4. Potentiation of RyR2 enhances SR Ca^{2+} leak and diminishes SR Ca^{2+} content.

There are some "blue" zones in the graph of Figure 2 in which it is difficult to intuitively visualize why they represent an arrhythmia-free zone, despite the high increase in RyR2 conductance. The interpretation of the zone below "the island" is similar to that given for S2814D myocytes. Figure 4 illustrates what occurs experimentally when there is an increase in SR Ca^{2+} leak without any corresponding increase in SR Ca^{2+} uptake, able to reestablish SR Ca^{2+} load to the threshold for SR Ca^{2+} leak. The experiment from Eisner´s group shows that administrating isolated myocytes low doses of caffeine is able to sensitize RyR2, producing an increase in the Ca^{2+} transient due to the

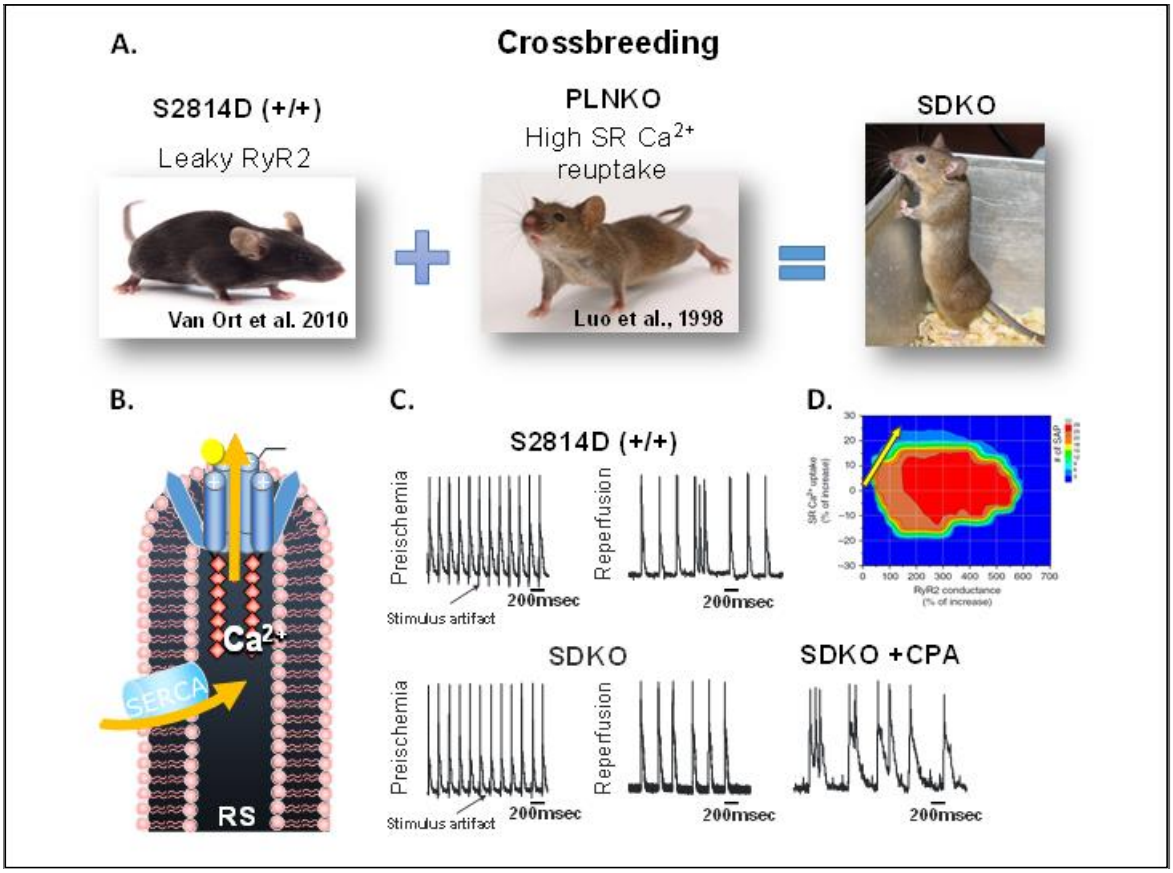

Figure 5.A. Mutants characteristics. **B.** Graphical representation of RS with an increase in both SR Ca^{2+} uptake and leak. **C.** Results obtained in S2814D and SDKO hearts ssubjected to a protocol of ischemia/reperfusion. **D.** Yellow arrow show possible trajectory from the non-arrhythmogenic to the arrhythmogenic area when S2814D mice were crossbred with PLNKO mice to produce SDKO animals.

increase in SR Ca^{2+} release, but which rapidly returns to control values as a result of the decrease in SR Ca^{2+} load [34]. However, the consequence of RyR2 sensitization can be seen: The SR was able to release the same amount of Ca^{2+}, despite the decrease in SR Ca^{2+} load. Thus, RyR2 sensitization, although unable to evoke a persistent increase in Ca^{2+} transient amplitude, produces a persistent increase in fractional Ca^{2+} release, i.e., the Ca^{2+} released at a given SR Ca^{2+} load [31].

The blue area above the "arrhythmogenic island" is more difficult to explain. In this case the increase in SR Ca^{2+} leak associated with an increase in SR Ca^{2+} uptake can hardly be visualized as a non-arrhythmogenic combination. To explore this paradox, we performed experiments in genetic modified mice obtained by crossbreeding S2814D mice with mice with PLN ablation mice (SDKO mice) (Figure 5A).

This provides an animal model with an increase in both SR Ca^{2+} uptake and leak (Figure 5B). Figure 5C shows the results obtained in S2814D hearts subjected to a protocol of ischemia/reperfusion. Immediately after ischemia, it is possible to observe the classic reperfusion arrhythmias found in S2814D hearts. PLN ablation avoids reperfusion arrhythmias in SDKO hearts. As a proof of concept, decreasing the activity of SERCA2a by perfusion of the hearts with the SERCA2a inhibitor cyclopiazonic acid (CPA) at the time of reperfusion, evokes again reperfusion arrhythmias. Figure 5D shows a possible trajectory of S2814D from the non-arrhythmogenic to the arrhythmogenic area when these mice were crossbred with PLNKO mice to produce SDKO animals.

Which is the mechanism by which PLN ablation avoids the arrhythmias that usually occur under stress conditions in mice with an increase in Po? We mentioned above that an increase in SR Ca^{2+} uptake increases SR Ca^{2+} load and SR Ca^{2+} leak (Figure 1). Is this mechanism absent in SDKO hearts? To test this idea, we measured Ca^{2+} sparks in SDKO hearts subjected to either a stress [22] or to the

ischemia/reperfusion protocol [12]. Figure 6 A is a representative example obtained using confocal microscopy in the intact heart, revealing that during reperfusion there is an increase in SR Ca^{2+} leak (Ca^{2+} sparks) in SDKO when compared with S2814D hearts.

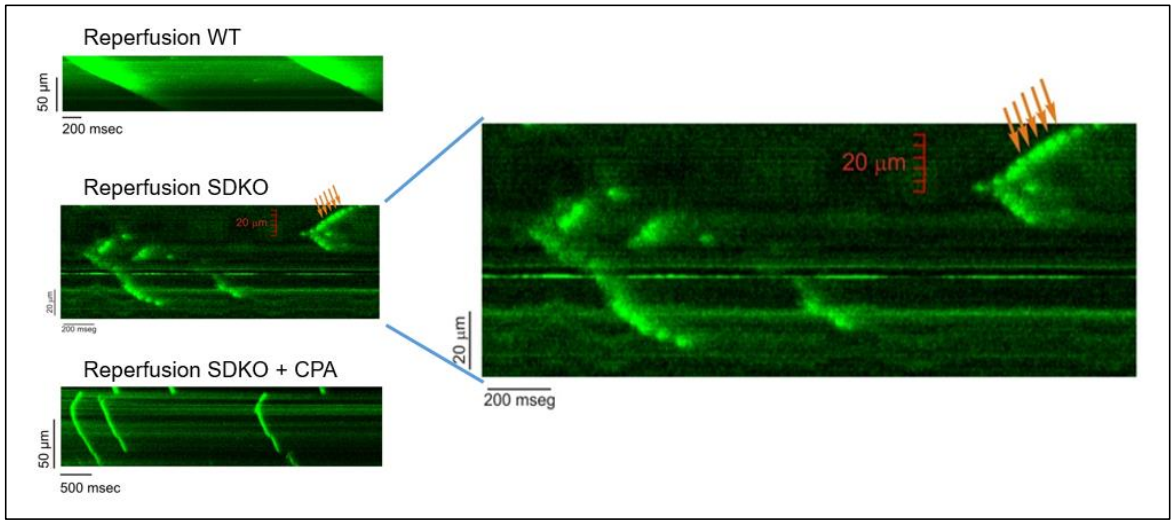

Figure 6. Protocol and typical examples of line scan recordings of epicardial Ca^{2+} waves obtained at the beginning of reperfusion in intact WT and SDKO hearts. Protocol and typical recordings of line scan before and after the addition of the SERCA2a inhibitor CPA in SDKO hearts. Red arrows indicate fragmented SR Ca^{2+} waves.

The question is then, why does this increase in SR Ca^{2+} leak fail to produce reperfusion arrhythmias as expected? As explained above, Ca^{2+} triggered arrhythmias occur because excess of Ca^{2+} leak from the SR, "travels" as Ca^{2+} waves in the cytosol and is extruded through the electrogenic NCX, producing a membrane depolarization that, if high enough, may lead to an ectopic beat. Figure 6B shows that whereas there are Ca^{2+} waves during reperfusion in S2814D hearts, in SDKO heart Ca^{2+} waves are aborted and converted into non-arrhythmogenic Ca^{2+} events, termed mini waves. Abortion of Ca^{2+} waves in these mice may occur due to the fact that the increase in SR Ca^{2+} uptake produced by PLN ablation, increases cytosolic Ca^{2+}buffer [34], that would hamper the traveling of the wave as a whole. Interestingly, this mechanism was also observed to be present in cardiac myocytes treated with Istaroxime, a cardiotonic steroid that is under clinical evaluation to treat heart failure [35]. Istaroxime, which combines Na+/K+ ATPase inhibition with SERCA-mediated uptake acceleration, increases SR Ca^{2+} load but brakes Ca^{2+} wave propagation leading to non- arrhythmogenic mini-wave occurrence [36]. In contrast, recent experiments show that the dual-action (CaMKII-dependent Ca^{2+}-leak and CaMKII-independent Ca^{2+}-uptake) of aging -induced increase in stress kinase JNK2 (c-Jun N-terminal kinase2) enhances atrial fibrillation vulnerability [37]. In this case, the increase in cytosolic Ca^{2+} buffer capacity produced by the enhanced SR Ca^{2+}-uptake seems not to be sufficient to break cytosolic Ca^{2+} waves. Instead, the increased SR Ca^{2+} uptake favors arrhythmia events by keeping SR Ca^{2+} load above the threshold necessary to enhance SR Ca^{2+} leak.

The comparison of the arrhythmic island obtained with mathematical model with the Goldilocks principle, may sound counterintuitive. In contrast to the Three Beer Story, the right combination of release and uptake is far from producing a pleasant effect as that evoked by the warm bowl of porridge. In this case, the right combination leads to unwanted results, like the enhanced possibility of threatening arrhythmias. Conversely, too much or too little, gives rise to a good result. This is not the case of some related phenomena. For instance, based on different previous works [38-40], Liu et al., [41] suggested a Goldilocks behavior in the relationship between Ca^{2+} release restitution (CRR) and arrhythmias: An increase in the velocity of CRR, predisposes to triggered arrhythmias but precludes Ca^{2+} alternans [40], whereas a slow recovery of CRR can increase the risk of alternans, i.e. recovery that is either too fast or too slow may be detrimental, whereas a rate of recovery between the

potentially dangerous extremes may be ideal. This relationship seems to hold true under a variety of conditions that exclusively affect CRR. However, our results showed that when the enhancement of CRR achieved by increasing SR Ca^{2+} uptake, as is the case of PLN ablation is high enough, triggered arrhythmias are prevented [12].

In summary, the results indicate that the arrhythmogenic effect of increasing RyR2 Po can only persist if there is an associated increase in SR Ca^{2+} uptake that allows SR Ca^{2+} load to be maintained above the necessary level (threshold) that produces leak. However, if the increase in SR Ca^{2+} uptake is high enough, it might prevent arrhythmias by aborting Ca^{2+} waves.

Perspectives

Based on the Goldilocks´principle, we can consider that two therapeutic strategies could reduce Ca^{2+} triggered arrhythmias. The first is the pharmacological reduction of RyR2 opening, with several compounds accumulating evidence about their capacity to prevent waves occurrence [42-45]. However, concerns about their "alternogenic" potential have been raised [46]. The second approach is to enhance SR Ca^{2+} uptake velocity to brake waves propagation and reduce their arrhytmogenic potential. Interestingly, it could also prevent alternans due to an acceleration of CRR. Drugs such as istaroxime, that circumvent the Goldilocks principle regarding SR Ca^{2+} release and reuptake [36], would be useful to treat heart failure, increasing contractility without the risk of triggering arrhythmias. Nevertheless, further studies are necessary to analyze the relationship not only between intracellular Ca^{2+} handling and arrhythmias, but also associated with CRR and alternans, to establish the best balance of Ca^{2+} management where electrical abnormalities are prevented.

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