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The cardiac IP3 receptor: Uncovering the role of "the other" calcium release channel

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Normal cardiac function relies on the tight coupling of functionally-related ion channels and transporters in the sarcolemma (plasma membrane and transverse-tubule network, TTs) with calcium-release channels (ryanodine receptors, type 2, RyR2) in the sarcoplasmic reticulum (SR), the intracellular Ca^{2+} storage organelle (reviewed in [1]). Cardiac excitation-contraction (EC) coupling is initiated by membrane depolarization during the action potential (AP) that activates voltage-gated L-type Ca^{2+} channels (LTCC) in the sarcolemma. The small increase in *local* $[Ca^{2+}]_i$ due to the Ca^{2+} flux through the plasma membrane Ca^{2+} channels is detected by nearby (15 nm) clusters of RyR2s in the junctional SR (jSR) to produce Ca^{2+} sparks. This amplification system (termed "Ca^{2+}-induced Ca^{2+} release" or CICR) operates at high gain with great stability and is referred to as "local control" because there is a high $[Ca^{2+}]_i$ only locally between the LTCC and the jSR (in small space between them called the "subspace") [2–4]. The synchronization of Ca^{2+} sparks by the AP produces the cell-wide $[Ca^{2+}]_i$ transient that activates contraction. Instability in cardiac Ca^{2+} management may be due to altered RyR sensitivity ("RyR2 tuning" - see [2,5]), altered spatial organization of local Ca^{2+} release sites, or mutations and variants of the RyR2 protein (as those found in specific diseases such as catecholaminergic polymorphic ventricular tachycardia). These changes in cardiac Ca^{2+} signaling may result in defects in myocyte electrical activity and multiple human cardiac disease phenotypes, including arrhythmia, myopathy, and heart failure [6].

While RyR2 Ca^{2+} release channels have received significant attention by molecular cardiologists, in the past five years the role of a second pathway for internal Ca^{2+}-release has largely been ignored. Specifically, the cellular role(s) for inositol 1,4,5-trisphosphate receptors (IP_3R) have remained elusive. However, there is great and growing interest in cardiac IP_3 signaling due to the known importance of several IP_3-inducing agonists (e.g. angiotensin II, endothelin, and norepinephrine) in hypertrophy and heart failure [7–15].

While agonist-induced IP_3-dependent Ca^{2+} release is readily observed in most tissues, the role of IP_3Rs in cardiac tissue is less clear. The subcellular localization of IP_3Rs in cardiac myocytes has received increasing attention as the field attempts to define the function of these channels. In ventricular myocytes, immunofluorescence studies show that IP_3Rs are found at the Z-lines, in the perinuclear region and in the nuclear membrane [7,16,17]. Moreover, IP_3Rs are found

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in similar locations in both atrial [12] and Purkinje myocytes [18–20]). The role(s) played by these IP_3Rs have yet to be convincingly demonstrated, but provocative suggestions for their function include modulation of transcription [21], amplification of RyR2 Ca^{2+} signals [9], and independent activation through diverse pathways that generate IP_3 [22,23]. While expression of IP_3Rs (mainly type 2 in atrial and ventricular myocytes and type 1 in Purkinje myocytes [18–20]) is about 50-fold less than RyR2s in ventricular myocytes [24], there are still about 20,000 copies per ventricular myocyte and likely more per cell in both atrial and Purkinje myocytes [18].

In this issue of *The Journal of Molecular and Cellular Cardiology*, Hirose and colleagues identify a small population of wide long-lasting Ca^{2+}-release events (WLE) in isolated canine cardiac Purkinje cells that are triggered from subsarcolemmal and perinuclear domains [25]. The biophysical basis of these unusual Ca^{2+} release events is unclear. Do they arise from clusters of RyR2s with some IP_3Rs nearby? Furthermore, what is the stoichiometry and organization of the RyR2s and the IP_3Rs? How is Ca^{2+} release terminated? What role is played by the SR/ER/nuclear envelope Ca^{2+} content? How important are the various lumenal Ca^{2+} buffers such as calsequestrin and calreticulin? What is the biophysical basis for Ca^{2+} wave generation and propagation? How do IP_3Rs contribute to the origin and propagation of Ca^{2+} waves? The answers to these questions are paramount for understanding Purkinje fiber Ca^{2+} signaling and also for understanding the contributions of IP_3Rs to all myocyte Ca^{2+} signaling.

In their new manuscript, Hirose and colleagues demonstrate that wide long-lasting Ca^{2+}-release events are augmented by the IP_3-generating alpha-adrenergic agonist phenylephrine but not in the presence of a phospholipase C inhibitor U73122 (or the putative IP_3R blocker 2APB). Consistent with their previous findings [20], Hirose and colleagues describe type 1 IP_3Rs in the subsarcolemma. However, in their new manuscript, with a new antibody, Hirose et al. identify a second population of perinuclear IP_3Rs not previously observed [20]. Specifically, they show IP_3Rs localize with RyR2s near the nucleus. This proximity of IP_3Rs to RyR2s near the nucleus may underlie the amplification of perinuclear IP_3R Ca^{2+} signals and support a regional Ca^{2+} wave or mini-wave at those sites. Hirose and colleagues also show that on rare occasions wide long-lasting Ca^{2+}-release events may generate cell-wide waves.

Mounting evidence suggests there may be two classes of organized SR Ca^{2+} release sites in myocytes (See Fig. 1). To date, however, high resolution electron micrographs have not specifically revealed such an organization - nor have they denied it. Nevertheless, RyR2s are organized tightly with LTCCs as shown by the Moore group [26], a requirement for local control of EC coupling [27–29], while our group and others have demonstrated that IP_3Rs, ankyrin-B, Na^+/K^+ ATPase, Na^+/Ca^{2+} exchanger and Na^+ channels ($Na_v1.5$) are co-localized nearby at the Z-line [16,17,30,31]. These findings suggest a spatial organization shown in Fig. 1 which, while based on information in the literature, remains somewhat speculative. In this paradigm, the parajunctional SR (pjSR), a region of the SR near the jSR, contains IP_3Rs, and only a few RyR2s. The pjSR is positioned near Na^+ channels, the Na^+/Ca^{2+} exchanger, the Na^+/K^+ ATPase and stabilized by ankyrin-B. In cells with few TT, such as atrial and Purkinje fiber cells, there may be a "corbular" SR (cSR) structure and a "para-corbular" structure (pcSR) in the cell core and in and around the nucleus and ER.

Under normal conditions in cardiac Purkinje fiber cells, depolarization triggers an increase in subsarcolemmal (SSL) Ca^{2+} followed by passive diffusion of Ca^{2+} into the non-SSL region (cellular core) [32] without much CICR amplification. This unique Ca^{2+} signal characterized by restriction of AP-triggered Ca^{2+} release to the SSL region and absence of propagated CICR is attributable to the lack of TTs and the relatively small amount of Ca^{2+} released in the SSL region combined with the insensitivity of core RyR2s in cSR/ER/nuclear regions. Activation of other Ca^{2+} release channels (e.g. IP_3Rs) may have a large effect in myocytes with few TTs

such as atrial and Purkinje fiber cells. Under these conditions, we envision five scenarios governing the cellular impact of Ca^{2+} release from IP_3R-rich sites: 1) It may increase local $[Ca^{2+}]$ at RyR2 clusters in jSR/cSR/ER/nuclear regions, thereby increasing the probability of triggering a Ca^{2+} spark; 2) It may increase the Ca^{2+} spark duration due to the fact that IP_3Rs have unique channel kinetics; 3) It may increase the spatial extent of a single Ca^{2+} spark because Ca^{2+} release occurs away from the Ca^{2+} spark center; (4) It may enhance instability because a Ca^{2+} spark site will be more distant from the central site of elevated $[Ca^{2+}]_i$ (spatial disarray); (5) Additional Ca^{2+} release triggers may be possible due to the sensitivity of the pjSR or pcSR collection of RyR2s and IP_3Rs. The overall actions of IP_3R-dependent altered Ca^{2+} spark rate, and size or likelihood of Ca^{2+} wave propagation, will be constrained by the requirements of pump-leak balance of the SR/ER/nuclear Ca^{2+} stores (see [9]). The provocative studies of Hirose et al [25] in Purkinje fiber cells appear to be consistent with the model described in Fig. 1 and may also be relevant to atrial and ventricular myocytes.

Purkinje fibers constitute a specialized conduction system in the heart allowing for the rapid and coordinated transfer of the propagating depolarization wave through the large ventricular mass. This special role played by Purkinje fibers in heart combined with their spatially intermittent isolation from the ventricular mass make them potential arrhythmogenic sources as illustrated by mapping studies in idiopathic ventricular fibrillation, long-QT and Brugada syndromes, and following myocardial infarction [33–35]. Several groups have explored the link between Ca^{2+} waves and afterdepolarizations in Purkinje fiber cells as a possible mechanism for triggered arrhythmias [36–38]. The findings by Hirose and colleagues raise the possibility that activation of IP_3Rs promotes the development of Ca^{2+} sparks and waves in Purkinje fiber cells and suggests that IP_3R activation may be pro-arrhythmic. The role of IP_3R-dependent enhanced Ca^{2+} release in the ER and nuclear and peri-nuclear regions may affect EC coupling and may also contribute to Ca^{2+}-dependent transcription modulation. Despite provocative and suggestive work to date [21,39], details of targeted Ca^{2+}-dependent transcriptional regulation are missing in heart and represent an important experimental and conceptual challenge. Perhaps the greatest outstanding questions about IP_3Rs in cardiac myocytes remain the most practical: What do they do? Why are they placed where they are? How do they influence Ca^{2+} signaling?

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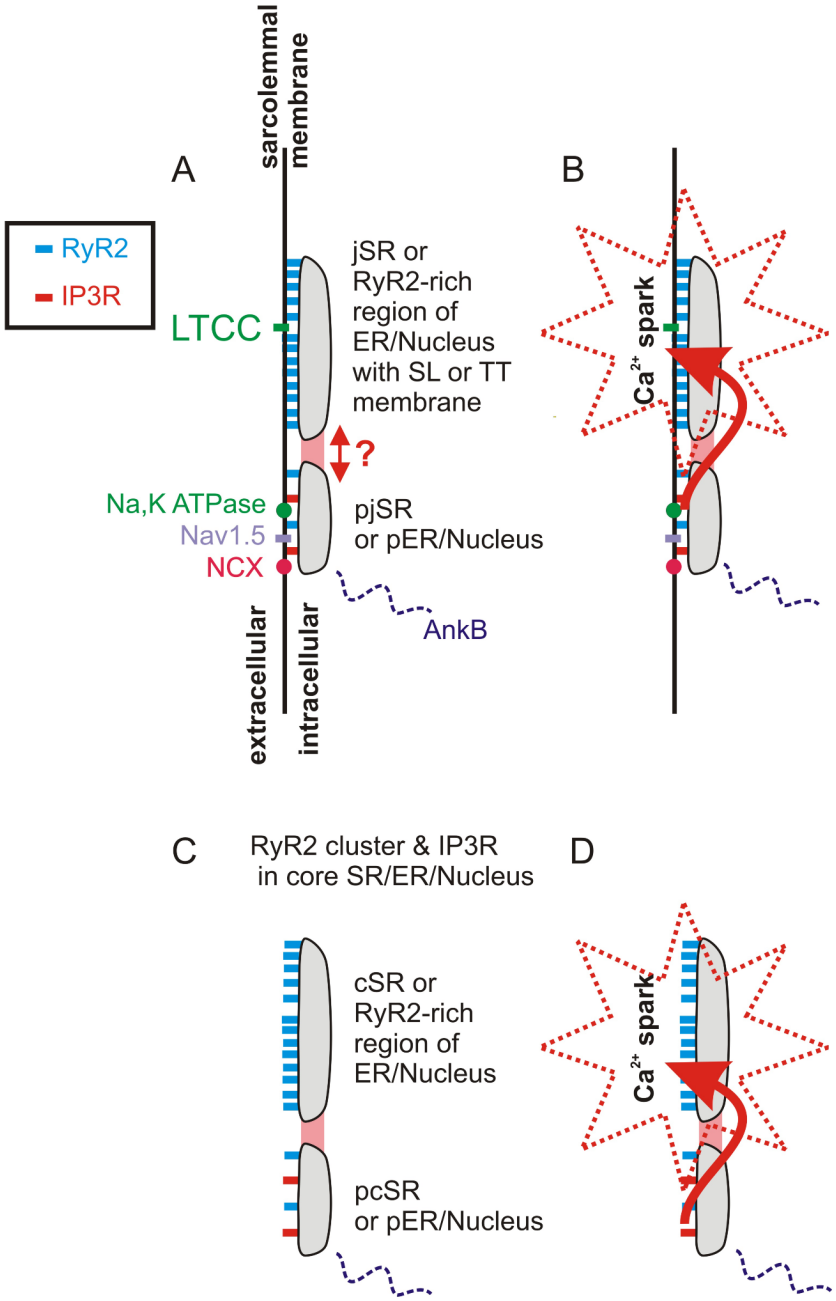

Figure 1. Hypothesized organization of the RyR2 and IP_3Rs in cardiac myocytes

Recent studies suggest the network of intracellular Ca^{2+} storage organelles consists of two components, one containing a large cluster of only RyR2s (labeled here as the junctional SR or corbular SR) and another containing RyR2s mixed with IP_3Rs (labeled the para-junctional SR or para-corbular SR). Because the SR, ER and nuclear envelope Ca^{2+} storage organelles are interconnected, they are represented as a single element in each of the drawings. The interconnecting "network SR" or "free SR" is not shown in these drawings but serves to connect all jSR and cSR and pjSR and pcSR to the ER and nuclear components. **A**. The jSR and pjSR are located along the SL and TT (when present) membranes that contain diverse channels and transporters. **B**. Close proximity of the pjSR to the jSR and the distinct kinetics of pjSR Ca^{2+}

release channels enable the pjSR to influence Ca^{2+} signaling and Ca^{2+} sparks in the jSR. **C**. RyR2 clusters not associated with an extracellular membrane are here loosely termed the corbular SR (cSR) and its nearby para-corbular SR (pcSR). **D**. Close proximity of the pcSR to the cSR and the distinct kinetics of pcSR Ca^{2+} release channels enable the pcSR to influence Ca^{2+} signaling and Ca^{2+} sparks in the cSR.