J Physiol 587.13 (2009) pp 3113–3115

The Journal of Physiology

PERSPECTIVES

Store overload-induced Ca^{2+} release as a triggering mechanism for CPVT and MH episodes caused by mutations in *RYR* and *CASQ* genes

David H. MacLennan[1]
and S. R. Wayne Chen[2]
[1] *Banting and Best Department of Medical Research, University of Toronto, C. H. Best Institute, 112 College St, Toronto, Ontario, Canada M5G 1L6*
[2] *Libin Cardiovascular Institute of Alberta, Departments of Physiology & Biophysics and Biochemistry & Molecular Biology, University of Calgary, Calgary, Alberta, Canada T2N 4N1*

Email: david.maclennan@utoronto.ca

The disorders

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an arrhythmogenic cardiac disorder caused, in part, by dominant mutations in *RYR2*, the cardiac ryanodine receptor (RyR2) gene (Liu *et al.* 2009). The disorder manifests as syncopal events and sudden cardiac death, usually in response to intensive exercise; elevated catecholamines induce arrhythmogenic episodes that respond to β-blockers.

In other CPVT families arrhythmia is caused by recessive mutations in *CASQ2* that cause truncation and/or misfolding of cardiac calsequestrin (CASQ2). In a mouse model, *casq2*-null or partially null mice also exhibit arrhythmias (Knollmann *et al.* 2006; Chopra *et al.* 2007; Knollmann, 2009, this symposium).

Malignant hyperthermia (MH) is an acute response to volatile anaesthetics, such as halothane, and depolarizing muscle relaxants, characterized by hypermetabolism, skeletal muscle contracture and fever (Loke & MacLennan, 1998) and caused, in part, by dominant mutations in *RYR1*. MH episodes can be reversed by dantrolene, an RyR1 Ca^{2+} release channel blocker. RyR1 MH mutant channels are activated by low levels of caffeine or halothane (Tong *et al.* 1999).

As a corollary of CPVT, ablation of *casq1* causes MH (Dainese *et al.* 2009; Protasi *et al.* 2009, this symposium). *CASQ1* mutations have not yet been reported to cause human MH.

Characteristics of RyR2 mutants in CPVT

It is accepted that CPVT arrhythmias result from repetitive, premature activation of RyR2. It has long been known that RyR is activated by elevation of luminal Ca^{2+} beyond a certain threshold (Palade *et al.* 1983; Venetucci *et al.* 2008). Here, we refer to this phenomenon as store overload-induced Ca^{2+} release (SOICR). RyR2 CPVT mutant channels expressed in the human embryonic kidney cell line HEK-293 were activated by much lower luminal Ca^{2+} levels than wild-type (wt) (Jiang *et al.* 2004, 2005). Critically, the frequency of Ca^{2+} oscillations was markedly increased and the size of the Ca^{2+} store was decreased. When single channel activities of RyR2 CPVT mutants were measured in planar lipid bilayers with 45 nM cytoplasmic Ca^{2+}, incremental elevation of luminal Ca^{2+} from 0 to 1000 μM increased the probability of opening to 0.3–0.4 for CPVT mutant RyR2 *vs.* 0.02 for wt RyR2. These results demonstrate that RyR2 CPVT mutant channels, in isolation from other sarcoplasmic reticulum (SR) proteins, manifest a lower SOICR threshold than wt RyR2.

Characteristics of RyR1 mutants in MH

In comparable studies, RyR1 MH mutant channels expressed in HEK-293 cells are activated by much lower luminal Ca^{2+} levels than wt (Jiang *et al.* 2008). Furthermore, in single channel measurements, the MH-triggering agent, halothane, enhanced the frequency of activation of RyR1 MH mutant channels in the presence of luminal Ca^{2+}, but had no effect in the absence of luminal Ca^{2+}. Appropriately, dantrolene suppressed SOICR in HEK-293 cells expressing an RyR1 MH mutant. These results demonstrate that RyR1 MH mutations lower the threshold for luminal Ca^{2+} activation, that volatile anaesthetics further lower the SOICR threshold, and that dantrolene suppresses SOICR.

Characteristics of CASQ mutants in CPVT and MH

Calsequestrin plays at least three prominent roles: (1) it increases the SR luminal total Ca^{2+} content through its highly cooperative, high capacity, low affinity Ca^{2+} binding; (2) it buffers free Ca^{2+} levels in the SR lumen; and (3) it may convey bound Ca^{2+} into the RyR pore through its strategic localization in relation to triadin (Tri) and RyR (Wang *et al.* 1998; MacLennan & Reithmeier, 1998; Park *et al.* 2003; Royer & Ríos, 2009, this symposium). Mutations in CASQ can reduce that portion of the total Ca^{2+} binding and buffering capacity of the lumen that can be ascribed to CASQ in two ways: (1) through complete or partial ablation, as in *casq*-null mice or (2) through point or truncation mutations that affect key residues involved in such mechanisms as 'domain swapping' required for proper CASQ oligomerization, leading to cooperative, high capacity Ca^{2+} binding (Kim *et al.* 2007).

Common features of CPVT and MH

First, CPVT- and MH-susceptible individuals do not display abnormalities in muscle structure or function at rest. A CPVT episode is triggered in response to physical or emotional stress. A fulminant MH episode is triggered in response to halothane, stress or elevated temperature. As in arrhythmias, skeletal muscle contracture is a manifestation of abnormal, repetitive Ca^{2+} cycling. Second, causal *RYR* mutations are dominant, whereas causal *CASQ* mutations are recessive.

A unifying theory identifying SOICR as the common triggering mechanism for CPVT and MH episodes arising from mutations in *RYR* and *CASQ* genes

We propose that the aetiology triggering the arrhythmias caused by *RYR2* or *CASQ2* CPVT mutations or the muscle contracture caused by *RYR1* or *CASQ1* MH mutations is the same – the free SR luminal Ca^{2+} level repetitively exceeds the threshold for SOICR (Fig. 1). In CPVT, RyR2 mutants have a lower threshold for SOICR so that enhanced Ca^{2+} uptake, induced by β-adrenergic stimulation of the sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA), causes the level of free Ca^{2+} to overshoot its *lowered* SOICR threshold. CASQ2 mutants have reduced Ca^{2+} binding and buffering capacity, allowing free luminal Ca^{2+} levels to overshoot the *normal* RyR2 SOICR threshold when SERCA-dependent Ca^{2+}

DOI: 10.1113/jphysiol.2009.172155

uptake is increased. In either case, repetitive SOICR manifests as arrhythmia.

In MH, RyR1 mutants have a lowered threshold for SOICR. In the presence of halothane, which *further lowers its mutation-lowered* SOICR threshold, RyR1 will be triggered to open when the free SR luminal Ca^{2+} level overshoots its *lowered* SOICR threshold. CASQ1 mutants have a reduced Ca^{2+} binding and buffering capacity, allowing free luminal Ca^{2+} levels to overshoot the *halothane-lowered* SOICR threshold of wt RyR1. In either case, a cycle is initiated in which SERCA pumps only partially refill the luminal Ca^{2+} store before SOICR is triggered. Repetitive SOICR elevates cytosolic Ca^{2+} to provide the signal for skeletal muscle contracture and hypermetabolism, whereas fever arises from enhanced ATP hydrolysis by SERCA and myosin.

An alternative proposal for the relationship between RyR and CASQ in CPVT and MH

The unifying theory proposed above has a simplicity not found in an alternate explanation of the aetiology of CPVT and MH. Experiments by Györke & Terentyev (2008; Györke *et al.* 2009, this symposium) and Qin *et al.* (2008) lead them to postulate that a CASQ2–Tri–RyR2 complex regulates RyR2 function and to assign a 4th and even a 5th role to CASQ in this complex: (4) the deactivation of RyR in low luminal Ca^{2+}; and (5) the activation of RyR by elevated luminal Ca^{2+}. Their theory is that mutations in CASQ2 (and, by extension, mutations in CASQ1) alter protein–protein interactions within the complex, resulting in the abnormal openings of RyR2 (or RyR1) that cause episodes of CPVT (or MH). There is general agreement in both theories and for both disorders, whether caused by mutations in RyR or CASQ, that manifestations of disease are ultimately caused by the abnormal response of RyR channels to elevations in luminal Ca^{2+}.

To evaluate the relative merits of the hypotheses that SOICR acts directly through RyR or through a RyR–Tri–CASQ complex, let us consider critical features of the diseases. First, the dominant inheritance of RyR mutations in CPVT and MH is expected, since openings of abnormal channels in the presence of normal

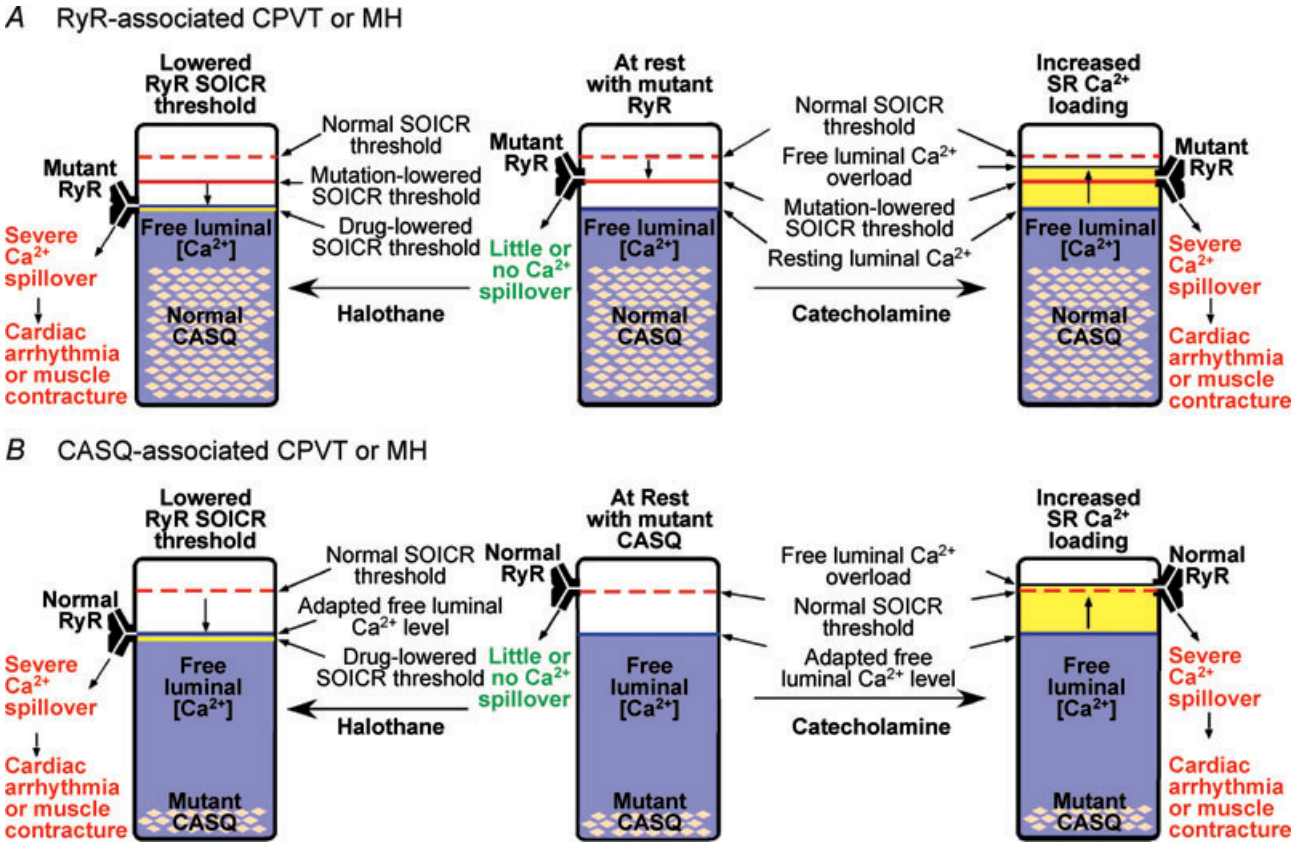

Figure 1. A model unifying the aetiology of CPVT and MH arising from mutations in *RYR* or *CASQ* genes
The different thresholds for SOICR and the free SR luminal Ca^{2+} levels in CPVT or MH, associated with mutations in *RYR* (*A*) or *CASQ* genes (*B*), are illustrated in the resting state (middle panels) and stimulated states (left and right panels). The normal SOICR threshold is depicted as a dashed red bar. The SOICR threshold that is lowered as a consequence of RyR mutations, is depicted as a continuous red bar. The SOICR threshold that is lowered by drugs (halothane or caffeine) is depicted as a yellow bar in the left panels. The SR free luminal Ca^{2+} level is represented as a blue area. The pink lozenges within the blue areas represent functional Ca^{2+}–CASQ complexes, which are reduced as a result of point or truncation mutants (as depicted) or abolished as a result of null mutations (not depicted). The yellow areas above the blue areas in the right panels represent an elevation, even if only transient, in the free Ca^{2+} levels, which, we propose, will occur when SERCA activity is enhanced by catecholamines. When the SOICR threshold is lowered below the SR free luminal Ca^{2+} level (left panels), or when the SR free luminal Ca^{2+} level surpasses, even transiently, the normal SOICR threshold (right panels), SOICR occurs, leading to a spillover of SR Ca^{2+} that can trigger spontaneous Ca^{2+} release, cardiac arrhythmias or skeletal muscle contracture.

closed channels can cause full disease manifestations. Why then are all cases of CPVT caused by CASQ2 mutations inherited recessively? Surely if dysregulation of a CASQ2–Tri–RyR2 complex were causal, then mutation of one CASQ2 allele would be sufficient to trigger abnormal activation in abnormal complexes, manifesting as an arrhythmia – but this is not the case.

Second, CASQ2 CPVT causal mutations have diverse properties: R33Q activates wt RYR2 channels in response to elevated luminal Ca^{2+}, whereas L167H did not (Qin *et al.* 2008); R33Q is an unstable molecule and may be degraded (Rizzi *et al.* 2008); R33Q causes a compensatory elevation of the expression of RyR2 and calreticulin (Song *et al.* 2007); R33Q, but not $CASQ^{del}$ lowers both basal and nadir SR Ca^{2+} levels (Terentyev *et al.* 2008). In contrast to those papers that relate *differences* among CASQ2 mutants to CPVT, one paper pinpoints the *common* feature of all CASQ2 CPVT mutants – their loss of the ability to oligomerize properly and therefore to bring about cooperative high capacity Ca^{2+} binding (Kim *et al.* 2007). Thus, the critical *common* feature in CASQ2-associated CPVT is a decrease in luminal Ca^{2+} binding and Ca^{2+} buffering that would permit an overshoot of luminal Ca^{2+} when SERCA pumps are stimulated.

Final questions are: why, if CASQ is critical to the regulation of normal RyR channels, do *CASQ2*-null humans or *casq2*-null mice, susceptible to CPVT, only display arrhythmias under stressful conditions that elevate luminal Ca^{2+} loading; and why do *casq1*-null mice only display MH symptoms in the presence of MH-triggering anaesthetics or heat? The answers cannot lie in CASQ–Tri–RyR interactions, since CASQ is absent. Therefore, the answer is most likely to lie in normal RyR itself, which, we propose, is intrinsically programmed to open in response to a tsunami of Ca^{2+} in a luminal space depleted of its capacity to buffer a surge of incoming Ca^{2+} (CPVT), or to open in response to halothane, which lowers its SOICR threshold (MH).

References

Chopra N, Kannankeril PJ, Yang T, Hlaing T, Holinstat I, Ettensohn K, Pfeifer K, Akin B, Jones LR, Franzini-Armstrong C & Knollmann BC (2007). Modest reductions of cardiac calsequestrin increase sarcoplasmic reticulum Ca^{2+} leak independent of luminal Ca^{2+} and trigger ventricular arrhythmias in mice. *Circ Res* **101**, 617–626.

Dainese M, Quarta M, Lyfenko AD, Paolini C, Canato M, Reggiani C, Dirksen RT & Protasi F (2009). Anesthetic- and heat-induced sudden death in calsequestrin-1-knockout mice. *FASEB J*; DOI: 10.1096/fj.08-121335.

Györke S, Stevens SCW & Terentyev D (2009). Cardiac calsequestrin: quest inside the SR. *J Physiol* **587**, 3091–3094.

Györke S & Terentyev D (2008). Modulation of ryanodine receptor by luminal calcium and accessory proteins in health and cardiac disease. *Cardiovasc Res* **77**, 245–255.

Jiang D, Chen W, Xiao J, Wang R, Kong H, Jones PP, Zhang L, Fruen B & Chen SR (2008). Reduced threshold for luminal Ca^{2+} activation of RyR1 underlies a causal mechanism of porcine malignant hyperthermia. *J Biol Chem* **283**, 20813–20820.

Jiang D, Wang R, Xiao B, Kong H, Hunt DJ, Choi P, Zhang L & Chen SRW (2005). Enhanced store overload-induced Ca^{2+} release and channel sensitivity to luminal Ca^{2+} activation are common defects of RyR2 mutations linked to ventricular tachycardia and sudden death. *Circ Res* **97**, 1173–1181.

Jiang D, Xiao B, Yang D, Wang R, Choi P, Zhang L, Cheng H & Chen SRW (2004). RyR2 mutations linked to ventricular tachycardia and sudden death reduce the threshold for store-overload-induced Ca^{2+} release (SOICR). *Proc Natl Acad Sci U S A* **101**, 13062–13067.

Kim E, Youn B, Kemper L, Campbell C, Milting H, Varsanyi M & Kang C (2007). Characterization of human cardiac calsequestrin and its deleterious mutants. *J Mol Biol* **373**, 1047–1057.

Knollmann BC (2009). New roles of calsequestrin and triadin in cardiac muscle. *J Physiol* **587**, 3081–3087.

Knollmann BC, Chopra N, Hlaing T, Akin B, Yang T, Ettensohn K, Knollmann BE, Horton KD, Weissman NJ, Holinstat I, Zhang W, Roden DM, Jones LR, Franzini-Armstrong C & Pfeifer K (2006). *Casq2* deletion causes sarcoplasmic reticulum volume increase, premature Ca^{2+} release, and catecholaminergic polymorphic ventricular tachycardia. *J Clin Invest* **116**, 2510–2520.

Liu N, Rizzi N, Boveri L & Priori SG (2009). Ryanodine receptor and calsequestrin in arrhythmogenesis: What we have learnt from genetic diseases and transgenic mice. *J Mol Cell Cardiol* **46**, 149–159.

Loke JCP & MacLennan DH (1998). Malignant hyperthermia and central core disease: disorders of Ca^{2+} release channels. *Am J Med* **104**, 470–486.

MacLennan DH & Reithmeier RA (1998). Ion tamers. *Nat Struct Biol* **5**, 409–411.

Palade P, Mitchell RD & Fleischer S (1983). Spontaneous calcium release from sarcoplasmic reticulum. General description and effects of calcium. *J Biol Chem* **258**, 8098–8107.

Park H, Wu S, Dunker AK & Kang C (2003). Polymerization of calsequestrin. Implications for Ca^{2+} regulation. *J Biol Chem* **278**, 16176–16182.

Protasi F, Paolini C & Dainese M (2009). Calsequestrin-1: a new candidate gene for malignant hyperthermia and exertional/environmental heat stroke. *J Physiol* **587**, 3095–3100.

Qin J, Valle G, Nani A, Nori A, Rizzi N, Priori SG, Volpe P & Fill M (2008). Luminal Ca^{2+} regulation of single cardiac ryanodine receptors: insights provided by calsequestrin and its mutants. *J Gen Physiol* **131**, 325–334.

Rizzi N, Liu N, Napolitano C, Nori A, Turcato F, Colombi B, Bicciato S, Arcelli D, Spedito A, Scelsi M, Villani L, Esposito G, Boncompagni S, Protasi F, Volpe P & Priori SG (2008). Unexpected structural and functional consequences of the R33Q homozygous mutation in cardiac calsequestrin: a complex arrhythmogenic cascade in a knock in mouse model. *Circ Res* **103**, 298–306.

Royer L & Ríos R (2009). Deconstructing calsequestrin. Complex buffering in the calcium store of skeletal muscle. *J Physiol* **587**, 3101–3111.

Song L, Alcalai R, Arad M, Wolf CM, Toka O, Conner DA, Berul CI, Eldar M, Seidman CE & Seidman JG (2007). Calsequestrin 2 (CASQ2) mutations increase expression of calreticulin and ryanodine receptors, causing catecholaminergic polymorphic ventricular tachycardia. *J Clin Invest* **117**, 1814–1823.

Terentyev D, Kubalova Z, Valle G, Nori A, Vedamoorthyrao S, Terentyeva R, Viatchenko-Karpinski S, Bers DM, Williams SC, Volpe P & Gyorke S (2008). Modulation of SR Ca release by luminal Ca and calsequestrin in cardiac myocytes: effects of CASQ2 mutations linked to sudden cardiac death. *Biophys J* **95**, 2037–2048.

Tong J, McCarthy TV & MacLennan DH (1999). Measurement of resting cytosolic Ca^{2+} concentrations and Ca^{2+} stores in HEK-293 cells transfected with malignant hyperthermia or central core disease mutant Ca^{2+} release channels. *J Biol Chem* **274**, 693–702.

Venetucci LA, Trafford AW, O'Neill SC & Eisner DA (2008). The sarcoplasmic reticulum and arrhythmogenic calcium release. *Cardiovasc Res* **77**, 285–292.

Wang S, Trumble WR, Liao H, Wesson CR, Dunker AK & Kang CH (1998). Crystal structure of calsequestrin from rabbit skeletal muscle sarcoplasmic reticulum. *Nat Struct Biol* **5**, 476–483.

Acknowledgements

This work was supported by Grants to D.H.M. from the Canadian Institutes of Health Research (CIHR) and to S.R.W.C. from the National Institutes of Health (HL75210) and CIHR and the Alberta Heritage Foundation for Medical Research.