Mechanisms of SR calcium release in healthy and failing human hearts

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Abstract Normal heart contraction and rhythm relies on the proper flow of calcium ions (Ca^{2+}) into cardiac cells and between their intracellular organelles, and any disruption can lead to arrhythmia and sudden cardiac death. Electrical excitation of the surface membrane activates voltage-dependent L-type Ca^{2+} channels to open and allow Ca^{2+} to enter the cytoplasm. The subsequent increase in cytoplasmic Ca^{2+} concentration activates calcium release channels (RyR2) located at specialised Ca^{2+} release sites in the sarcoplasmic reticulum (SR), which serves as an intracellular Ca^{2+} store. Animal models have provided valuable insights into how intracellular Ca^{2+} transport mechanisms are altered in human heart failure. The aim of this review is to examine how Ca^{2+} release sites are remodelled in heart failure and how this affects intracellular Ca^{2+} transport with an emphasis on Ca^{2+} release mechanisms in the SR. Current knowledge on how heart failure alters the regulation of RyR2 by Ca^{2+} and Mg^{2+} and how these mechanisms control the activity of RyR2 in the confines of the Ca^{2+} release sites is reviewed.

Keywords Ryanodine receptor · Heart failure · Calcium signalling · Calcium release units · Single channel recordings

Introduction

In cardiac muscle, the sarcoplasmic reticulum (SR) is the calcium store from which the release of calcium ions (Ca^{2+}) through ryanodine receptors (RyRs), a family of Ca^{2+} release

channels, is the key determinant of muscle force. Excitation–contraction (E–C) coupling describes the mechanisms linking depolarisation of the surface membrane and the release of Ca^{2+} from the SR, which provides the signal for muscle contraction. Action potentials generated in the surface membrane can propagate into the cell interior along intracellular networks of transverse and axial tubules (Asghari et al. 2009). This causes voltage-dependent L-type Ca^{2+} channels [dihydropyridine receptors (DHPRs)] in these membranes to open and allow Ca^{2+} to enter the cytoplasm. The subsequent increase in cytoplasmic Ca^{2+} concentration [Ca^{2+}] activates RyRs, which releases Ca^{2+} from the SR. The subsequent binding of cytosolic Ca^{2+} to the myofilament protein, troponin C, activates muscle contraction. RyR2 is the sole RyR isoform expressed in cardiac muscle and has been dubbed the cardiac RyR. Allosteric regulation of RyR2 by intracellular Ca^{2+} , Mg^{2+} and ATP (Eisner et al. 1998; Laver and Honen 2008; Meissner 1994; Meissner and Henderson 1987) plays a key role in determining normal cardiac contraction and rhythmicity (Bers 2002b; Meissner 1994), and its disruption can lead to arrhythmia and sudden cardiac death (George 2008).

Heart failure (HF) is a complex disorder that involves changes in the expression of Ca^{2+} handling proteins, Ca^{2+} dynamics and tissue remodelling (George 2008). HF development involves the reduction in cardiac output, which is partly compensated by a chronic increase in sympathetic nervous system activity. Approximately half of HF-related deaths result from cardiac arrhythmia (mainly during early stage HF), while the remainder result from insufficient muscle force (Mozaffarian et al. 2007). Heart dysfunction associated with HF is generally associated with aberrant Ca^{2+} transport across the sarcolemma and SR of cardiac cells (Bers et al. 2003) and the abnormal regulation of RyR2 (Marks 2001; Wehrens et al. 2003). Here, we review how intracellular Ca^{2+} transport is altered in HF, with an emphasis on the Ca^{2+} release mechanisms in the SR. We examine how HF alters the

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regulation of RyR2 by Ca^{2+} and Mg^{2+} and how these ions control the activity of RyR2 in the confines of the Ca^{2+} release sites.

Ca^{2+} transport and its alteration in HF

During heart contraction (systole), E–C coupling utilises a chemical amplifier by which a small influx of Ca^{2+} into the cell through the DHPRs triggers a much larger Ca^{2+} release from the SR. The Ca^{2+} released from the SR contributes between 60 and 95 % to the rise in cytoplasmic $[\text{Ca}^{2+}]$ during E–C coupling, depending on the species (Bers 2002b). During diastole, the cytoplasmic Ca^{2+} level falls and the heart muscle relaxes as Ca^{2+} is either sequestered by the SR via the SR Ca^{2+} -ATPase (SERCA2a) or extruded from the cell by the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) (Bers 2002b). Under normal conditions, SERCA2a takes up approximately 70 % of the Ca^{2+} released by the SR, whereas nearly 30 % is extruded from the cytoplasm via NCX, and approximately 1 % each via sarcolemmal Ca -ATPase and the mitochondrial Ca^{2+} uniporter (Bers 2002b).

Thus, during E–C coupling, the main transport routes for Ca^{2+} across the sarcolemma are the NCX for extrusion and the DHPR for Ca^{2+} entry. In the SR, SERCA2a and RyR2 serve as the main uptake and release pathways, respectively. The concept of auto-regulation posited by Eisner et al. (1998) is that the $[\text{Ca}^{2+}]$ in the cytoplasm and SR lumen take on values such that the inward and outward Ca^{2+} fluxes balance across the SR and sarcolemmal membranes. Therefore, the concentration of Ca^{2+} in the cytoplasm is ultimately determined by the external $[\text{Ca}^{2+}]$ and the sarcolemmal Ca^{2+} pathways, whereas $[\text{Ca}^{2+}]$ in the SR is determined by the cytoplasmic $[\text{Ca}^{2+}]$ and the SR Ca^{2+} pathways.

In HF, these mechanisms for calcium transport across the SR and sarcolemma are altered, and for the reasons given above, the $[\text{Ca}^{2+}]$ in the SR and cytoplasm are also altered (Bers 2006; Bers et al. 2003). Across the sarcolemma, Ca^{2+} extrusion through the sarcolemma by NCX and influx through the DHPR are both increased (Bers et al. 2003), and despite the tendency of these changes to cancel, their net effect is to load cells with Ca^{2+} (Schwinger et al. 1999). Across the SR membrane, Ca^{2+} uptake by SERCA2a is decreased and the Ca^{2+} leak via RyR2 is increased, leading to depletion of SR Ca^{2+} load (luminal $[\text{Ca}^{2+}]$) and an increase of diastolic cytoplasmic $[\text{Ca}^{2+}]$ (Shannon et al. 2003). Enhanced Ca^{2+} extrusion by NCX in HF is partly due to increased NCX expression (Studer et al. 1994) and partly due to gain of function because of increased cytoplasmic $[\text{Ca}^{2+}]$ and the tendency for the NCX-mediated efflux to balance an increased Ca^{2+} influx through DHPRs (Bers 2001; Bers et al. 2003; Hasenfuss and Pieske 2002). Ca^{2+} influx through DHPRs is increased during HF because of increased phosphorylation even though the

density of DHPRs in the membrane is decreased (Chen et al. 2002).

The authors of several studies attribute the reduced SR Ca^{2+} uptake in the failing human heart to a 30–50 % decrease in SERCA2a expression (Jiang et al. 2002; Piacentino et al. 2003; Schmidt et al. 1999; Studer et al. 1994). However, these findings have been challenged in other studies showing no change in SERCA2a expression in failing hearts (Frank et al. 2002; Schwinger et al. 1995), suggesting that changes in SERCA2a activity are due to changes in the regulation of SERCA2a by diminished phosphorylation of phospholamban (Bers 2001; Dash et al. 2001; Schmidt et al. 1999; Schwinger et al. 1999) due to excess protein phosphatase 1 (PP1) activity (Pathak et al. 2005). These contradictory findings on SERCA2a expression may be reconciled by the findings that alteration of SERCA2a expression depends on the type of HF (e.g. ischaemic vs. non-ischaemic idiopathic dilated cardiomyopathy) as well as on the sections from which failing tissues are sampled (e.g. sub-endocardium vs. sub-epicardium) (Lou et al. 2011; Prestle et al. 1999). Although RyR2 expression in HF decreases by 30–35 % (Go et al. 1995; Jiang et al. 2002), HF is associated with an increased diastolic SR Ca^{2+} leak as a consequence of RyR2 remodelling, which is reviewed in detail in the following section.

Structure of Ca^{2+} release sites

The release of Ca^{2+} from the SR occurs mainly at the synapse between the t-tubule and the SR (the dyad). The t-tubule is approximately 125 nm in diameter (McGrath et al. 2009) and is separated from the SR by a gap of 15 nm (the dyad cleft) (Radermacher et al. 1994). Junctophilin-2 is an essential component of the dyadic cleft that spans the gap from the sarcolemma to the SR, and it plays a key role in the development and maintenance of that space (Nishi et al. 2003; Takeshima et al. 2000). The SR is an extensive cytoplasmic network of interconnected tubules [network SR (n-SR)] around the myofibrils (Franzini-Armstrong and Protasi 1997) and junctional SR (j-SR; approx. 26 nm thick with an average diameter about 592 nm) which form extended flattened cisternae that synapse with the t-tubules (Brochet et al. 2005). Electron micrographs show that the j-SR contains an electron-dense material that is formed by calsequestrin (Ca^{2+} binding protein). Each j-SR cisterna carries 10–20 RyRs (Baddeley et al. 2009) arranged in a loose “checkerboard” array with a mean centre-to-centre separation of 37 nm (Asghari et al. 2014) which permit flux of Ca^{2+} from the lumen of the j-SR into the dyad cleft. Ca^{2+} uptake into the SR is mediated by SERCA2a located throughout the n-SR (Tijssens et al. 2003). The DHPRs are randomly distributed in the t-tubule membrane in juxtaposition with the RyRs at a density four to tenfold lower than that of the RyR2, depending on species (Bers 2001; Bers and Stiffel 1993).

Cardiomyocytes in the failing human heart display heterogeneous disruption of t-tubule architecture, with some regions showing normal cell structure (Crossman et al. 2010). T-tubule remodelling is associated with a small loss of RyR2 clusters and approximately a 20–30 % displacement of DHPR colocalization with RyR2 (Crossman et al. 2010; Song et al. 2006), indicating a separation of SR and sarcolemma membranes at the dyad junctions. To date, the process of t-tubule remodelling is not well understood. A clue to the loss of dyads in HF is the recent finding that chronically elevated cytoplasmic Ca^{2+} activates proteases known as calpains that cleave junctophilin-2 (Murphy et al. 2013), which would lead to separation of the t-tubule and SR membrane and loss of RyR2 function.

The RyR2 molecular complex

The RyRs are homotetramers of subunits containing approximately 5,035 amino acids. They form a macromolecular complex with various cytoplasmic proteins, such as the FK506 binding protein (FKBP12.6 and FKBP12), calmodulin (CaM), phosphodiesterase 4D3 (PDE4D3) as well as with protein kinases (protein kinase A, (PKA) and CaM-dependent protein kinase II (CaMKII)) and phosphatases (PP1, PP2A) (Mohler and Wehrens 2007). The luminal protein calsequestrin (CSQ2, the cardiac isoform) is a high-capacity Ca^{2+} buffer in the SR lumen and forms a luminal multi-protein complex with RyR2 and the trans-membrane proteins, triadin and junctin, which also bind to RyR2 (Gyorke et al. 2004; Zhang et al. 1997).

HF results in a remodelling of the RyR2 molecular complex in which there is a reduction in the stoichiometric ratio of various cytoplasmic proteins (e.g. FKBP12.6, PP1, PP2A, PDE4D3) in the RyR2 complex (Marx et al. 2000). The mechanisms and causal relationships responsible for the progression of RyR2 remodelling have not yet been elucidated. It has been proposed that excessive reactive oxygen species (ROS) production by mitochondrial Ca^{2+} overload in the early stages of HF (Ide et al. 1999) contributes to subsequent RyR2 remodelling (Belevych et al. 2011; Gyorke and Carnes 2008). RyR2 contains approximately 84 free thiols (Xu et al. 1998) and several cysteine residues that are susceptible to modulation by redox modifications, including disulfide crosslinking, S-nitrosylation, and S-glutathionylation (Sun et al. 2001). There is evidence that these redox modifications destabilise inter-domain interactions (domain-unzipping) in the RyR2, leading to elevated Ca^{2+} leakage from the SR, which in turn exacerbates the mitochondrial Ca^{2+} overload and ROS production (Shan et al. 2010; Yano et al. 2005). How much of the RyR2 remodelling can be attributed to ROS production and domain-unzipping is not clear, but co-immunoprecipitation assays have revealed that oxidation of RyR2 by H_2O_2 or

RyR2 mutations that cause domain-unzipping leads to a 25–50 % reduction in FKBP12.6 binding to RyR2 (Wehrens et al. 2005; Zissimopoulos et al. 2007).

Although FKBP12.6 association with RyR2 is reduced in HF [50 % in paced dog and 65 % in human (Marx et al. 2000), and 38 % in rabbit (Ai et al. 2005)], the role of FKBP12.6 in RyR2 modulation remains highly controversial (Eisner and Trafford 2002; Jiang et al. 2002; Li et al. 2002; Marks 2003; Marks et al. 2002; Marx et al. 2000). Fluorescent assays indicate that FKBP12.6/RyR2 stoichiometry in mouse myocytes is too low (10–20 %) for it to play a significant role in modulating SR Ca^{2+} release (Guo et al. 2010). However, using pacing-induced failing dog hearts, Shan et al. (2010) showed that RyR2 domain-unzipping (detected by the increase in the accessibility of labelled RyR2 to a fluorescence quencher) was associated with both a loss of FKBP12.6 from the RyR2 complex and increased Ca^{2+} leakage.

HF is accompanied by a chronic increase in sympathetic nerve system activity, increased levels of noradrenaline and chronic activation of β -adrenergic receptors (Braunwald and Chidsey 1965; Bristow et al. 1982; Cohn et al. 1984; Kinugawa et al. 1996). PKA hyperphosphorylation of most of the Ca^{2+} signalling proteins, including the RyR2, has been reported in HF (Kushnir et al. 2010; Lehnart et al. 2005; Wehrens et al. 2006). Although there is a general consensus that increased RyR2 phosphorylation in HF underlies pathological Ca^{2+} signalling, research groups are divided on the phosphorylation mechanism and whether RyR2 phosphorylation causes dissociation of FKBP12.6 from RyR2 (Bers et al. 2003; Dulhunty et al. 2007). One proposal is that RyR2 hyperphosphorylation in failing human hearts is exacerbated by a shift in the balance between the phosphorylation action of PKA and the dephosphorylating actions of PP1 and PP2A due to a reduction in PP1 and PP2A levels in the RyR2 macromolecular complex (Marx et al. 2000).

RyR regulation by Ca^{2+} , Mg^{2+} and ATP

RyR2 is activated by Ca^{2+} at an, as yet, unidentified site on the cytoplasmic side of the membrane (A-site) (Hymel et al. 1988; Smith et al. 1986). In the absence of Ca^{2+} , RyR2 is virtually closed with an open probability (P_o) of less than 10^{-4} (Laver and Honen 2008). RyR2 is activated by Ca^{2+} with a half-maximal activating concentration (K_a) of approximately 5 μM to a maximal P_o of 0.6 (Sitsapesan and Williams 1994a; Xu et al. 1996). The presence of ATP (5 mM in the cytoplasm; Godt et al. 1988) potentiates activation by Ca^{2+} , but it cannot open the channel in the absence of Ca^{2+} . ATP enhances RyR activation with a K_a of 0.22 mM (Kermode et al. 1998) so that at concentrations of >1 mM, ATP has a near-maximal effect. In the presence of ATP (2 mM), RyR2 is activated to a maximal P_o of approximately 1 by cytoplasmic Ca^{2+} with

K_a ranging from 1 to 4 μM depending on the species (Laver and Honen 2008; Walweel et al. 2014). RyR2 is inhibited by Mg^{2+} in the cytoplasm (1 mM) because Mg^{2+} binds to the A-site, occluding this site and preventing Ca^{2+} from binding and activating the RyR, and unlike Ca^{2+} , Mg^{2+} does not cause channel opening (Laver et al. 1997; Meissner 1986). Thus, cytoplasmic Mg^{2+} acts as a competitive non-agonist that shifts the K_a for Ca^{2+} -activation to 36 μM (in the presence of 2 mM ATP) (Laver et al. 2013; Walweel et al. 2014).

RyR2 is also activated by Ca^{2+} on the luminal side of the SR membrane (Sitsapesan and Williams 1994b). The precise mechanism for this is controversial, and various studies have attributed luminal Ca^{2+} activation to two different mechanisms (see reviews by Gyorke et al. 2002; Sitsapesan and Williams 1997). The first, initially proposed by Sitsapesan and Williams (1994b), is that Ca^{2+} activates RyR2 by binding to a luminal-facing Ca^{2+} site that triggers channel opening. In support of this hypothesis, it was demonstrated that the effect of luminal Ca^{2+} can be abolished by tryptic digestion of RyR2 for the luminal side only (Ching et al. 2000). A recent study has identified the amino acids comprising the luminal activation site and confirms that they do face the luminal side of the membrane (Chen et al. 2014). The second mechanism, proposed by Tripathy and Meissner (1996), is that luminal Ca^{2+} passes through the RyR2 pore and activates RyR2 via its cytoplasmic Ca^{2+} activation site. This hypothesis is supported by the strong correlation between the degree of luminal Ca^{2+} activation of RyR2 and the magnitude of the Ca^{2+} flow through the pore (called Ca^{2+} feed-through) and how Ca^{2+} chelators in the cytoplasmic solution can influence this correlation. These disparate findings have been reconciled by a model of Ca^{2+} activation that includes both Ca^{2+} feed-through and luminal Ca^{2+} site (L-site) mechanisms (Laver 2007, 2009, 2010; Laver and Honen 2008). Experimental dissection of these mechanisms was based on a detailed analysis of the duration of the open and closed states of RyR2 (open and closed durations, respectively). Analysis of RyR2 closed durations permitted a study of the L-site-mediated activation in the absence of Ca^{2+} feed-through because feed-through is blocked during channel closed periods. This analysis revealed an allosteric mechanism whereby RyR2 activation has a synergistic dependence on Ca^{2+} binding to luminal and cytoplasmic Ca^{2+} activation sites (Laver and Honen 2008). Analysis of RyR2 open durations revealed a Ca^{2+} feed-through-dependent mechanism of activation similar to that identified by Tripathy and Meissner (1996). The resulting model accounts fully for the Ca^{2+} - and Mg^{2+} -dependent gating of RyR2 (Laver 2010; Laver and Honen 2008). In brief, RyR2 is inhibited by Mg^{2+} in the SR lumen (Laver and Honen 2008), partly because Mg^{2+} binds to the L-site and prevents Ca^{2+} from activating RyR2 and partly because feed-through of luminal Mg^{2+} to the cytoplasmic facing A-site causes RyR2 inhibition (see preceding text). Thus, as a competitive non-

agonist, Mg^{2+} increases the K_a for luminal Ca^{2+} activation from <0.1 mM up to approximately 1 mM (Li et al. 2013; Walweel et al. 2014) which puts the K_a in the physiological range of luminal Ca^{2+} and renders luminal Ca^{2+} a physiologically significant regulator of RyR2 activity.

Two mechanisms for the cytoplasmic Ca^{2+} inhibition of RyR2 have been identified in isolated RyR2, with channels being inhibited by millimolar concentrations of cytoplasmic Ca^{2+} at the I_1 -site (Laver et al. 1995; Meissner et al. 1986) and partially inhibited by micromolar concentrations at the I_2 -site (Laver 2007). Mg^{2+} inhibits RyR2 at the I_1 -site because it acts as a surrogate for Ca^{2+} (Laver et al. 1997). The roles of these inhibition processes in RyR2 function in the cell are not clear. Intracellular $[\text{Mg}^{2+}]$ (1 mM) is below the K_i for this type of Mg^{2+} inhibition (2–6 mM; Walweel et al. 2014) and therefore should only have a minor effect on RyR2 activity.

Thus, RyR2 channels isolated from animal hearts and recorded in artificial bilayers have provided valuable insights into the regulation of RyR2 by intracellular Ca^{2+} , Mg^{2+} and ATP. These studies have provided evidence for at least four different Ca^{2+} -dependent mechanisms, controlled by four $\text{Ca}^{2+}/\text{Mg}^{2+}$ sites (A-, L-, I_1 - and I_2 -sites) on each RyR2 subunit. Although the regulation of RyR2 by intracellular Ca^{2+} and Mg^{2+} is central to our understanding of SR Ca^{2+} release in humans, studies on human RyR2 in healthy and failing hearts are relatively few (Holmberg and Williams 1992; Jiang et al. 2002; Marx et al. 2000; Walweel et al. 2014; Wehrens et al. 2004). The first single channel recordings of human RyR2 were of RyR2 isolated from hearts with myopathies, including ischaemic- and idiopathic dilated cardiomyopathies (Holmberg and Williams 1992). These authors found that RyR2 demonstrated apparently normal activation by cytoplasmic Ca^{2+} (i.e. similar to that seen in sheep and canine hearts), suggesting that heart remodelling in HF had no effect on RyR2 function. Wehrens et al. (2004) showed similar cytoplasmic Ca^{2+} activation properties in recombinant human RyR2. Jiang et al. (2002) also reached the same conclusion based on the finding that the probability of the open state of RyR2 and the gating properties of human RyR2 from healthy hearts in the presence of systolic (5 μM) cytoplasmic Ca^{2+} were no different to those observed in failing hearts with dilated cardiomyopathy and ischaemic cardiomyopathy. However, Marx et al. (2000) did find differences between RyRs taken from healthy and failing hearts in the presence of diastolic cytoplasmic $[\text{Ca}^{2+}]$ (50 nM). These authors reported that 95 % of RyR2 from healthy hearts were inactive under these conditions, whereas only 30 % of RyR2s from failing hearts were inactive, with half of the active ones showing full activity (i.e. P_o approximates 1); this behaviour was not seen at all in RyR2s from healthy hearts. This RyR2 heterogeneity may reflect heterogeneity in structural remodelling of the SR and t-tubular systems recently observed in failing hearts (Crossman et al. 2010). The activation of calpains is known

to disrupt dyad junctions (Murphy et al. 2013) and also to cleave RyR2s (Pedrozo et al. 2010; Rardon et al. 1990); consequently, it is likely that the high activity group of RyR2 consists of calpain-degraded channels.

Only recently has a detailed comparison of human and animal (rat and sheep) RyR2 function been completed (Walweel et al. 2014). These authors found that RyR2s from human, rat and sheep are similarly activated by cytoplasmic Ca^{2+} and inhibited by Mg^{2+} , suggesting that the A-sites are strongly conserved between species. Luminal regulation of RyR2 from human and sheep was very similar but differed from that of the rat, where RyR2 function was less activated by luminal Ca^{2+} and more inhibited by luminal Mg^{2+} , suggesting species differences exist in the L-site. As yet, a full survey of RyR2 function in failing hearts has not been published.

Control of Ca^{2+} release in the dyad

In late diastole, the $[\text{Ca}^{2+}]$ in the dyad cleft (cytoplasmic) is approximately 50–100 nM, and in the j-SR the free $[\text{Ca}^{2+}]$ is approximately 1 mM (Bers 2002b). In this condition, cytoplasmic Ca^{2+} is 40-fold lower than the K_a for the A-site, and cytoplasmic Mg^{2+} exceeds the concentration required to produce half maximum inhibition (K_i , inhibitor constant) for inhibition at the A-site also by approximately 40-fold (see preceding text). Consequently, RyR2 is strongly inhibited, and in the absence of sarcolemmal excitation, each RyR2 in the cell is estimated to have an opening rate of approximately one opening per hour (Cannell et al. 2013). Excitation of the sarcolemma triggers the opening of DHPRs. Computational models predict that the Ca^{2+} flux through a single DHPR will raise $[\text{Ca}^{2+}]$ in the confines of the dyad cleft to 100 μM in <1 ms (Soeller and Cannell 1997). This concentration of Ca^{2+} exceeds the K_a for Ca^{2+} activation of RyR2 (30 μM in the presence of Mg^{2+} , see above), hence triggering the opening of the adjacent RyR2 channels, also within 1 ms. Computer simulations of subsequent Ca^{2+} release from the j-SR (Laver et al. 2013) shows that $[\text{Ca}^{2+}]$ throughout the dyad cleft further increases to 200–300 μM , causing the activity of all RyR2 in the cluster to attain an open probability of approximately 0.5 through a self-regenerative process called Ca^{2+} -induced Ca^{2+} release (CICR). RyR2 activity remains at this level for approximately 20 ms until $[\text{Ca}^{2+}]$ in the j-SR declines to approximately 50 μM (Ca^{2+} release depletes the j-SR of Ca^{2+}), the flux through the RyRs falls and $[\text{Ca}^{2+}]$ in the dyad cleft falls. Once $[\text{Ca}^{2+}]$ falls below this threshold, the computer model predicts a rapid decay and ultimate failure of CICR—a process called induction decay (Laver et al. 2013). Experimental evidence (Guo et al. 2012) supports such a mechanism for termination of CICR (dubbed ‘pernicious attrition’ by these authors). The process of Ca^{2+} release then enters a refractory

period, which continues for approximately 200 ms until the j-SR is replenished (Cannell et al. 2013; Sobie et al. 2005; Zima et al. 2008). Thus, even though SR Ca^{2+} release is inherently self-regenerating because of CICR, it is kept under the control of sarcolemma excitation because of the negative feedback in induction decay. It is now understood that the graded response of SR Ca^{2+} release to excitation of the sarcolemma is due to recruitment of different numbers of these Ca^{2+} release events that are localised at the dyads. These release events are called Ca^{2+} sparks because they appear as bursts of light (approx. 2 μm diameter) in the presence of fluorescent Ca^{2+} indicators. The fluorescence time-course of these Ca^{2+} sparks has an exponential rising phase lasting 10–20 ms, which corresponds to the time that the RyRs are open and releasing Ca^{2+} . When the RyRs close, the fluorescence declines to baseline within approximately 50–100 ms.

The fact that Ca^{2+} on the luminal side of the membrane can also trigger the opening of RyR2 means that Ca^{2+} release from the SR can also be triggered in response to an increase in $[\text{Ca}^{2+}]$ in the SR lumen. Experimental studies demonstrate that the frequency of Ca^{2+} sparks increases in proportion to the second power of luminal $[\text{Ca}^{2+}]$ (Zima et al. 2010). The ability of luminal Ca^{2+} to regulate Ca^{2+} release is an important mechanism underlying the timing of sarcolemmal excitation and heart rhythm. The mechanisms for this were first identified in lymphatic smooth muscle (van Helden 1993; van Helden and Imtiaz 2003) and then more recently in the sinoatrial node of the heart (the pacemaker region) (Ju and Allen 1998; Ju and Allen 2007; Rigg and Terrar 1996; Vinogradova et al. 2005). During diastole, SERCA2a loads the SR to a point at which the elevated luminal $[\text{Ca}^{2+}]$ causes spontaneous opening of RyR2 channels and Ca^{2+} release by the process described above. The subsequent rise in cytoplasmic $[\text{Ca}^{2+}]$ activates the NCX to extrude Ca^{2+} from the cell, causing depolarisation of the sarcolemma (i.e. 3 Na^+ enter for every Ca^{2+} extruded). When this depolarisation is sufficiently large, it triggers an action potential. In the sinoatrial node, this provides a mechanism for adjusting heart rate in response to adrenergic stimulation. However, when this occurs in the ventricle, it may lead to arrhythmias, such as ectopic beats and polymorphic ventricular tachycardia (Bers 2002a).

Control of Ca^{2+} in the failing heart

In human HF, the increased RyR2 activity at diastolic cytoplasmic Ca^{2+} (Marx et al. 2000) should substantially increase the frequency of Ca^{2+} sparks and SR Ca^{2+} leakage. This notion is supported by animal models of HF in which cardiomyocytes from mouse, rabbit and dog all show increased Ca^{2+} spark frequency and SR Ca^{2+} leak compared to healthy controls despite a reduction in SR luminal $[\text{Ca}^{2+}]$ (Kubalova et al. 2005; Maier et al. 2003; Shannon et al.

2003; Song et al. 2005). This has been attributed to a shift in RyR2 sensitivity to luminal Ca^{2+} towards lower concentrations in comparison with controls (Kubalova et al. 2005; Shannon et al. 2003). As yet, there is no published data on the effects of human HF on the luminal Ca^{2+} sensitivity of RyR2.

Abnormalities in Mg^{2+} metabolism and low intracellular $[\text{Mg}^{2+}]$ play a key role in the aetiology of many heart diseases, including HF, and can lead to cardiac arrhythmias (Chakraborti et al. 2002). The authors of a number of studies have reported Mg^{2+} deficiency in patients with HF (Iseri et al. 1952; Ralston et al. 1989). A paced dog model (Haigney et al. 1998) showed a 50 % decrease in intracellular $[\text{Mg}^{2+}]$ in cardiomyocytes compared with healthy controls. Since Mg^{2+} is a competitive inhibitor at RyR2 Ca^{2+} activation sites, a reduction in intracellular $[\text{Mg}^{2+}]$ will activate RyR2 and cause an increase in SR Ca^{2+} release in HF. In addition, low intracellular $[\text{Mg}^{2+}]$ induces an increase in end-diastolic cytoplasmic $[\text{Ca}^{2+}]$ from 50 to 200 nM and the formation of ROS (Chakraborti et al. 2002) which will further increase RyR2 activity and SR Ca^{2+} leakage. As yet, there is no published data on the effects of human HF on the Mg^{2+} sensitivity of RyR2.

Finally, remodelling of the structure of the Ca^{2+} release sites will have a major effect on E–C coupling. Computational models that simulate Ca^{2+} sparks also predict that the amount of Ca^{2+} released during a spark is proportional to the area of the dyad junction (Cannell et al. 2013; Lee et al. 2013). Surprisingly, one model predicts that the ability of DHPRs to trigger RyR2 in the dyad cleft is critically dependent on the separation of the t-tubule and j-SR membranes (Cannell et al. 2013). If the dyad gap increases, as would be the case when junctophilin-2 is degraded, then there would be a proportional decrease in the dyad $[\text{Ca}^{2+}]$, which if it falls below a threshold of 50 μM would effectively terminate SR Ca^{2+} release. Therefore, the increased separation of t-tubule and j-SR membranes at the Ca^{2+} release sites observed by Crossman et al. (2010) and Song et al. (2006) could substantially inhibit SR Ca^{2+} release.

Conclusion

In conclusion, it appears that animal models have provided valuable insights into how intracellular Ca^{2+} transport is altered in human HF. RyR2 channels isolated from human hearts are now known to be subject to similar regulation by intracellular Ca^{2+} , Mg^{2+} and ATP as those isolated from animal hearts. As yet, there has been no complete survey of how HF alters RyR2 regulation in human heart or even in animals for that matter.

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Compliance with Ethical Standards

Conflict of interest Kafa Walweel declares that she has no conflict of interest. Derek R. Laver declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human or animal subjects performed by the any of the authors.

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