Unraveling the Mechanisms of Catecholaminergic Polymorphic Ventricular Tachycardia

Vivek Iyer and Antonis A. Armoundas, *Member, IEEE*

***Abstract*— Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a heritable arrhythmia unmasked by exertion or stress, characterized by triggered activity and sudden cardiac death in affected patients. In this study we used a mathematical model to simulate two mutations linked to CPVT, in cardiac calsequestrin (CSQN2) and the ryanodine receptor (RyR2). The aim of the present study is to characterize the mutations responsible for CPVT and establish the mechanistic basis for spontaneous Ca^{2+} release events that lead to delayed afterdepolarizations (DADs) and triggered arrhythmias.**

Simulated calcium transients in the mutant CSQN2 model recapitulated the smaller amplitude and time to peak, as well as accelerated recovery from inactivation seen in experiments. When simulated CSQN2-mutant myocytes were paced in current-clamp mode, DADs were observed, suggesting that accelerated recovery of RyR2 induced by impaired luminal Ca^{2+} sensing can lead to the triggered activity observed in the mutant CSQN2. Simulations of mutant RyR2 suggest that the hyperactive, "leaky" receptors characteristic of reduced FKBP12.6 function may be centrally involved in triggering DADs.

These results provide plausible mechanisms by which defects in RyR2 gating may lead to the cellular triggers of CPVT, with implications for the development of targeted therapies.

I. Introduction

CATECHOLAMINERGIC polymorphic ventricular tachycardia (CPVT) is a heritable arrhythmia unmasked by exertion or stress. CPVT is a genetically heterogeneous disease, and mutations in the sarcoplasmic reticulum (SR) Ca^{2+} release channel (RyR2) and the SR calcium buffer protein calsequestrin (CSQN2) have been reported in families affected by this disorder (see [2] for review). The precise mechanisms by which these mutations cause arrhythmias are as of yet unclear, although many hypotheses have been advanced on the basis of experimental characterizations in transgenic animal models [1, 3-7].

CPVT likely arises through disrupted regulation of Ca^{2+} release from the SR through the RYR2. RyRs are activated by cytosolic Ca^{2+} that enters through the voltage-gated L-type calcium channels. The release process is likely also regulated by Ca^{2+} in the SR, or luminal side of the release complex.

Mutations in the RyR2 gene cause an autosomal dominant form of CPVT. Although the idea is controversial [8], it has been advanced that disruption of the FKBP12.6–RyR2 interaction [5, 7] is responsible for hyperactive "leaky" release receptors, which in turn promote the spontaneous Ca^{2+} release events that lead to arrhythmogenic delayed afterdepolarizations (DADs). This theory was supported by experiments in FKBP12.6 (-/-) mice, which experienced high incidence of ventricular tachyarrhythmias and sudden cardiac death [7].

CSQN2 mutations responsible for CPVT are inherited in an autosomal dominant or autosomal recessive fashion. Experiments in ventricular myocytes expressing mutant CSQN2 [1] and under- and over-expressing CSQN2 [9] have suggested that impaired luminal Ca2+ sensing may promote the spontaneous SR release events and DADs underlying the ventricular tachyarrhythmias characteristic of the disease.

At present, the precise mechanistic basis for the genesis of DADs in CSQN2 and RyR2 mutations has not been determined. This study uses a detailed mathematical model of excitation contraction coupling [10] to investigate specific theories of DAD formation. Models for mutant CSQN2 and RyR2 are created on the basis of experiments in ventricular myocytes. The model is then used to analyze the mechanism of formation of the DADs, the cellular triggers of arrhythmia in affected patients.

II. Methods

Simulations were performed using a model of the human left ventricular myocyte developed by us (see [10] for details). This model incorporates a RyR2 gating model with explicit representations of opening (from resting state C1 to O1), release termination via adaptation (from open state O1 to C2), and recovery upon further increases in Ca^{2+} (from C2 to O1 and O2) [11].

A. Modeling luminal Ca^{2+}-sensing in wild-type and CSQN2 mutants

RyR2 channels are activated by free Ca^{2+} on the luminal (intra-SR) face of the protein, as channel open probability rises with increases in SR Ca^{2+} in recordings of the RyR2 Ca^{2+} flux in lipid bilayers [12]. The model opening

Manuscript received April 14, 2006. The work was supported by an American Heart Association Beginning Grant-in-Aid to AAA (#0365304U).

A. A. Armoundas is with the Cardiovascular Research Center, Massachusetts General Hospital, Charlestown, MA 02139, USA (617-726-0930; fax: 617-726-5806; e-mail: aarmoundas@partners.org).

V. Iyer, was with the Cardiovascular Research Center, Massachusetts General Hospital, Charlestown, MA 02139, USA. He is now with the Columbia University College of Physicians and Surgeons, Department of Medicine, New York, NY 10039 (e-mail: viyer@post.harvard.edu).

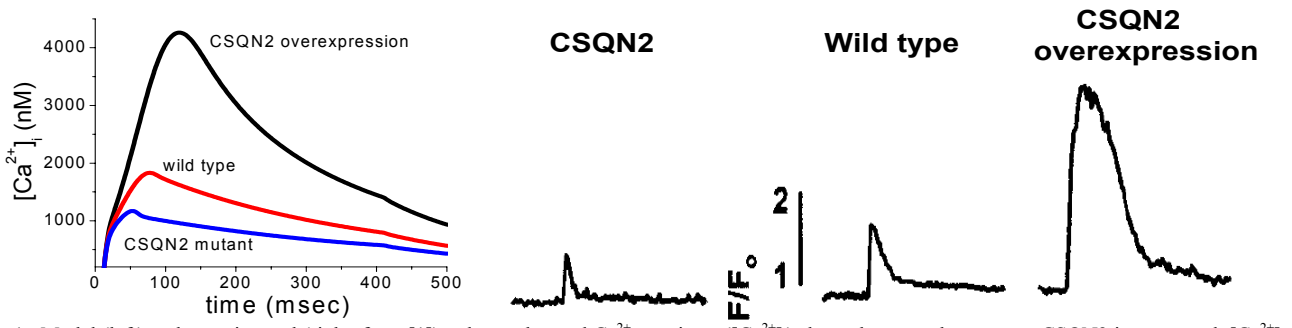

Fig. 1. Model (left) and experimental (right, from [1]) voltage-clamped Ca^{2+} transients ($[Ca^{2+}]_i$) show changes when mutant CSQN2 is expressed; $[Ca^{2+}]_i$ have smaller amplitudes and shorter rise times, in both the experiments and for simulations. Overexpression of CSQN2 leads to dramatically larger $[Ca^{2+}]_i$ with a dome-like peak, in both the experiments and the simulations.

transition was therefore multiplied by a Hill function of SR Ca^{2+} concentration, k_{lumen}, providing a graded increase in

$$k_{lumen} = H_{Max} - (H_{Max} - H_{Min}) / 1 + \left(\frac{H_{50}}{[Ca^{++}]_{JSR}} \right)^{HN}$$

opening rate with increase in free SR Ca^{2+}, where

Also, RyR2 channels enter a refractory state more quickly upon reduction in luminal Ca^{2+}, possibly by adjusting their responsiveness to cytosolic Ca^{2+} [13]. Consequently, the adaptation step of the model (O1->C2) was scaled by $1/k_{lumen}$. As this process is faster in mutant CSQN2-expressing myocytes compared to wild-type [1, 9], the adaptation transition is accelerated in the mutant CSQN2 model.

Restitution of RYR2 appears also to be dependent on restoration of SR Ca^{2+} content ([14]). Features of the RyR2 restitution can be assessed with imperatoxin A, an activator of RyR2 [15]. Ventricular myocytes simultaneously loaded with imperatoxin A and intra-SR Ca^{2+} buffers showed increased spark frequency in the absence of SR Ca^{2+} buffering, suggesting enhanced recovery dynamics at higher luminal Ca^{2+} [6]. The simulated RyR2 recovery from the adapted state (transition C2->O1) was therefore also scaled by k_{lumen}. In similar experiments in CSQN2-underexpressing myocytes, recovery from inactivation was shown to be faster [16]; thus, this transition is accelerated in the mutant CSQN2 model.

Mutant CSQN2 also reduces SR buffering capacity for Ca^{2+} [17]. This was simulated by adjusting the rapid buffering approximation of Wagner and Keizer [18] by scaling the effective CSQN2 concentration by k_{CSQN2}.

Initial parameter choices for k_{lumen} were taken from a recent simulation study [19] and adjusted to ensure physiologic transition scaling rates at typical SR Ca^{2+} content generated by pacing the virtual myocyte at 1 Hz.

B. Modeling RyR2 mutations

Several experiments investigating impaired calcium cycling with mutant RyR2 channels have demonstrated reduced FKBP12.6 binding to RyR2 upon PKA phosphorylation [5, 7], leading to a destabilized release mechanism. FKBP12.6 binds adjacent RyR2 subunits, and is responsible for the cooperativity of activation of subunits by dyadic Ca^{2+} [20, 21]. Thus, RyR2 mutations leading to CPVT were modeled by scaling the parameters responsible for RyR2 cooperativity (m_{coop} and n_{coop} in C1->O1 and O1->O2) by a constant k_{RyR2}.

Parameter choices for k_{lumen} for each model are shown in the table:

Parameter	Luminal Ca^{2+} model	mutant CSQN2 – release termination	mutant CSQN2– release recovery	mutant RyR2
H_{Max}	0.5	0.2	0.5	0.5
H_{Min}	5	5	5	5
H_{50}	1 mM	1 mM	0.5 mM	1 mM
HN	2.5	2.5	2.5	2.5
k_{RyR}	1	1	1	0.8
k_{CSQN2}	1	0.5	0.5	1
k_{beta}	1.5	1.5	1.5	1.5

Table 1. Parameters used in the model for k_{lumen} (comprised of parameters H_{Max} – the maximal k_{lumen} value, H_{Min} – the minimum k_{lumen} value, H_{50} – the SR Ca^{2+} concentration at which k_{lumen} value is half-maximal, HN – the parameter that controls the gradedness of k_{lumen} increase across SR Ca^{2+} concentrations), k_{CSQN2} (the scaling factor reflecting reduced CSQN2-Ca^{2+} binding), k_{RyR} (the scaling factor for cooperativity of the RyR2 gating), and k_{beta} (the factor that scales L-type current and SR uptake flux in the isoproterenol model).

III. Results

In order to validate the mutant CSQN2 simulation, the model output was compared against available experimental data. The virtual myocyte was paced using voltage-clamp steps, and the initial response of the model was plotted alongside experimental recordings of Ca^{2+} transients ($[Ca^{2+}]_i$) in rat ventricular myocytes. As shown in Figure 1, experimentally-obtained $[Ca^{2+}]_i$ in myocytes expressing mutant CSQN2 are characterized by smaller amplitude with an earlier peak with a similar initial rate of rise; these features are reproduced well by the mutant CSQN2 model.

Furthermore, the effect of CSQN2 overexpression was simulated by reversing the changes implemented in the mutant (ie, increasing CSQN2 concentration and modifying RyR2 transitions to incorporate delayed termination of release and prolonged recovery from inactivation). Simulated $[Ca^{2+}]_i$ recapitulate the larger amplitude of the experimental Ca^{2+} waveform with a delayed, dome-like peak. Taken together, these data reproduce the altered RyR2 gating properties in the mutant CSQN2 seen in vitro.

Figure 2 shows the ability of the two CPVT mutant models to produce DADs in the presence of simulated isoproterenol. Both the mutant CSQN2 (panel A) and RyR2 (panel B) models, exhibited small amplitude DADs in current clamp mode at 2 Hz stimulation frequency. Notably, DADs were only observed in the presence of simulated isoproterenol – a behavior observed also in vitro (see Figure 1). Recordings from rat ventricular myocytes show small amplitude diastolic DADs which measure roughly 8% of the action potential amplitude [1].

The DAD mechanism in myocytes expressing the mutant RyR2 was investigated by comparing the occupancy of the RyR2 first open state (state O1) as a function of time for both the wild-type and mutant RyR2 models, shown in Figure 3. Mutant RyR2 channels gate into state O1 more rapidly and show a higher diastolic open probability, leading to a slow accumulation of dyadic Ca^{2+}, eventually triggering a premature spontaneous activation event toward the end of the diastolic interval. This behavior is consistent with the hyperactive, "leaky" RyR2s reported in myocytes with reduced FKBP12.6-RyR2 binding.

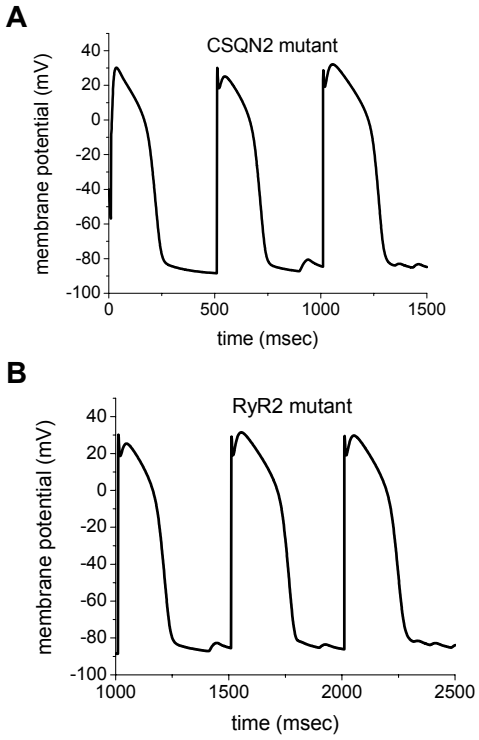

Fig. 2. Representative DADs formed in the mutant CSQN2 (panel A) and the mutant RyR2 (panel B) models. DADs were elicited by pacing the model at 2 Hz in the presence of β-stimulation. No DADs are observed in the absence of isoproterenol.

IV. Discussion

This study investigated the mechanisms of DAD generation in CPVT using a mathematical model of human excitation-contraction coupling. We have found first, that the accelerated termination and recovery of RyR2 release in mutant CSQN2 results in DAD formation in the presence of isoproterenol; second, that the free SR Ca^{2+} content and rate of recovery from inactivation are the key parameters in controlling the stability of Ca^{+} release from the RyR2s due to CSQN2 mutations; and third, that impaired FKBP12.6 binding is likely to generate DADs through hyperactive RyR2s that exhibit larger diastolic open probability. Overall, our results provide plausible mechanisms by which defects in RyR2 gating induced by CPVT related gene mutations may lead to multiple cellular triggers of arrhythmias.

A. Mechanisms of DADs in the mutant CSQN2 model

A critical test of the mutant CSQN2 model was its ability to reproduce experimental data that it has not been constrained on. Thus, the demonstration that the mutant CSQN2 minimal order model (as a result of CSQN2-dependent altered RyR2 Ca^{2+} release termination and recovery) that produced DADs only in the presence of isoproterenol, provides evidence that the proposed mechanisms are plausible explanations for the aberrant Ca^{2+} release in the presence of the mutation.

How these DADs arise is a question that is particularly well-suited for analysis using this model. To isolate the effect of each of the two modifications in the minimal order model of the mutant CSQN2, additional simulations were conducted by (i) changing the rate of termination alone, and leaving recovery unchanged from that in the wild-type, and (ii) changing the rate of recovery from inactivation alone, holding the adaptation rate at wild-type.

Simulations in which only termination of Ca^{2+} release was incrementally accelerated reveals that DADs could be elicited as SR Ca^{2+} reached a critical level. This

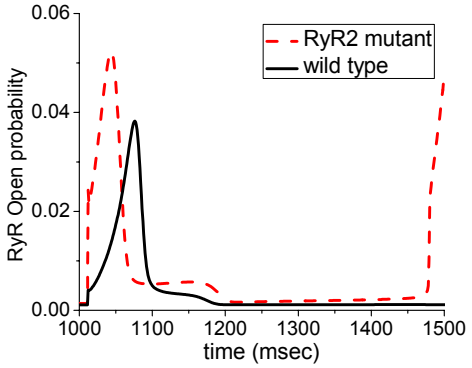

Fig. 3. Analysis of the RyR2 open probability of the first open state reveals a larger peak, longer release duration, and larger diastolic value for the mutant model (shown in dashed trace), leading to a large spontaneous, aberrant RyR2 opening.

phenomenon is likely a consequence of "autoregulation", whereby smaller release events raise free SR Ca^{2+} resulting in enhanced luminal Ca^{2+}-dependent aberrant RyR2 openings [19]. These simulations add further support to the central role of free SR Ca^{2+} in the genesis of DADs.

When the recovery step is accelerated sufficiently (in the absence of changes to the rate of termination of release), DADs result. This finding suggests that primary alteration in RyR2 gating that provides premature recovery of the

RyR2s from a luminal Ca^{2+}-dependent refractory state is a plausible mechanism for producing spontaneous Ca^{2+} release events. This provides theoretical support to the scheme presented by Györke and colleagues [9], who suggested that CPVT might arise from accelerated recharge of receptors associated with a "smaller" functional SR Ca^{2+} store. Isoproterenol may unmask DADs by accelerating the recharge of this smaller store.

B. Mechanisms of DADs in the mutant RyR2 model

The results of this investigation show that reducing cooperativity of RyR2 activation is sufficient to reproduce the triggered activity seen in RyR2 mutations (Figure 4).

Each RyR2 subunit in the tetrameric release assembly binds one FKBP12.6 protein, coupling their activity and stabilizing the closed conformation [21]. Recent simulation studies [14] have shown that increased coupling between adjacent RyR2 subunits in a "sticky cluster model" through more synchronous activation, has shorter duration of opening, and stabilized release. Conversely, decreased cooperativity increases heterogeneity of release across release sites, prolonging overall release duration, as seen in the present study. The additional finding that reduced cooperativity produces DADs in a whole cell model supports the idea advanced by the Marks group [5, 7], which proposes that PKA-mediated hyperphosphorylation of RyR2 reduces FKBP12.6 binding and leads to hyperactive RyR2 channels that are prone to triggered activity in CPVT.

Other studies have not been able to demonstrate reduced FKBP12.6 binding to mutant RyR2 in ventricular myocytes [3, 4, 8], suggesting that DADs in RyR2 mutants arise from FKBP12.6-independent mechanisms, such as impaired luminal Ca^{2+} sensing. Jiang proposed one such scheme that attributes DADs in RyR2 mutants to a reduced threshold for functional recovery of the RyR2s, a process they have termed "store-overload induced calcium release"[8]. In the present model, accelerating functional recovery produces DADs, providing support for this mechanism. Such a scheme is furthermore attractive since it provides a common mechanistic framework for understanding different mutations in CSQN2 and RyR2 that lead to a similar phenotype, i.e., through accelerated recovery of RyR2.

V. CONCLUSIONS

In this study we have explored the mechanistic basis for DADs in mutations linked to CPVT. We have demonstrated that CSQN2 mutations may give rise to DADs through accelerated recovery from inactivation, and that free SR Ca^{2+} is an important variable in determining the propensity for DADs. We have further demonstrated that reduced cooperativity of RyR2 gating, as might be found with reduced FKBP12.6-RyR2 binding in CPVT mutants, can also cause DADs associated with the mutant RyR2s via hyperactive release channels. These findings can potentially be used to guide further experiments in characterizing these mutants, and assist in the development of targeted therapies.

REFERENCES

1. Viatchenko-Karpinski, S., et al., *Abnormal calcium signaling and sudden cardiac death associated with mutation of calsequestrin.* Circ Res, 2004. **94**(4): p. 471-7.
2. Kontula, K., et al., *Catecholaminergic polymorphic ventricular tachycardia: recent mechanistic insights.* Cardiovasc Res, 2005. **67**(3): p. 379-87.
3. Cerrone, M., et al., *Bidirectional ventricular tachycardia and fibrillation elicited in a knock-in mouse model carrier of a mutation in the cardiac ryanodine receptor.* Circ Res, 2005. **96**(10): p. e77-82.
4. George, C.H., G.V. Higgs, and F.A. Lai, *Ryanodine receptor mutations associated with stress-induced ventricular tachycardia mediate increased calcium release in stimulated cardiomyocytes.* Circ Res, 2003. **93**(6): p. 531-40.
5. Lehnart, S.E., et al., *Sudden death in familial polymorphic ventricular tachycardia associated with calcium release channel (ryanodine receptor) leak.* Circulation, 2004. **109**(25): p. 3208-14.
6. Terentyev, D., et al., *Luminal Ca2+ controls termination and refractory behavior of Ca2+-induced Ca2+ release in cardiac myocytes.* Circ Res, 2002. **91**(5): p. 414-20.
7. Wehrens, X.H., et al., *FKBP12.6 deficiency and defective calcium release channel (ryanodine receptor) function linked to exercise-induced sudden cardiac death.* Cell, 2003. **113**(7): p. 829-40.
8. Jiang, D., et al., *Enhanced store overload-induced Ca2+ release and channel sensitivity to luminal Ca2+ activation are common defects of RyR2 mutations linked to ventricular tachycardia and sudden death.* Circ Res, 2005. **97**(11): p. 1173-81.
9. Terentyev, D., et al., *Calsequestrin determines the functional size and stability of cardiac intracellular calcium stores: Mechanism for hereditary arrhythmia.* Proc Natl Acad Sci U S A, 2003. **100**(20): p. 11759-64.
10. Iyer, V., R. Mazhari, and R.L. Winslow, *A computational model of the human left-ventricular epicardial myocyte.* Biophys J, 2004. **87**(3): p. 1507-25.
11. Keizer, J. and L. Levine, *Ryanodine receptor adaptation and Ca2+(-)induced Ca2+ release-dependent Ca2+ oscillations.* Biophys J, 1996. **71**(6): p. 3477-87.
12. Sitsapesan, R. and A.J. Williams, *Regulation of the gating of the sheep cardiac sarcoplasmic reticulum Ca(2+)-release channel by luminal Ca2+.* J Membr Biol, 1994. **137**(3): p. 215-26.
13. Gyorke, I. and S. Gyorke, *Regulation of the cardiac ryanodine receptor channel by luminal Ca2+ involves luminal Ca2+ sensing sites.* Biophys J, 1998. **75**(6): p. 2801-10.
14. Sobie, E.A., et al., *Termination of cardiac Ca(2+) sparks: an investigative mathematical model of calcium-induced calcium release.* Biophys J, 2002. **83**(1): p. 59-78.
15. Shtifman, A., et al., *Effects of imperatoxin A on local sarcoplasmic reticulum Ca(2+) release in frog skeletal muscle.* Biophys J, 2000. **79**(2): p. 814-27.
16. Szentesi, P., et al., *Sarcoplasmic reticulum Ca2+ refilling controls recovery from Ca2+-induced Ca2+ release refractoriness in heart muscle.* Circ Res, 2004. **95**(8): p. 807-13.
17. Houle, T.D., M.L. Ram, and S.E. Cala, *Calsequestrin mutant D307H exhibits depressed binding to its protein targets and a depressed response to calcium.* Cardiovasc Res, 2004. **64**(2): p. 227-33.
18. Wagner, J. and J. Keizer, *Effects of rapid buffers on Ca2+ diffusion and Ca2+ oscillations.* Biophys J, 1994. **67**(1): p. 447-56.
19. Shannon, T.R., F. Wang, and D.M. Bers, *Regulation of cardiac sarcoplasmic reticulum Ca release by luminal [Ca] and altered gating assessed with a mathematical model.* Biophys J, 2005. **89**(6): p. 4096-110.
20. Ahern, G.P., P.R. Junankar, and A.F. Dulhunty, *Subconductance states in single-channel activity of skeletal muscle ryanodine receptors after removal of FKBP12.* Biophys J, 1997. **72**(1): p. 146-62.
21. Brillantes, A.B., et al., *Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein.* Cell, 1994. **77**(4): p. 513-23.