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Review article

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".

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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^{+} 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

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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 an active 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 be minimal 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