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Store-dependent deactivation: Cooling the chain-reaction of myocardial calcium signaling

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Abstract

In heart cells, Ca^{2+} released from the internal storage unit, the sarcoplasmic reticulum (SR) through ryanodine receptor (RyR2) channels is the predominant determinant of cardiac contractility. Evidence obtained in recent years suggests that SR Ca^{2+} release is tightly regulated not only by cytosolic Ca^{2+} but also by intra-store Ca^{2+} concentration. Specifically, Ca^{2+}-induced Ca^{2+} release (CICR) that relies on auto-catalytic action of Ca^{2+} at the cytosolic side of RyR2s is precisely balanced and counteracted by RyR2 deactivation dependent on a reciprocal decrease of Ca^{2+} at the luminal side of RyR2s. Dysregulation of this inherently unstable Ca^{2+} signaling is considered to be an underlying cause of triggered arrhythmias, and is associated with genetic and acquired forms of sudden cardiac death. In this article, we present an overview of recent advances in our understanding of the regulatory role luminal Ca^{2+} plays in Ca^{2+} handling, with a particular emphasis on the role of Ca^{2+} release refractoriness in aberrant Ca^{2+} release. This article is part of a Special Issue entitled “Calcium Signaling in Heart”.

Keywords

Luminal calcium; Sarcoplasmic reticulum; Ryanodine receptor; Calsequestrin; Calcium-induced calcium-release

1. Introduction

During each heartbeat, coordinated contraction and subsequent relaxation of the billions of cardiomyocytes in the mammalian heart is attained through a synchronized effort of two high fidelity signaling systems: The first is mediated by the action potential (AP) and the other by intracellular Ca^{2+} transient. In the former case, opening of voltage-dependent Na^{+}

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channels generates a depolarizing inward Na^+ current that initiates the AP [1]. In the latter, Ca^{2+} influx through voltage-sensitive L-type Ca^{2+} channels in response to electrical depolarization activates Ca^{2+}-sensitive ryanodine receptors channels (RyR2) on the surface of the sarcoplasmic reticulum (SR), leading to a self-regenerating process known as Ca^{2+}-induced Ca^{2+} release (CICR), which initiates contraction [2]. Although the intracellular Ca^{2+} storage site, the SR, takes up only 3.5% of the total myocyte volume, it contains sufficient Ca^{2+} not only for the generation of systolic contraction but also for a sizable physiological SR Ca^{2+} reserve [3]. This in part is due to the presence of the SR Ca^{2+}-adenosine triphosphatase (SERCA) pump on the SR membrane that raises the free intra-SR (luminal) Ca^{2+} concentration ($[Ca^{2+}]$) close to 1 mM [3], as well as to the low affinity, high capacity luminal Ca^{2+} buffering protein calsequestrin (CASQ2) that virtually doubles the SR Ca^{2+} storage capacity [4].

It is important to note that both membrane depolarization and CICR, because of their positive feedback mechanisms, are intrinsically prone to instabilities and spontaneous activation. For this reason, these high fidelity, self-regenerating signaling processes require effective means for signal termination and containment. For instance, the termination of the AP and the resultant restoration of the resting membrane potential is achieved by the inactivation of Na^+ and Ca^{2+} channels as well as by the repolarizing currents carried by multitude of K^+ channels that include the delayed rectifier current with its multiple components [5]. Resting membrane potential between APs, on the other hand, is stabilized by the inward rectifier K^+ current. Analogously, termination of SR Ca^{2+} release and restoration of the diastolic Ca^{2+} level within the cytosol of the cardiomyocyte is predominantly attained through inactivation/deactivation of RyR2s as well as by SERCA-dependent resequestration of Ca^{2+} into the SR, and to a lesser extent by extrusion of Ca^{2+} by the sarcolemmal Na^+-Ca^{2+} exchanger (NCX) [6]. These processes responsible for signal termination ensure that the membrane potential and CICR remain functionally silent or refractory during the diastolic period preventing thereby inappropriately timed APs or contractions.

The concepts of refractoriness and repolarization reserve have been key to understanding the pathophysiology and treatment of rhythm disorders associated with abnormal membrane excitability [7,8]. Despite, growing evidence indicating that regulation of RyR2 by luminal Ca^{2+} is critical for controlling physiologic SR Ca^{2+} release (for recent reviews see references [4,9–11]) key questions as to the molecular components involved in luminal Ca^{2+} regulation of SR Ca^{2+} release and its refractoriness in the development of cardiac disease remains to be resolved. In this review, we will highlight recent advances that have furthered our understanding of the mechanisms underlying the control of SR Ca^{2+} release, termination and refractoriness by the interaction of luminal Ca^{2+} with RyR2 complex. We will also discuss some of the important unresolved issues regarding these processes in normal physiologic and cardiac disease conditions.

2. Luminal Ca^{2+} governs its own release

In cardiomyocytes during contractions, where most of the Ca^{2+} required for contractile activation is supplied by the SR, luminal $[Ca^{2+}]$ itself is an important determinant of

contractility. However, as suggested by the initial studies, intra-SR Ca^{2+} is not merely acting as a passive reservoir of available Ca^{2+}, but plays anactive role in controlling the Ca^{2+} release process [4,12–15]. More precisely, high luminal [Ca^{2+}] (load) facilitates Ca^{2+} release from the SR, while a sharp decrease of release is observed at reduced SR [Ca^{2+}] load [16,17]. Two principal mechanisms have been proposed by which this regulation could be accomplished. The first is the modulation of RyR2 activity via RyR2 luminal Ca^{2+} sensing sites. The other, termed feed-through, posits that leak of Ca^{2+} from the SR (via RyR2) then alters cytosolic [Ca^{2+}] in the vicinity of the "leaky" RyR2 and thereby affects the open probability of that very same and neighboring RyR2s via cytosolic Ca^{2+} sensing sites [4]. Delineating the contributions of these mechanisms in isolated myocytes is complicated by the difficulty of controlling [Ca^{2+}] on both sides of the RyR2 channel and by the possibility that these mechanisms may operate in tandem.

Compelling evidence for the regulation of RyR2 gating by luminal Ca^{2+} was obtained in planar lipid bilayers, which allowed not only a direct control over the luminal environment surrounding RyR2s but also prevented the possibility of the feed-through effects [18,19]. It was possible in these studies to isolate the true luminal Ca^{2+} effects and minimize the possibility of feed-through mechanism(s) by either setting a cytosolic-to-luminal electrochemical Ca^{2+} gradient [18] or by verifying the luminal localization of the gating effect by trypsin digestion of the luminal aspects of RyR2 [20]. These studies demonstrated that RyR2 open probability changes as a function of luminal Ca^{2+} with a K_d value of about 1 mM, which corresponds to the SR [Ca^{2+}] in a cardiomyocyte at rest [18,21]. Taken together, these studies suggest that in addition to CICR, luminal effects contribute to the regulatory role of SR Ca^{2+} load on Ca^{2+} transient/release by potentiating this effect at elevated SR Ca^{2+} loads. Importantly, these studies also raised the possibility that reduced luminal [Ca^{2+}] could serve as a negative regulator of RyR2 by inhibiting Ca^{2+} release at decreased SR Ca^{2+} content [18].

3. Luminal Ca^{2+} alters the sensitivity of cytosolic Ca^{2+} activation site

A number of studies using isolated cardiomyocytes and reconstituted RyR2s demonstrated that luminal Ca^{2+} alters the sensitivity of RyR2 to cytosolic Ca^{2+}. Single channel studies demonstrated that rather than uniformly scaling RyR2 activity at different cytosolic [Ca^{2+}], increases in luminal Ca^{2+} shift the cytosolic Ca^{2+} sensitivity to lower [Ca^{2+}] [18,22,23]. As already mentioned, such investigation of the interplay between the cystosolic and luminal Ca^{2+} on RyR2 function is challenging in the setting of isolated myocytes. To better understand the mechanisms of SR Ca^{2+} release regulation by Ca^{2+} on both sides of the SR membrane, Stevens et al. [24] investigated the effects of a wide range (1–100 μM) of cytosolic [Ca^{2+}] on SR Ca^{2+} release in permeabilized cardiomyocytes by monitoring luminal [Ca^{2+}]with Fluo-5N, a low-affinity Ca^{2+} indicator. At any given cytosolic [Ca^{2+}], including levels as high as 50 μM, luminal Ca^{2+} evidenced periodic oscillations. Since feed-through effects of luminal Ca^{2+} on SR Ca^{2+} release should beminimal at such high cytosolic [Ca^{2+}], these intra-SR Ca^{2+} oscillations were attributed to RyR2 alternating between low and high cytosolic Ca^{2+}-sensitivity states as determined by the filling status of the SR (low and high Ca^{2+} load, respectively). A similar conclusion regarding dynamic allosteric regulation of RyR2 functional activity by cytosolic and luminal Ca^{2+} was also reached by

MacQuaide et al. [25] in their analysis of the effects of tetracaine on spontaneous Ca^{2+} release in cardiomyocytes. Of note, a recent study by Tencerová et al. [26] demonstrated that luminal Ca^{2+} influences RyR2 gating by allosterically modulating affinity of the adenosine-5′-triphosphate (ATP) binding site and thereby RyR2 activation by ATP and low levels of cytosolic [Ca^{2+}]s (<500 nM). Taken together, these results are consistent with the view that RyR2 is an allosteric protein whose activity is regulated not only by luminal Ca^{2+} but also by numerous ligands, both endogenous (Mg^{2+}, ATP, calmodulin) and exogenous (caffeine, ryanodine, tetracaine) that modulate each others' effects on RyR2 activity [27].

Conversely, Jiang et al. [28] using recombinant channels expressed in HEK cells reported that luminal Ca^{2+} acts without influencing cytosolic Ca^{2+} sensitivity of RyR2. Furthermore, these investigators reported that the application of caffeine, considered a sensitizer of cytosolic activation sites [27], actuated RyR2 Ca^{2+} release by sensitizing the luminal sites without affecting cytosolic sensitivity. However, these results were challenged by the work of Porta et al. [29] which reaffirmed previous observations that caffeine indeed acts by sensitizing the RyR2 cytosolic activation sites [27]. Thus, most available evidence suggests that luminal Ca^{2+} allosterically influences the sensitivity of RyR2 to cytosolic Ca^{2+}. As discussed below, the mode of action by which RyR2 is regulated by luminal Ca^{2+} is relevant to understanding the mechanistic control of SR Ca^{2+} release by intra-store Ca^{2+} in normal physiology and disease.

4. Luminal Ca^{2+} and SR Ca^{2+} release termination

Although Ca^{2+} release from the SR should be a self-limiting process because of the restricted size of the Ca^{2+} store, only a fraction of available Ca^{2+} is released during a regular Ca^{2+} transient, thus leaving a substantial Ca^{2+} reserve in the SR [30,31]. Growing evidence, both theoretical and experimental, points to the importance of luminal Ca^{2+} for release termination. Computer simulations by Sobie et al. [15] demonstrated the feasibility of local depletion of SR Ca^{2+} as a signal for the closure of RyR2. At the same time, Terentyev et al. [12,13] used low affinity Ca^{2+} buffers directed to the SR to demonstrate that changes in luminal [Ca^{2+}] play a critical role in Ca^{2+} release termination and refractoriness, whereby a decline in the SR Ca^{2+} store resulted in functional deactivation of RyR2s. Subsequent introduction of luminal Ca^{2+} measurement techniques (with SR-entrapped low-affinity Ca^{2+} indicators) made possible direct detection of Ca^{2+} depletion during both unitary Ca^{2+} release events (Ca^{2+} sparks) as well as during cell-wide Ca^{2+} transients [32,33]. Through paired measurements of cytosolic and luminal [Ca^{2+}], it has been demonstrated that Ca^{2+} spark termination occurs at a certain steady-state threshold of intra-SR [Ca^{2+}] [34–36]. These findings thus offered further support for the role of luminal [Ca^{2+}] in release termination. However, it is important to note that this seemingly premature termination of SR Ca^{2+} release in cardiac myocytes that does not completely deplete the SR of Ca^{2+} can also have other explanations. For example, activation of only a fraction of release sites, a scenario where all the activated sites become fully depleted while idle sites maintain their corresponding SR Ca^{2+} content, could also account for the partial release of the total SR [Ca^{2+}]. Consistent with this notion, Hake et al. [37] using mathematical modeling to compare the true extent of local junctional SR depletion as reported by Ca^{2+} blinks concluded that blinks may underestimate the extent of local SR depletion. Critical to the role

of SR depletion in termination of local Ca^{2+} release is the concept of Ca^{2+} mobility within the SR. Indeed, high Ca^{2+} mobility would tend to prevent severe depletion by hastening Ca^{2+} redistribution from adjacent SR elements. On the other hand, low Ca^{2+} mobility would facilitate SR emptying by restricting the aforementioned redistribution of Ca^{2+} within the SR network. However, studies of the intra-SR diffusion yielded conflicting results with nearly an order of magnitude difference in the apparent diffusion coefficients (8 $\mu m^2/s$ [38] vs. 60 $\mu m^2/s$ [39,40]). Resolving the precise role of local SR Ca^{2+} depletion in termination of Ca^{2+} release will require future investigation into Ca^{2+} diffusion through the SR network as well as further advances in the techniques for measurement of luminal Ca^{2+} in cardiac myocytes.

5. Refractoriness of SR Ca^{2+} release

Following the discharge of Ca^{2+} from the SR, the release mechanism enters a refractory period when it is unresponsive to stimulation by cytosolic Ca^{2+} [41,42]. There is a growing consensus that this refractoriness of Ca^{2+} signaling involves a change of $[Ca^{2+}]$ on the luminal side of the SR [4,12,14,24,42,43]. For example, experimental interventions that either accelerate or slow SR refilling corresponding accelerate or slow Ca^{2+} release restitution, respectively, independent of changes in cytosolic Ca^{2+} [12,13,43]. Importantly, the functional recovery of release lags significantly behind refilling of the SR Ca^{2+} store, an observation made on the level of focal as well as global SR Ca^{2+} release using both direct and indirect methods for assessing intra-SR Ca^{2+} recovery [14,43–46].

The specific components of Ca^{2+} signaling restitution following SR Ca^{2+} release were recently defined by Belevych et al. [45] by concomitant assessment of L-type Ca^{2+} current along with cytosolic and luminal Ca^{2+} recorded from myocytes isolated from normal and post-infarction canine hearts. As illustrated in Fig. 1, SR Ca^{2+} release is accompanied by a transient decrease in luminal Ca^{2+} content. The total time from this initial release to a spontaneous Ca^{2+} release event is composed of a period of refilling of the SR Ca^{2+} stores and a subsequent latency period during which the intra-SR Ca^{2+} levels remain constant. Two-pulse experiments demonstrated that the latency period is comprised of a refractory phase and an “idle” phase. As suggested by theoretical analysis performed by Maltsev et al. [47], the idle period can be attributed to a delay required for synchronization of stochastic release sites that have recovered from refractoriness through a process resembling a phase transition from unitary events to global Ca^{2+} release. Of note, the refractoriness and not the idle period are significantly shortened in post-infarction myocytes thereby accounting for increased vulnerability of these myocytes to diastolic spontaneous Ca^{2+} waves. The distinction between a refilling time and a latency period has also been described on the level of Ca^{2+} spark. Using both isolated cardiomyocytes as well as computational simulations, Ramay et al. [48] separated the recovery of Ca^{2+} spark amplitude from recovery of spark triggering probability for recurring events triggered by ryanodine. These investigators concluded that the former depended only on local SR refilling whereas the latter depended on both refilling and on recovery from RyR2 refractoriness. Accordingly, mathematical theory developed by Rovetti et al. [49] proposed that luminal Ca^{2+}-dependent refractoriness of release units is one of the important variables required for stability of CICR that governs the appearance of arrhythmogenic phenomena such Ca^{2+} waves and alternans. Thus, in

contrast to Ca^{2+} release termination for which the role of functional effects of luminal Ca^{2+} on RyR2 gating is yet to be distinguished from SR Ca^{2+} depletion, release refractoriness does have a distinct component due to the effects upon RyR2 gating (i.e. deactivation).

6. The intermediary luminal Ca^{2+} sensor

Consistent with the presence of a RyR2 Ca^{2+}-mediated modulation site on the luminal side of the SR, it is possible that luminal Ca^{2+} can directly regulate SR Ca^{2+} release by binding directly to the luminal side of RyR2 or via intermediate luminal auxiliary proteins such as triadin (TRD), junctin (JNT) and CASQ2. The notion of direct luminal regulation was further advanced in experiments using recombinant RyR2 variants (expressed in HEK cells devoid of RyR2 luminal auxiliary proteins) with altered responsiveness to luminal Ca^{2+} [28,50,51]. Based on these experiments it was proposed that direct activation of RyR2 by luminal Ca^{2+} independent of accessory proteins and effects of cytosolic Ca^{2+} causes the release of Ca^{2+} from the SR [28,50]. However, a body of evidence has accumulated, suggesting that intermediate regulatory proteins play a role in modulation of SR Ca^{2+} release by luminal Ca^{2+} [13,22,52–57]. Moreover as noted above, most available evidence indicates that luminal Ca^{2+} acts by allosterically affecting RyR2's responsiveness to cytosolic Ca^{2+}, and hence CICR, rather than by directly activating the RyR2 [24–27].

Studies from several laboratories have demonstrated that CASQ2 is capable of conferring luminal Ca^{2+} sensitivity to RyR2s reconstituted in planar lipid bilayers [22,52–54]. The effects of CASQ2 are dependent on the presence of luminal TRD and JNT and are influenced by cytosolic factors such as ATP. In RyR2s activated by cytosolic Ca^{2+} and ATP association with CASQ2 has been reported to decrease RyR2 activity at low luminal Ca^{2+} (<1 mM) and facilitate the channel response to high luminal Ca^{2+} [52,54]. The effects of CASQ2 were mediated by TRD (and/or JNT) as they relied on the presence of luminal TRD and JNT [52]. In the absence of activating ATP, CASQ2 conferred luminal Ca^{2+} dependency by increasing RyR2 activity at high (>1 mM) but not low luminal Ca^{2+} (at which open probability was close to zero) [22]. In both settings (i.e. in the presence or absence of ATP) the ability of CASQ2 and luminal Ca^{2+} to modulate RyR2 function was compromised by arrhythmogenic mutations linked to Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) [22,35]. Future RyR2 reconstitution studies will have to address the precise contributions of direct Ca^{2+} binding and intermediate luminal Ca^{2+} sensor(s) on the RyR2 as well as the role of intracellular affecters (including ATP, Mg^{2+}, calmodulin) in modulation of RyR2 complex gating by luminal Ca^{2+}.

The role of CASQ2 in Ca^{2+} handling has been further demonstrated in studies utilizing myocytes deficient in CASQ2 [13,55,56] and those expressing inhibitory peptides targeting CASQ2-TRD interaction [57]. These studies revealed that the absence of CASQ2 or specifically targeting CASQ2 interaction with the RyR2 complex leads to dysregulated SR Ca^{2+} release. Such alterations in the function of the RyR2 complex manifest an increased frequency of diastolic Ca^{2+} sparks and Ca^{2+} waves. Taken together these results provided further evidence that CASQ2 can act as a luminal Ca^{2+} sensor that cross-talks (possibly through TRD) with RyR2, thereby regulating SR Ca^{2+} release and its termination and/or refractoriness by stabilizing the deactivation of RyR2.

Based on the combination of studies conducted using single RyR2 and myocyte reviewed above it was proposed that RyR2 modulation by CASQ2 and luminal Ca^{2+} involves the following steps [24]: At low luminal [Ca^{2+}], CASQ2 interacts with the RyR2 complex rendering the cytosolic activation site on RyR2 allosterically inhibited (Fig. 1). Refilling the SR Ca^{2+} stores by SERCA during the recovery (restitution) phase once again increases luminal Ca^{2+}, causing CASQ2 to dissociate from the RyR2s rendering these SR Ca^{2+} release channels once more sensitized for yet another activation by cytosolic Ca^{2+}. There is also evidence that has implicated other proteins such as TRD [56,58], JNT [59] and histidine-rich Ca^{2+} binding protein [60] in regulation of RyR2. The potential role of these proteins in mediating the effects of luminal Ca^{2+} on RyR2 function, as well as that of Ca^{2+}-dependent CASQ2 polymerization-depolymerization as part of the luminal signaling cascade [35] requires further investigation.

7. The luminal Ca^{2+} threshold level for spontaneous SR Ca^{2+} release revisited

Over forty years ago Fabiato & Fabiato [2] reported that in addition to CICR, mechanically skinned cardiac cells can exhibit another form of SR Ca^{2+} release that occurs secondary to increased SR Ca^{2+} load, independent of a cytosolic Ca^{2+} trigger. These unprovoked, aberrant SR Ca^{2+} releases, also visualized as propagating Ca^{2+} waves, have been examined in many subsequent studies. A common observation that emerged from these investigations was that whenever luminal Ca^{2+} reached a “threshold” level, this resulted in spontaneous Ca^{2+} release [61,62] Moreover, this apparent intra-SR threshold has been reported to be reduced in cardiac disease as a consequence of either genetic mutations or acquired modifications in the RyR2 channel complex thus accounting for the increased arrhythmogenic propensity in these cell types [28,61,63,64]. These results have sometimes been taken as an indication that spontaneous Ca^{2+} release is directly activated by luminal Ca^{2+} independent of canonical CICR, a process referred to as store-overload-induced Ca^{2+} release (SOICR) [28,50,51]. This view however may not be sufficiently justified.

As recent studies have demonstrated with the aid of direct luminal Ca^{2+} monitoring, spontaneous Ca^{2+} release does not occur once the intra-SR Ca^{2+} returns to its baseline level, but a distinct time delay is present before another spontaneous Ca^{2+} release event may occur (Fig. 1) [45,48,65]. Importantly, this latency to spontaneous Ca^{2+} release is shortened in disease despite lowered diastolic intra-SR [Ca^{2+}] [45,65]. Thus direct monitoring of [Ca^{2+}] SR failed to confirm existence of a luminal [Ca^{2+}] threshold as a sufficient factor for activation of diastolic Ca^{2+} release. The concept of threshold SR Ca^{2+} content while being useful for characterization of SR Ca^{2+} release at steady-state, it may be less applicable to beat-to-beat Ca^{2+} cycling dynamics as occurs in the beating heart and in arrhythmias. The aforementioned behavior further challenges the notion of direct activation of Ca^{2+} release by luminal Ca^{2+}, and as alluded to earlier instead points to recovery from refractoriness as the underlying mechanism responsible for the timing of spontaneous Ca^{2+} release. A correlation between incidents of diastolic release and SR refilling rate consistent with a role for store-dependent refractoriness has been also demonstrated in intact beating hearts [46].

Considering the intrinsic instability of CICR, which requires mechanisms able to exquisitely control it, it is difficult to envision a physiologically useful role for direct luminal Ca^{2+} activation of RyR2. Rather than operating as a stimulator of Ca^{2+} release, luminal modulation appears to constrain RyR2 activity when intra-SR Ca^{2+} level is low such as following SR Ca^{2+} release. As growing evidence suggest [12–14,43,45], following Ca^{2+} release the decrease of luminal Ca^{2+} provides a signal for deactivation of RyR2s thereby contributing to diastolic refractoriness of Ca^{2+} release. Thus CICR that relies on auto-catalytic action of Ca^{2+} at the cytosolic side of the SR is precisely balanced and counteracted by a deactivation mechanism dependent on a reciprocal decrease of Ca^{2+} inside the SR. Importantly, the impairment of this regulatory mechanism accounts for the spontaneous Ca^{2+} release observed in various pathologies as discussed below.

8. Genetic and acquired arrhythmias associated with altered luminal Ca^{2+} control of SR Ca^{2+} release

The importance of RyR2 modulation by luminal Ca^{2+} in healthy heart is highlighted by recent findings indicating that genetic defects in RyR2 and its auxiliary luminal proteins including CASQ2 and TRD are associated with inherited arrhythmias such CPVT [66–68]. CPVT has been attributed to “leaky” RyR2s resulting in spontaneous Ca^{2+} release during diastole [51,69,68].Whereas the link between spontaneous Ca^{2+} release and the consequent membrane depolarization (delayed after depolarization, DAD) is well established and involves NCX stimulated by elevated cytosolic Ca^{2+} [62,70–72], the mechanism responsible for spontaneous Ca^{2+} release in CPVT has been an area of intense investigation. Based on studies using recombinant RyR2, Chen and colleagues suggested that spontaneous release associated with CPVT is due to direct activation of SR Ca^{2+} release by luminal Ca^{2+} [28,51]. However, CASQ2 and other auxiliary luminal proteins were absent from the experimental preparations used by these investigators making their interpretation of the role of luminal Ca^{2+} in a physiological milieu difficult. Furthermore, as already stated the concept of direct activation of RyR2 by luminal Ca^{2+} is not supported in cardiomyocytes by direct experimental measurements of spontaneous Ca^{2+} release either on the level of cytosolic or luminal Ca^{2+} dynamics [45,65]. Conversely, evidence provided by other laboratories suggests that rather than acting by direct luminal Ca^{2+} activation, CPVT-associated genetic mutations of CASQ2 destabilize the deactivation of RyR2 [44,68,73,74]. This is accomplished by an alteration of luminal Ca^{2+} control of RyR2 and thereby shortening Ca^{2+} release refractoriness resulting in premature spontaneous Ca^{2+} release (Fig. 1).

Growing evidence suggests that similar alterations in RyR2 function as those ascribed to CPVT-associated mutations accompany some of the much more common acquired forms of cardiac diseases including both ischemic and non-ischemic cardiomyopathy. In the case of these acquired diseases, post-translational modification of RyR2s by either phosphorylation (via Protein Kinase A and/or Calmodulin-dependent Protein Kinase II) and/or by reactive oxygen and nitrogen species have been implicated as molecular mechanisms of altered Ca^{2+} release function [45,64,65,73,75]. Moreover, research into alterations of RyR2 complex in these acquired forms of cardiac disease provided new mechanistic insight into the role of

altered Ca^{2+} signaling and its refractoriness in arrhythmogenesis. Specifically, RyR2 phosphorylation and oxidation evidenced in post-infarction cardiomyocytes resulted in shortened Ca^{2+} signaling refractoriness that contributed to the increased rate of spontaneous Ca^{2+} waves observed in these cells [45]. Thus it appears that genetic arrhythmogenic mutations within the components of the RyR2 complex along with posttranslational modifications of the Ca^{2+} release channel associated with acquired cardiac disease act through a common mechanism, which affects the RyR2 luminal to cytosolic Ca^{2+} regulatory axis (Fig. 1).

Taken together these studies highlight the importance of RyR2 function and its regulation by its auxiliary luminal proteins and luminal Ca^{2+} in the maintenance of dynamic stability of SR Ca^{2+} release. The loss of such regulatory mechanism results in shortened Ca^{2+} release refractoriness that in turn underlies arrhythmogenic spontaneous Ca^{2+} release and DADs. Refractoriness has been extensively investigated in relation to electrical repolarization and its reserve [8,76]. By analogy it appears Ca^{2+} release refractoriness and CICR stability reserve hold similar promise for understanding and treatment of arrhythmias. Future studies will need to elucidate the role of Ca^{2+} release refractoriness as well as other mechanism(s) responsible for synchronization of Ca^{2+} waves and the ensuing membrane potential depolarization between individual cardiac cells.

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Glossary

AP	Action potential
ATP	Adenosine-5′-triphosphate
[Ca^{2+}]	Ca^{2+} concentration
CICR	Ca^{2+}-induced Ca^{2+} release
CASQ2	Calsequestrin
JNT	Junctin
NCX	Na^{+}–Ca^{2+} exchanger
RyR2	Ryanodine receptors channels
SR	Sarcoplasmic reticulum
SERCA	SR Ca^{2+}-adenosine triphosphatase
SOICR	Store-overload-induced Ca^{2+} release
TRD	Triadin

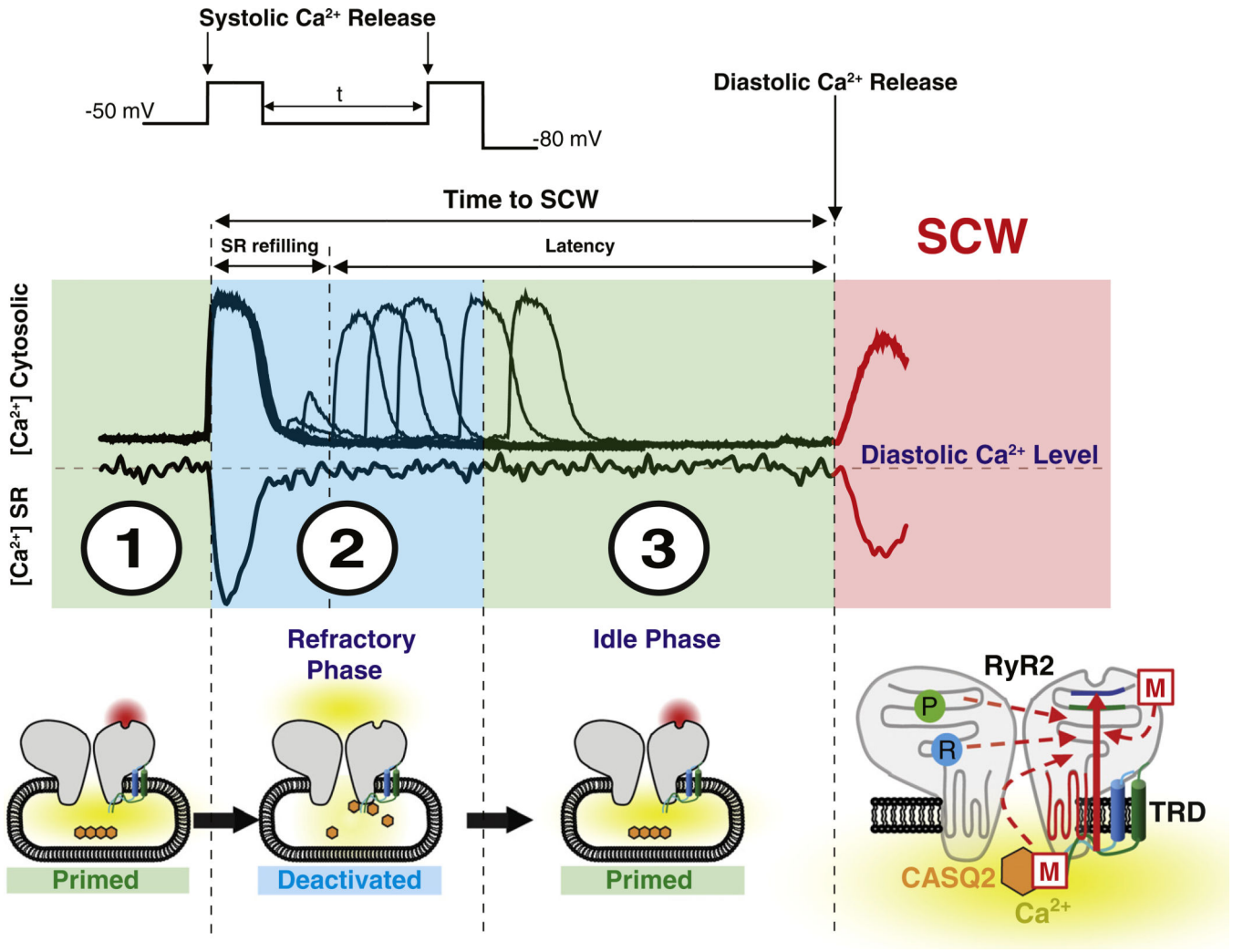

Fig. 1.

Prior to membrane depolarization, the SR Ca^{2+} interacts with CASQ2 functionally detaching it from the RyR2 complex rendering RyR2 primed for activation (primed cytosolic RyR2 activation site highlighted in red) by cytosolic Ca^{2+} (1). During RyR2 activation and the resulting systolic Ca^{2+} release, SR Ca^{2+} content is partially reduced thereby facilitating CASQ2 interaction with the RyR2 complex [via triadin (TRD) and/or junctin (JNT) (2). This interaction results in an allosteric inhibition of cytosolic activation site of the RyR2. The time between systolic SR Ca^{2+} release and spontaneous diastolic Ca^{2+} release is composed by refilling of SR Ca^{2+} store and latency during which the Ca^{2+} within the SR remains constant (2 and 3). The refractory phase reflects the time required for recovery of RyR2 from luminal Ca^{2+} deactivation and can be determined by a 2-pulse protocol (insets 1 and 2). At the completion of the refractory phase, refilling of the SR Ca^{2+} stores leads to dissociation of CASQ2 rendering RyR2 again functionally primed (primed cytosolic RyR2 activation site highlighted in red, 3) underlying thereby an idle phase during which stochastic activation of the recovered SR Ca^{2+} release sites triggers spontaneous diastolic Ca^{2+} waves (SCW). Phosphorylation (P) and redox modification (R) of RyR2 along with mutations in CASQ2 or the SR Ca^{2+} release complex (M) promote shortened recovery of

RyR2 from luminal Ca^{2+} deactivation reducing thereby the refractory period and the time to spontaneous diastolic Ca^{2+} waves.