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PERSPECTIVES

Cardiac calsequestrin: quest inside the SR

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Although calsequestrin (CASQ), a major sarcoplasmic reticulum (SR) Ca^{2+} binding protein, was discovered more than 30 years ago, its precise roles and modes of operation in both skeletal and cardiac muscle remain to be elucidated (Rios, 2009, this issue; Knollmann, 2009, this issue). Recent years witnessed a significant surge of interest in this Ca^{2+} binding protein after mutations in the gene encoding the cardiac isoform of calsequestrin (CASQ2) were linked to exercise-induced cardiac death due to catecholaminergic polymorphic ventricular tachycardia (CPVT) (Postma *et al.* 2002; Eldar *et al.* 2003). Surprisingly, humans and genetically altered mice devoid of CASQ2 preserve nearly normal cardiac structure and function but develop lethal arrhythmias under conditions of adrenergic stimulation (Postma *et al.* 2002; Knollmann *et al.* 2006; Song *et al.* 2007). Here we provide a brief overview of our current understanding of the functional role and mode of operation of CASQ2 in the heart, of how hearts missing CASQ2 cope in the absence of this protein and of the mechanisms of CPVT.

Role of luminal Ca^{2+} in controlling SR Ca^{2+} release

In cardiac myocytes, Ca^{2+}-induced Ca^{2+} release (CICR) evoked by the action potential (AP) results in a rapid and temporary rise of the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) from ~100 nM to ~1 μM, i.e. the cardiac Ca^{2+} transient. Because of the limited size of the SR Ca^{2+} store, CICR in principle is a self-limiting process which must extinguish once the SR Ca^{2+} store is depleted. However, it is well known that during a regular Ca^{2+} transient only a portion of the total SR Ca^{2+} is released (40–60%; Bassani *et al.* 1993; Delbridge *et al.* 1997), leaving a sizable Ca^{2+} reserve in the SR. This implies that some mechanism is working to terminate the Ca^{2+} release process before the SR Ca^{2+} store is emptied. It is believed that such a restraining mechanism would also be required to stabilize the inherently unstable, self-regenerating CICR and to prevent it from spontaneous activation during diastole.

Growing evidence suggests that changes in Ca^{2+} concentration in the SR lumen ($[Ca^{2+}]_{SR}$) during the exodus of SR Ca^{2+} play an important role in controlling the release process by influencing the functional activity of the cardiac ryanodine receptor (RyR2) channels (Györke & Terentyev, 2008). RyR2 open probability changes as a monotonic function of luminal Ca^{2+} with an EC_{50} ~1 mM (Györke & Györke, 1998). Since resting $[Ca^{2+}]$ in the SR is ~1 mM, declining $[Ca^{2+}]_{SR}$ reduces RyR2 activity forcing, upon reaching a critical level of $[Ca^{2+}]_{SR}$, CICR to terminate. This mechanism, termed luminal Ca^{2+}-dependent deactivation (Terentyev *et al.* 2002) leaves the Ca^{2+} store in a temporarily unresponsive, refractory state preventing untimely SR Ca^{2+} release before the next AP. Mechanistically, luminal Ca^{2+} seems to act on RyR2s by allosterically affecting RyR2s' sensitivity to cytosolic Ca^{2+}, such that lowering luminal Ca^{2+} reduces the sensitivity of RyR2s to activation by cytosolic Ca^{2+} whereas increasing the SR Ca^{2+} load sensitizes the RyR2s (Endo, 1975; Fabiato & Fabiato, 1979; Györke & Györke, 1998; Qin *et al.* 2008; Stevens *et al.* 2009). Additionally, SR Ca^{2+} store size reportedly regulates RyR2 activity by influencing the access of luminal Ca^{2+} to the cytosolic activation sites of the channel (Laver, 2007). Thus, the functional status of RyR2s at any time is determined by combined inputs from cytosolic and luminal Ca^{2+}. While the cytosolic Ca^{2+} activation site has been localized to certain residues of RyR2, the molecular nature of the luminal regulatory side, i.e. the Ca^{2+} sensor, is less clear.

CASQ2 as a luminal Ca^{2+} sensor

Being Ca^{2+} dependent and strategically localized at the points of SR Ca^{2+} release, CASQ2 presents itself as a putative luminal Ca^{2+} sensor for the RyR2. As suggested by crosslinking studies (Froemming & Ohlendieck, 1998), CASQ2 exists in the junctional SR as a mixture of monomers, dimers and multimers. While the multimeric form of CASQ2 functions as a Ca^{2+} buffer (see below), the monomers appear to be responsible for the regulatory function of the protein (Qin *et al.* 2008) (although the role of multimers in RyR2 regulation cannot be excluded). Consistent with its regulatory role, CASQ2 inhibits RyR2 activity at low luminal Ca^{2+} and this inhibition is relieved at elevated luminal Ca^{2+} in reconstitution studies (Györke & Terentyev, 2008). Although direct effects of CASQ2 on RyR2 have been described, according to most reports Ca^{2+}-dependent interactions of CASQ2 with RyR2 are mediated by the integral membrane proteins triadin (TRD) and/or junctin (JN). In further support of the role of CASQ2 as a Ca^{2+} sensor for the termination of SR Ca^{2+} release, expression of a dominant negative CASQ2 mutant (R33Q) has been shown to compromise SR Ca^{2+} release termination and shorten store refractoriness in cardiac myocytes (Terentyev *et al.* 2006). Based on these findings, CASQ2 is thought to modulate RyR2 function in the following manner (Györke & Terentyev, 2008): when $[Ca^{2+}]_{SR}$ is low, CASQ2 is bound to TRD and/or JN and inhibits the activity of RyR2; with SR Ca^{2+} load restored, increased $[Ca^{2+}]$ inhibits binding of CASQ2 to TRD (JN), thereby relieving the inhibitory action of CASQ2 on the RyR2 channel activity. This Ca^{2+}-dependent modulation of RyR2s by CASQ2 has been suggested as the molecular basis for deactivation of RyR2s and store refractoriness following SR Ca^{2+} release. However, modulation through CASQ2 may not be the only mechanism through which SR loading status influences RyR2 function.

CASQ2 as a Ca^{2+} storage reservoir

Since its discovery, calsequestrin has been presumed to play a major role as a Ca^{2+} storage site in the SR. This long-held view, however, is now being re-examined by several investigators (Royer & Ríos, 2009, this issue; Knollmann, 2009, this issue). The amount of Ca^{2+} bound to CASQ2 in cardiac SR has not been determined and is likely to vary with species and experimental conditions. Bers (2001) estimated that

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approximately 50% of Ca^{2+} taken up by the SR is bound to CASQ2 in cardiac muscle. We have shown that an acute, ~3-fold reduction of CASQ2 in quiescent rat myocytes results in a ~2-fold decrease in the SR Ca^{2+} content (Terentyev *et al.* 2003) which would imply that approximately 75% of Ca^{2+} was bound to CASQ2 in myocytes with a complete set of the protein. The reduction in SR Ca^{2+} content was not accompanied by changes in free intra-SR $[Ca^{2+}]$ (Kubalova *et al.* 2004) indicating that the reduced SR Ca^{2+} content indeed reflected a decrease in the amount of Ca^{2+} bound to CASQ2 rather than being caused by Ca^{2+} leaking out from the SR through RyR2s. However, the ~75% of total SR Ca^{2+} bound to CASQ2 suggested by our study is likely to present an upper estimate because of the tendency of the SR to gain Ca^{2+} in rat myocytes at rest (Bers, 2001). Interestingly, ectopic expression of certain CPVT-causing CASQ2 mutants (e.g. CASQ2(G112+5X)) in cardiac myocytes diminishes the ability of the SR to store Ca^{2+} and disrupts myocyte Ca^{2+} handling by disrupting CASQ2 polymerization (Terentyev *et al.* 2008). These results are consistent with the notion that CASQ2 polymers shown to form at high Ca^{2+} levels *in vitro* (Park *et al.* 2004) and found in the SR as filamentous structures (Tijskens *et al.* 2003), represent the high Ca^{2+}-binding capacity form of CASQ2 (Park *et al.* 2004).

In myocytes from genetically modified mice deficient of CASQ2, the total SR Ca^{2+} content was found to be either decreased 2-fold (Song *et al.* 2007) or preserved on the background of a compensatory increase in the SR volume (Knollmann *et al.* 2006). These results also seem to support the buffering role of CASQ2 in the heart. However, such a compensatory SR remodelling was not observed in mice with a 55% reduction of CASQ2 protein secondary to gene-targeted modification of CASQ2 (Casq2-R33Q) (Rizzi *et al.* 2008) or in TRD-deficient mice that exhibited an increase in the SR Ca^{2+} content despite a ~60% reduction in CASQ2 (Knollmann, 2009, this issue). As noted by Knollmann (this issue), one possible interpretation of these findings is that CASQ2 may not play as important a role in storing Ca^{2+} as previously thought. Another possibility, however, is that the changes in expression of other SR proteins such as TRD and JN that occur in these genetically modified mice increase the SR Ca^{2+} content by affecting Ca^{2+} release through RyR2s (see below). Clearly, future studies are needed to define the exact Ca^{2+} buffering role of CASQ2 in the heart.

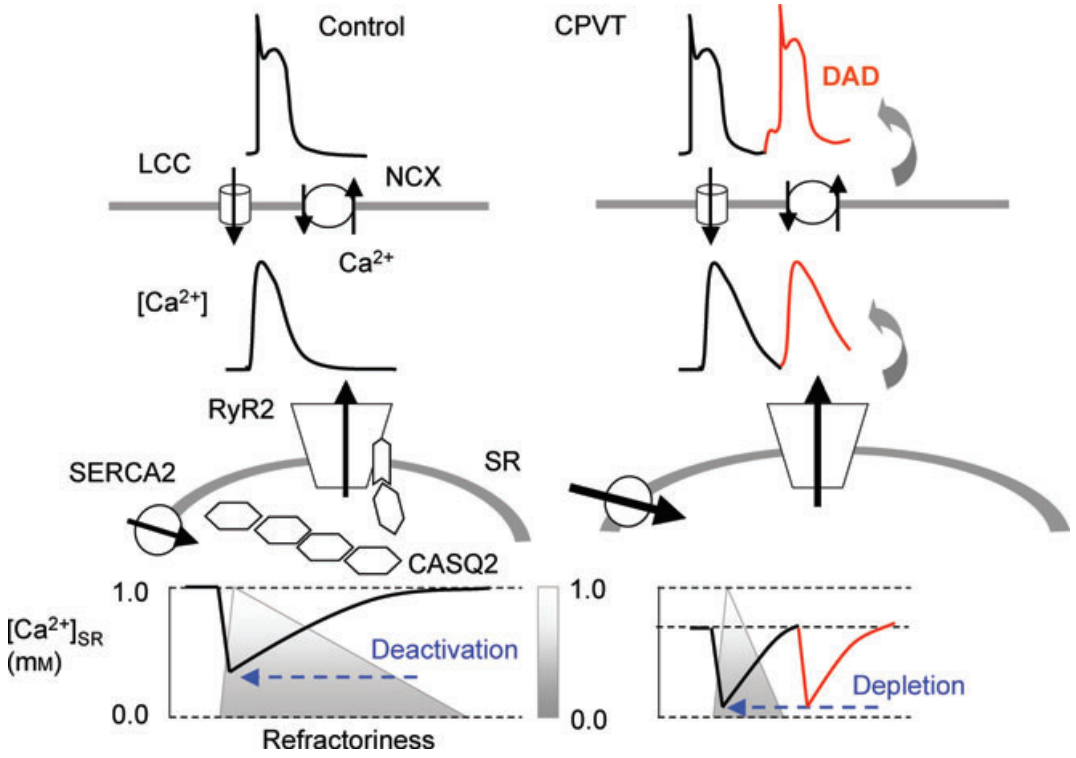

Figure 1. Ca^{2+} handling in myocytes from normal hearts and hearts lacking CASQ2
In a normal myocyte, Ca^{2+} release from the SR evoked by Ca^{2+} entry through voltage-dependent Ca^{2+} channels (LCCs) results in a decline of $[Ca^{2+}]$ in the SR. As $[Ca^{2+}]_{SR}$ declines, Ca^{2+} dissociates from CASQ2 and the Ca^{2+}-free CASQ2 monomers bind to and inhibit RyR2s causing Ca^{2+} release to terminate at a certain threshold $[Ca^{2+}]_{SR}$. The SR Ca^{2+} store stays refractory for some time after luminal Ca^{2+} is recovered by the SERCA2 pump and Ca^{2+}-bound CASQ2 dissociates from TRD. This prevents spontaneous Ca^{2+} release during diastole. In myocytes lacking CASQ2, reduced SR Ca^{2+} binding capacity is compensated by increased SR Ca^{2+} volume. In the absence of the inhibitory influence of CASQ2 increased leak through the RyR2 results in reduced basal $[Ca^{2+}]_{SR}$. In addition, the RyR2s lack the capacity to deactivate until nearly complete depletion stops CICR and store refractoriness is substantially shortened predisposing the SR to premature SR Ca^{2+} release. Extrasystolic elevation of $[Ca^{2+}]_c$ evokes pro-arrhythmic delayed afterdepolarizations (DADs) by stimulating the Na^+/Ca^{2+} exchanger (NCX).

Life without CASQ2: the beat goes on albeit disturbed

Absence of CASQ2 in both humans (homozygous 62delA and 532+1 G/A) and mice ($CASQ2^{-/-}$) have been shown to be associated with CPVT in subjects with otherwise functionally and structurally normal hearts (Postma *et al.* 2002; Knollmann *et al.* 2006; Song *et al.* 2007; Knollmann, 2009, this issue). The finding that the absence of CASQ2 did not cause major alterations in cardiac function and structure is surprising given the presumed importance of CASQ2 to Ca^{2+} handling. Studies in CASQ2-deficient mice by Knollmann *et al.* (2006) and Song *et al.* (2007) provide some important answers in this regard. In the Knollmann *et al.* study, ablation of CASQ2 was accompanied by a striking expansion (∼50%) in SR volume and nearly complete loss of TRD and JN. The 50% increase in SR volume can to a large extent compensate for the loss of SR Ca^{2+} buffering by CASQ2 (Knollmann *et al.* 2006). Additionally, reduction in TRD and JN could contribute to the preserved SR Ca^{2+} content by reducing the leak through RyR2s (Yuan *et al.* 2007; Knollmann, 2009, this issue). In the Song *et al.* (2007) study, although wild-type (WT) and CASQ2-deficient mice had comparable myocyte shortening and Ca^{2+} transient amplitude, the SR Ca^{2+} content was significantly decreased (∼2-fold) in CASQ2-deficient myocytes with respect to WT myocytes. Whereas the SR volume was not assessed in this study, the expression of the SR/ER Ca^{2+}-binding protein calreticulin and RyR2 were increased nearly 10-fold with no apparent change in TRD and JN in CASQ2-deficient hearts. The reason for the different secondary changes in CASQ2-deficient mice found in these two studies is not clear. They may point to the existence of different cellular programmes to compensate for the absence of CASQ2 in the heart. These changes may have helped CASQ2-deficient mice adapt to the loss of this protein; however, they proved inadequate to avert predisposition to CPVT.

While these studies in CASQ2-deficient mice show how the storing function of the SR can be maintained in the absence of CASQ2 it is still difficult to understand how the hearts in these animals (or humans lacking CASQ2) can function without the regulatory influence of CASQ2 if this protein is indeed critical to the control of SR Ca^{2+} release. Recent results with simultaneous cytosolic and luminal Ca^{2+} measurements obtained in myocytes expressing CPVT CASQ2 mutants offer further insights as to how Ca^{2+} handling might be adjusted in the absence of CASQ2 and to the mechanisms of CPVT (Terentyev *et al.* 2008). See accompanying Fig. 1. As mentioned above, in control myocytes, SR Ca^{2+} release terminates at a certain threshold $[Ca^{2+}]_{SR}$ leaving a substantial Ca^{2+} reserve in the SR consistent with the role of luminal Ca^{2+}-dependent deactivation in termination of SR Ca^{2+} release. However, in myocytes expressing a CPVT CASQ2 mutant (R33Q) that disrupts RyR2 regulation by luminal Ca^{2+}, SR Ca^{2+} release does not terminate until the SR is depleted of Ca^{2+}. Since the baseline, diastolic $[Ca^{2+}]_{SR}$ is significantly reduced due to an enhanced leak in the R33Q-expressing myocytes, the overall amount of Ca^{2+} released to the cytosol and hence the amplitudes of the cytosolic Ca^{2+} signals are similar between the two types of cells. Thus, although Ca^{2+} transients (or Ca^{2} sparks) exhibit overall similar amplitudes and shapes in normal and CASQ2-deficient myocytes, the causes of release termination are different: luminal-Ca^{2+}-dependent deactivation in normal myocytes and depletion of Ca^{2+} from the SR in CASQ2-deficient myocytes. Consistent with the dominant role of SR Ca^{2+} depletion in the absence of the regulatory influence of CASQ2 in termination of SR Ca^{2+} release, the fractional SR Ca^{2+} release (portion of total Ca^{2+} released during a twitch) is significantly increased in myocytes from CASQ2-deficient mice (Knollmann *et al.* 2006; Song *et al.* 2007). Thus depletion may be an intrinsic fall-back condition that ensures release termination even in the absence of normal regulation by CASQ2. Although capable of effectively terminating SR Ca^{2+} release, CPVT myocytes lack a physiologically important SR Ca^{2+} reserve and become vulnerable to spontaneous bursts of release due to the absence of an effective refractory mechanism requiring the presence of CASQ2.

Summary

In recent years substantial progress has been made in understanding the roles of the molecular components of the Ca^{2+} release machinery, including CASQ2, in normal cardiac function and disease. In addition to serving as a Ca^{2+} buffer, CASQ2 plays an important part as a dynamic regulator of SR Ca^{2+} release. Ca^{2+}-dependent regulation of the RyR2 channel by CASQ2 contributes to Ca^{2+} release deactivation and Ca^{2+} store refractoriness, and alterations of this mechanism caused by genetic mutations in CASQ2 contribute to the genesis of arrhythmia in CPVT. Despite this substantial progress several important questions remain to be resolved. How do functionally different mutations in CASQ2 and RyR2 result in similar disease phenotypes and what is the role of different compensatory mechanisms and of genetic background in the manifestation of CPVT?

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