Targeting intracellular calcium cycling in catecholaminergic polymorphic ventricular tachycardia: a theoretical investigation

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1Institute of Life Sciences, National Central University, Taoyuan; 2Department of Financial and Computational Mathematics, Providence University, Taichung, Taiwan; and 3Division of Cardiovascular Medicine, Stanford University School of Medicine, Stanford, California

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Sung RJ, Lo CP, Hsiao PY, Tien HC. Targeting intracellular calcium cycling in catecholaminergic polymorphic ventricular tachycardia: a theoretical investigation. Am J Physiol Heart Circ Physiol 301: H1625–H1638, 2011. First published July 8, 2011; doi:10.1152/ajpheart.00696.2010.—Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a malignant arrhythmogenic disorder linked to mutations in the cardiac ryanodine receptor (RyR2) and calsequestrin, predisposing the young to syncope and cardiac arrest. To define the role of β-adrenergic stimulation (BAS) and to identify potential therapeutic targeted sites relating to intracellular calcium cycling, we used a Luo-Rudy dynamic ventricular myocyte model incorporated with interacting Markov models of the L-type Ca2+ channel (ICa,L) and RyR2 to simulate the heterozygous state of mouse RyR2 R4496C mutation (RyR2R4496C+/−) comparable with CPVT patients with RyR2 R4497C mutation. Characteristically, in simulated cells, pacing at 4 Hz or faster or pacing at 2 Hz under BAS with effects equivalent to those of isoproterenol at ≥0.1 μM could readily induce delayed afterdepolarizations (DADs) and DAD-mediated triggered activity (TA) in RyR2R4496C+/− but not in the wild-type via enhancing both ICa,L and sarcoplasmic reticulum (SR) Ca2+ ATPase (Ii, UP). Moreover, with the use of steady state values of isolated endocardial (Endo), mid-myocardial (M), and epicardial (Epi) cells as initial data for conducting single cell and one-dimensional strand studies, the M cell was more vulnerable for developing DADs and DAD-mediated TA than Endo and Epi cells, and the gap junction coupling represented by diffusion coefficient (D) of ≤0.000766*98 cm2/ms was required for generating DAD-mediated TA in RyR2R4496C+/−. Whereas individual reduction of Ca2+ release channel of SR and Na-Ca exchanger up to 50% was ineffective, 30% or more reduction of either ICa,L or Ii, UP could totally suppress the inducibility of arrhythmia under BAS. Of note, 15% reduction of both ICa,L and Ii, UP exerted a synergistic antiarrhythmic efficacy. Observations have shown that β-adrenergic blocking agents provide only incomplete protection and other antiarrhythmic agents such as quinidine, verapamil, and amiodarone are either ineffective or only partially efficacious (18, 19, 21, 23, 28). Of note, some patients need to resort to implantation of implantable cardioverter/defibrillators (ICD) and/or left cardiac sympathetic denervation and even to cardiac transplantation (23, 39).

Although more than 70 RyR2 mutations have been identified, mechanisms by which RyR2 mutations alter the properties of RyR2 thereby generating ventricular tachyarrhythmias in CPVT remain highly debatable (23). The three major ones speculated include reduced affinity of calstabin2 (FKBP12.6) to RyR2, defective interdomain interaction within RyR2, and increased Ca2+ sensitivity of RyR2. Regardless of the exact underlying mechanism, the general consensus is that RyR2 mutations lead to abnormally augmented Ca2+ release, which results in the genesis of delayed afterdepolarizations (DADs) and DAD-mediated triggered activity (TA), underpinning the development of ventricular tachyarrhythmias (23).

In efforts to elucidate electrophysiological mechanisms of ventricular tachyarrhythmias, few investigators have performed computational modeling of CPVT mutations. Using a human left ventricular epicardial myocyte model, Iyer and Armoundas (14) reduced the buffering capacity for Ca2+ alongside acceleration of adaptation transition in SR to simulate CASQ2 mutations and decreased the cooperability of RyR2 for enhancing its open probability to simulate RyR2 mutations; upon β-adrenergic stimulation (BAS), they could induce DADs in both CASQ2 and RyR2 mutants but not in wild-type. Applying a Luo-Rudy dynamic (LRd) ventricular myocyte model, which had incorporated dynamics of intracellular Ca2+ cycling (8, 9), Faber and Rudy (9) simulated the ventricular myocyte expressing homoyogous CASQ2 D307H mutation with 60% reduction of the total Ca2+ binding capacity of CASQ2; they illustrated that rapid pacing under BAS increased myocyte Ca2+ loading, culminating in spontaneous SR Ca2+ release followed by development of DADs. In the present study, we adopted the same LRd ventricular myocyte model (8, 9) to simulate RyR2 R4496C mutation (15, 23), in which mouse phenotype closely resembled clinical manifesta-

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tions of CPVT patients with RyR2 R4497C mutation (2, 3, 10, 22). Our goals were to further define the role of BAS in the arrhythmogenesis of RyR2 R4496C mutation with attendant gain-of-function (23) and, more importantly, to search for potential targeted sites involved in the dynamics of intracellular Ca$^{2+}$ cycling for effective suppression of ventricular tachyarrhythmias associated with CPVT.

**METHODS**

**Single LRd cell model.** We used the LRd ventricular myocyte model, which had incorporated interacting Markov models of the L-type Ca$^{2+}$ channel ($I_{Ca,L}$) and RyR2 in a restricted t-tubular subsarcolemmal space for Ca$^{2+}$ distribution (8, 9). The heterogeneity of ion channels was expressed in three cell types of the ventricular myocyte, i.e., the density ratios between slow and rapid delayed rectifiers (GK$_r$:GKr) were set at 23:7:15 in epicardial (Epi), mid-myocardial (M), and endocardial (Endo) cells, respectively. The Markov model of the $I_{Ca,L}$ channel possessed a conducting mode (ModeV) and a nonconducting mode (ModeCa). Additionally, there was a nonconducting Mode0 to be activated by BAS. Intracellular Ca$^{2+}$ cycling processes included Ca$^{2+}$ uptake and release by SR and a buffering system consisting of calmodulin and troponin, sarcosomal and SR Ca$^{2+}$ binding sites, and CASQ2. Ca$^{2+}$ release from SR ($I_{rel}$) was by way of both Ca$^{2+}$-induced and store-overload-induced components ($I_{rel,SOICR}$ and $I_{rel,SR}$, respectively) through RyR2.

Simultaneously, various ionic currents, $I_{ion}$, and SR Ca$^{2+}$ ATPase ($I_{ATP}$) and, besides cytoplasmic Ca$^{2+}$ transients (intracellular Ca$^{2+}$ concentration [Ca$^{2+}$]), those in the subspace ([Ca$^{2+}$]), net as well as junctional SR ([Ca$^{2+}$]$_{junct}$) and [Ca$^{2+}$]$_{cyto}$ were monitored. During BAS, transition rates were altered between states of $I_{Ca,L}$ and parameters of other currents including the slow component of delayed rectifier ($I_{k1}$), Na-K pump ($I_{NaK}$), inward rectifier ($I_{i}$), and $I_{Na}$ were formulated such that changes of these parameters would reflect effects of a saturating concentration of isoproterenol at $\pm 0.1 \mu$M. All formulations and source program codes of above described modifications of the LRd model can be found in References 8 and 9.

**Multicellular one-dimensional strand model.** The transmural heterogeneity of ion channel densities was incorporated in a theoretical strand with a total length of 1.65 cm composed by 165 LRd model cells to represent Endo (1st to 60th), M (61th to 105th), and Epi (106th to 165th) cells interconnected by resistive gap junctions (11). Barring cells to represent Endo (1st to 60th), M (61th to 105th), and Epi (106th to 165th) cells interconnected by resistive gap junctions (11).

**Modeling of wild-type RyR2 myocyte (RyR2wt) and RyR2 R4496C mutant cells.** In the process of formulation modifications, we first tried to fit into the cellular functional characteristics depicted by Jiang et al. (15) so that the R4496C mutant cell would exhibit enhanced sensitivity of RyR2 to luminal Ca$^{2+}$. However, we realized that the cell systems used by Jiang et al. (15) were those of heterologous human embryonic kidney (HEK)293 cell lines devoid of complex cardiac intracellular environment. Therefore, secondly, we attempted to conform to cellular phenotypic manifestations of the RyR2 R4496C knock-in mouse model described by Liu et al. (22) so that the R4496C mutant cell, but not RyR2wt, would manifest DADs and/or DAD-mediated TA under BAS. Finally, we considered that the LRd model being a simulated guinea pig ventricular myocyte should exhibit [Ca$^{2+}$]$_{cyto}$ and SR Ca$^{2+}$ load responses to increasing pacing rates different than those of the mouse model (see Correlation of [Ca$^{2+}$]$_{cyto}$ with induction of DADs and DAD-mediated TA) (7, 10). Taken together, we accordingly adjusted the open probability as well as other parameters relating to the mechanism of $I_{rel,SOICR}$ in the RyR2 Markov model. Detailed processes of formulation modification are explained in the Supplemental Methods:

$$I_{rel} = G_{rel} \cdot O([Ca^{2+}]_{junct} - [Ca^{2+}]_o)$$

$$G_{rel} = 250(\text{grad}redl/vgainrel + \text{soicr})$$

$$I_{rel,SR} = 250 \cdot \text{soicr} \cdot O([Ca^{2+}]_{junct} - [Ca^{2+}]_o)$$

$$\text{soicr} = \text{soicr}_{max} - (\text{soicr}_{max} - \text{soicr}(0)) \cdot \exp(-t/\text{soicr}_{t})$$

where $$\text{soicr}_{max} = 125$$ and for RyR2 R4496C mutant:

$$\text{soicr} = \frac{4500}{1 + \exp\left(\frac{5.2 - [Ca^{2+}]_{junct}}{10^{-8}}\right)}$$

$$\text{soicr}_{max} = 31.25; \alpha_{SR} = 2.100 \cdot [Ca^{2+}]_o/[Ca^{2+}]_{max}; \alpha_{SR} = \alpha_{SR} = \alpha_{SR} = 6.720 \cdot [Ca^{2+}]_o/[Ca^{2+}]_{max}$$

$$\gamma_{SR} = 0.05 \cdot [Ca^{2+}]_o/[Ca^{2+}]_{max}$$

$$\gamma_{SR} = 0.1 \cdot [Ca^{2+}]_o/[Ca^{2+}]_{max}$$

$$\gamma_{SR} = 0.2 \cdot [Ca^{2+}]_o/[Ca^{2+}]_{max}$$

$$\gamma_{SR} = 0.3 \cdot [Ca^{2+}]_o/[Ca^{2+}]_{max}$$

$$\gamma_{SR} = 0.4 \cdot [Ca^{2+}]_o/[Ca^{2+}]_{max}$$

where $$\text{max}_{sr} = 0.22, \text{min}_{sr} = 0.1, \kappa_{sr} = \text{max}_{sr} - (\text{max}_{sr} - \text{min}_{sr}) \cdot \frac{1}{1 + \left(\frac{1.5}{33 \cdot [Ca^{2+}]_{junct}}\right)^2}$$

The software of QuB (www.qub.buffalo.edu/wiki/index.php/Main_Page) was utilized to simulate single-channel activities of the RyR2wt and RyR2 R4496C mutant. The Ca$^{2+}$ concentration on the cytoplasmic face of RyR2 was adjusted to 45 nM Ca$^{2+}$ at control, and, to test the sensitivity of RyR2 to luminal Ca$^{2+}$ activity, luminal Ca$^{2+}$ concentration was increased to various levels. Comparative results of RyR2wt and RyR2 R4496C mutant at two settings of 45 nM Ca$^{2+}$ (cytoplasmic)/45 nM Ca$^{2+}$ (luminal) and 45 nM Ca$^{2+}$ (cytoplasmic)/300 nM Ca$^{2+}$ (luminal) are, respectively, illustrated in Fig. 1, A and B. The practical arithmetic mean values of mean open probability (P$_o$; i.e., total duration of open events per total time), mean open time ($T_o$; i.e., total duration of open events per number of open events), and mean close time ($T_c$; i.e., total duration of close events per number of close events) were also measured. Note that when compared with RyR2wt, there was a slight increase in the RyR2 activity associated with R4496C mutation, which became more prominent in response to an increase in luminal Ca$^{2+}$ concentrations. Because arithmetic mean values shown by Jiang et al. in their Fig. 3, A and C (15), were random numbers, these values carried little statistical meaning. Nevertheless, arithmetic mean values obtained in our model study did exhibit a trend similar to that shown by Jiang et al. (15), in which with an increase in the luminal Ca$^{2+}$ from 45 nM to 300 nM while keeping cytosolic Ca$^{2+}$ constantly at 45 nM, P$_o$ and $T_o$ were increased and $T_c$ was decreased, and the extent of these changes was significantly more noted in the R4496C mutant than in the wild-type. On the other hand, the graphic representation depicting the relationship between P$_o$ and luminal Ca$^{2+}$ concentrations was statistically meaningful (Fig. 3E of
Ref. 15), since these data were derived from an average with a standard derivation. When both data of our simulated R4496C mutant and those of Jiang et al. (15) were superimposed with each other, a standard derivation. When both data of our simulated R4496C mutant

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Consequent to our modification of formulations affecting the gating properties of RyR2 and the mechanism of SOICR \( (I_{\text{sol,SOICR}}) \), which included alteration of its threshold \( (i.e., 5.2 \text{ for the R4496C mutant and } 8.4 \text{ for RyR2wt}) \), we also could produce cellular phenotypic manifestations in close accord with those experimentally demonstrated by Liu et al. (22) in the R4496C knock-in mouse model. As manifested in Supplemental Figs. S1 and S2, supported that our simulated R4496C mutant could maintain the tendency of enhanced sensitivity to Ca\(^{2+}\) from a single-channel scale to a cell scale (e.g., 5,000 channels).

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attaining the steady state, [Na<sup>+</sup>], and SR Ca<sup>2+</sup> load of RyR2wt and RyR2<sup>R4496C+/−</sup> cells so obtained are listed in Supplemental Table S1.

After steady state values of isolated Endo, M, and Epi cells had been obtained, action potential duration (APD) of various percentages of repolarization, e.g., 90% (APD<sub>90</sub>) and 97% (APD<sub>97</sub>), was measured. To observe emergence of abnormal automaticity related to changes in the resting membrane potential (RMP) (e.g., that caused by I<sub>K1</sub> reduction), we waited for 40 s in a resting state in both RyR2wt and RyR2<sup>R4496C+/−</sup> before further pacing (33).

To assess effects of BAS, electrical stimulation was delivered to test the inducibility of DADs, and DAD-mediated TA after steady state values of each cell type at 2 Hz had been used as initial data. To determine contribution of each element of BAS to arrhythmia induction, we assessed effects of removing I<sub>Ca.L</sub>, I<sub>K1</sub>, I<sub>Ks</sub>, and I<sub>UP</sub> individually (34). Based on the mode of induction of DADs, and DAD-mediated TA, potential targeted sites for antiarrhythmic therapy relating to intracellular Ca<sup>2+</sup> cycling—I<sub>Ca.L</sub>, I<sub>UP</sub>, I<sub>rel</sub>, and Na-Ca exchange (I<sub>NaCa</sub>)—were each systematically decreased by 5% stepwise until 50% reduction was reached (34). Given that I<sub>NaK</sub> is strongly regulated by intracellular Na<sup>+</sup>, which depends on the pacing rate, and that transient accommodation of Na is a slow process (25), we opted not to evaluate its removal effect.

Finally, to determine the role of the gap junction coupling in generating DADs and/or DAD-mediated TA in the strand configuration, we tested and adjusted the diffusion coefficient (D) (19, 25, 27) in the monodomain model depicted in Multicellular one-dimensional strand model (11).

RESULTS

Correlation of [Ca<sup>2+</sup>]<sub>i</sub> with induction of DADs and DAD-mediated TA. Dynamic changes of [Ca<sup>2+</sup>]<sub>i</sub> of our simulated wild-type RyR2 and RyR2<sup>R4496C+/−</sup> cells were compared with experimental data obtained from the RyR2<sup>R4496C+/−</sup> knock-in mouse model (10). Notably, the findings that the induction of DADs and DAD-mediated TA was dependent on the pacing rate and BAS (Figs. 2 and 3) were in agreement with those elegantly reported by Fernandez-Velasco et al. (10). For example, with recording of [Ca<sup>2+</sup>]<sub>i</sub> alongside the membrane potential, Supplemental Fig. S3 shows that after cessation of pacing at 4 Hz (last paced beat denoted by a vertical arrow), the RyR2wt cell remains silent, whereas the RyR2<sup>R4496C+/−</sup> cell exhibits spontaneous [Ca<sup>2+</sup>]<sub>i</sub> activity consistent with DADs and DAD-mediated TA, matching those illustrated in Fig. 1 of Ref. 10. Moreover, while obtaining steady state values with recordings of [Ca<sup>2+</sup>]<sub>i</sub> and SOICR alongside the membrane potential, we noticed that during pacing at 2 Hz under BAS, spontaneous [Ca<sup>2+</sup>]<sub>i</sub> activity corresponding to DADs and/or DAD-mediated TA intermittently emerged in the RyR2<sup>R4496C+/−</sup> cell (Supplemental Fig. S4), matching findings of Fig. 6, A and B, of Ref. 10. This latter phenomenon correlates with the clinical setting whereby exercise provokes onset of arrhythmia in CPVT patients (10).

We also examined effects of the pacing frequency on [Ca<sup>2+</sup>]<sub>i</sub> and SR Ca<sup>2+</sup> load. Although pacing at 2 to 4 Hz, peak [Ca<sup>2+</sup>]<sub>i</sub>, and [Ca<sup>2+</sup>]<sub>jsr</sub> in RyR2<sup>R4496C+/−</sup> were lower in the R4496C mutant than in the wild-type (Supplemental Fig. S5). The differences in peak [Ca<sup>2+</sup>]<sub>i</sub>, and [Ca<sup>2+</sup>]<sub>jsr</sub> between RyR2wt and RyR2<sup>R4496C+/−</sup> became larger with faster pacing due to accumulation of intracellular Ca<sup>2+</sup> and the accompanying enhancement of open probability of RyR2<sup>R4496C+/−</sup> at high pacing rates. However, in contrast with findings in the ventricular myocyte of the R4496C knock-in mouse model reported by Fernandez-Velasco et al. (Fig. 4, A and F, of Ref. 10), both [Ca<sup>2+</sup>]<sub>i</sub> and [Ca<sup>2+</sup>]<sub>jsr</sub> simultaneously increased rather than decreased as the pacing rate became faster, a phenomenon that has been referred to as positive staircase response (7). We surmised that the latter phenomenon was related to the LRd model being based on guinea pig data (8, 9).

Despite differences in animal species (e.g., mouse vs. guinea pig) and the study methodology (experiment vs. simulation), we also compared data on Ca<sup>2+</sup> transients of our model study with those of experiments done by Fernandez-Velasco et al. (Fig. 4A of Ref. 10). The percentage of changes in Ca<sup>2+</sup> transients caused by RyR2<sup>R4496C+/−</sup> at various pacing rates as calculated by the formula: [(peak [Ca<sup>2+</sup>]<sub>i</sub> of RyR2wt) – (peak [Ca<sup>2+</sup>]<sub>i</sub> of RyR2<sup>R4496C+/−</sup>)]/(peak [Ca<sup>2+</sup>]<sub>i</sub> of RyR2wt) was 2% vs. 4%, 13% vs. 17%, and 22% vs. 18% for experiment versus simulation at 2 Hz, 3 Hz, and 4 Hz, respectively.

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**Fig. 2. Effects of pacing at various frequencies on action potentials of simulated heterozygous R4496C mutant (RyR2<sup>R4496C+/−</sup>) without β-adrenergic stimulation (BAS).** Action potentials of the last 5 of 64 driven stimuli at each pacing frequency from 1 to 5 Hz (A–E) interrupted by 5-s intermission are shown (see text). In A–C, pacing induced no delayed afterdepolarizations (DADs) or DAD-mediated triggered activity. In D, a single DAD is denoted by ∞, and in E, the first of a run of 9 DAD-mediated triggered activity beats by ●. In each panel, the arrow points to the last pacing stimulus.
**Effects of RyR2\(^{R4496C+/-}\) on RMP, APD, and the QT interval.** In either the single cell or one-dimensional strand model (Tables 1 and 2), simulated RyR2\(^{R4496C+/-}\) did not cause any significant change in RMP at control (without BAS) (e.g., −88.9 and −88.9 mV for RyR2wt and RyR2\(^{R4496C+/-}\), respectively, in single M cell recordings at 2 Hz) and with BAS (e.g., −85 and −85 mV for RyR2wt and RyR2\(^{R4496C+/-}\), respectively, in single M cell recordings at 2 Hz). In single cell recordings, APD\(_{90}\) of Endo, M, and Epi cells in RyR2\(^{R4496C+/-}\) closely matched that of RyR2wt at pacing frequency from 1 to 5 Hz (e.g., APD\(_{90}\) of the M cell without BAS was 187, 161, 149, 141, and 129 ms, respectively, for RyR2wt and 189, 159, 144, 137, and 130 ms, respectively, for RyR2\(^{R4496C+/-}\)). APD\(_{97}\) obtained from one-dimensional strand model showed that APD\(_{97}\) of all three cell types (30\(^{th}\), 80\(^{th}\), and 135\(^{th}\) cells for Endo, M, and Epi cells, respectively) of the 21st driven cell recordings, APD\(_{90}\) of Endo, M, and Epi cells respectively, in single M cell recordings at 2 Hz). In single cell recordings, APD\(_{90}\) of Endo, M, and Epi cells in RyR2\(^{R4496C+/-}\) closely matched that of RyR2wt at pacing frequency from 1 to 5 Hz (e.g., APD\(_{90}\) of the M cell without BAS was 187, 161, 149, 141, and 129 ms, respectively, for RyR2wt and 189, 159, 144, 137, and 130 ms, respectively, for RyR2\(^{R4496C+/-}\)). APD\(_{97}\) obtained from one-dimensional strand model showed that APD\(_{97}\) of all three cell types (30\(^{th}\), 80\(^{th}\), and 135\(^{th}\) cells for Endo, M, and Epi cells, respectively) of the 21st driven stimulus at 2 Hz was comparable between RyR2wt (154, 159, and 138 ms, respectively) and RyR2\(^{R4496C+/-}\) (140, 150, and 128 ms, respectively) in the absence of BAS (Fig. 4A and Table 1). and so was the corresponding QT interval (164 and 154 ms for RyR2wt and RyR2\(^{R4496C+/-}\), respectively, in Table 1).

Although pacing at 2 Hz under BAS, APD\(_{97}\) of the three cell types in both RyR2wt and RyR2\(^{R4496C+/-}\) became slightly prolonged compared with the control (without BAS) due to BAS-induced enhancement of I\(_{Ca,L}\) (178, 186, and 170 ms for RyR2wt and 178, 183, and 167 ms for RyR2\(^{R4496C+/-}\); Fig. 4B and Table 2). Nevertheless, under BAS, APD\(_{97}\) of RyR2wt and that of RyR2\(^{R4496C+/-}\) remained comparable and so were their corresponding QT intervals (186 and 184 ms for RyR2wt and RyR2\(^{R4496C+/-}\), respectively, in Table 2).

**Abnormal automaticity.** No emergence of abnormal automaticity (33) relating to any change of RMP in either RyR2wt or RyR2\(^{R4496C+/-}\) was observed at rest (without any pacing) for 40 s without BAS and under BAS (not shown).

**Intracellular events underlying mechanisms of BAS-facilitated induction of DAD-mediated TA in RyR2\(^{R4496C+/-}\).** During pacing for obtaining steady state values of isolated cell simulations at 2 Hz under BAS, DADs, and/or DAD-mediated TA intermittently emerged in RyR2\(^{R4496C+/-}\) (bottom presence (Fig. 4B) and in the presence (top) of BAS. Note that although BAS exhibited no effects on RyR2wt, it facilitated initiation of a sustained run of DAD-mediated triggered activity (10 beats are shown) in RyR2\(^{R4496C+/-}\) (at control, pacing induced only 1 such beat). In each panel, the arrow points to the last pacing stimulus.

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**Table 1. Comparison of \([Ca^{2+}]_i\), RMP, APD\(_{97}\), and QT of simulated wild-type RyR2 myocyte and heterozygous RyR2 R4496C mutant at control**

<table>
<thead>
<tr>
<th>RyR2 Mutation, %</th>
<th>([Ca^{2+}]_i), Peak/Diastolic, (\mu M)</th>
<th>RMP, mV</th>
<th>APD(_{97}), ms</th>
<th>QT, ms</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>2.48/0.179</td>
<td>−88.7</td>
<td>159</td>
<td>164</td>
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<tr>
<td>50</td>
<td>1.17/0.128</td>
<td>−89.0</td>
<td>150</td>
<td>154</td>
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</table>

Rybyodine receptor 2 (RyR2) R4496C mutation, 0% = RyR2 wild-type and 50% = RyR2R4496C+/−; [Ca\(^{2+}\)]_i, intracellular Ca\(^{2+}\) concentration; RMP, resting membrane potential; APD\(_{97}\), action potential duration at 97% repolarization; [Ca\(^{2+}\)]_i, RMP, and APD\(_{97}\) were obtained from the mid-myocardial cell (80th cell of the multicellular strand) of the 21st pseudo-ECG beat where QT interval was also measured at a pacing frequency of 2.0 Hz.

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**Table 2. Comparison of \([Ca^{2+}]_i\), RMP, APD\(_{97}\), and QT of simulated wild-type RyR2 myocyte and heterozygous RyR2 R4496C mutant under β-adrenergic stimulation**

<table>
<thead>
<tr>
<th>RyR2 Mutation, %</th>
<th>([Ca^{2+}]_i), Peak/Diastolic, (\mu M)</th>
<th>RMP, mV</th>
<th>APD(_{97}), ms</th>
<th>QT, ms</th>
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<tbody>
<tr>
<td>0</td>
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<td>186</td>
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<td>50</td>
<td>2.81/0.169</td>
<td>−87.7</td>
<td>183</td>
<td>184</td>
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[Ca\(^{2+}\)]_i, RMP, and APD\(_{97}\) were obtained from the mid-myocardial cell (80th cell of the multicellular strand) of the 21st pseudo-ECG beat where QT interval was also measured at a pacing frequency of 2.0 Hz.
Without BAS, a stimulus (vertical arrow) captured the M cell and induced no DADs or DAD-mediated TA in either RyR2wt or RyR2R4496C+/−, because [Ca2+]jsr of both RyR2wt and RyR2R4496C+/− did not attain their threshold values of 8.4 and 5.2 (diagonal arrow), respectively (Fig. 5A). However, under BAS, the stimulus (vertical arrow), which also captured the M cell, elicited a sustained run of DAD-mediated TA in RyR2R4496C+/− (10 TA beats are shown in Fig. 5B), but not in RyR2wt (even no DADs were discernible).

Of note, when compared with RyR2wt, RyR2R4496C+/− had smaller systolic Irel due to effect of Ca2+-dependent inactivation, but had larger diastolic Irel because of enhanced open probability (see Irel plot of Fig. 5A), resulting in lower [Ca2+]jsr. With lower [Ca2+]jsr, [Ca2+]ss and [Ca2+]i also became less in the absence of BAS. Consequently, the driving force for Irel,SOICR ([Ca2+]jsr − [Ca2+]ss) in RyR2R4496C+/− was weak, and [Ca2+]jsr remained less than the threshold value of 5.2 (denoted by a diagonal arrow in Fig. 5A) in RyR2R4496C+/−. To paraphrase, after one stimulus, Irel,SOICR remained low, the resultant contribution of which to the Irel conductance was negligible and the threshold of Irel,SOICR (i.e., 5.2 in [Ca2+]jsr) was not attained. Adding up, despite an increase in the open probability of RyR2R4496C+/− up to 40%, the total conductance of Irel was too low to induce release of Ca2+ for the generation of DADs and/or DAD-mediated TA.
In contrast, BAS significantly enhanced both $I_{Ca,L}$ and $I_{UP}$, which in turn raised the level of $[Ca^{2+}]_{int}$ to be above the threshold value of 5.2 (denoted by a diagonal arrow in Fig. 5B) during diastole, thereby increasing the driving force to activate $I_{rel,SOICR}$ in RyR2R4496C+/−. The resultant increase in $[Ca^{2+}]_{ls}$ and $[Ca^{2+}]_{int}$ then activated the forward mode $I_{Na,ca}$ to generate $I_{f1}$ (transient inward currents denoted by asterisks in Fig. 5B) and thereby triggered onset of DADs/DAD-mediated TA (17). Of note also, pacing did not induce DADs and/or DAD-mediated TA in RyR2wt at control and under BAS, because $[Ca^{2+}]_{int}$ of RyR2wt never attained the threshold value of 8.4 under both circumstances (Fig. 5, A and B).

Generation of DAD-mediated TA under BAS among different cell types and role of gap junction coupling (diffusion current) in the RyR2R4496C+/− strand configuration. After having applied steady state values of isolated (Endo, M, and Epi) cell simulations as initial data for the strand model study, we noted that a critical range of gap junction coupling represented by the diffusion coefficient ($D$) was required for the generation of DAD-mediated TA in RyR2R4496C+/− at 2 Hz under BAS: 1) if $D > 0.000766*98 cm^2/ms$ DADs but no DAD-mediated TA beats could be induced or 2) if $D ≤ 0.000766*98 cm^2/ms$ DAD-mediated TA beats became inducible.

As illustrated in Supplemental Fig. S6, with $D = 0.000766*0.005 cm^2/ms$, DAD-mediated TA beats first appeared in the M cell, then in the Endo cell followed by the Epi cell (time of appearance, t = 18, 97, and 206 ms, respectively, under BAS without pacing). There was transmission of one action potential of DAD-mediated TA from the M to the Endo but not to the Epi region, likely resulting from the preset fivefold decrease in the gap junction coupling between M and Epi cells (103th to 107th cells; see Multicellular one-dimensional strand model).

To clarify the role of gap junction coupling in the generation of DAD-mediated TA, two different settings of $D$: $D = 0.000766*0.005 cm^2/ms$ vs. $D = 0.000766*99 cm^2/ms$, with and without generation of DAD-mediated TA, respectively, were compared. As shown in Supplemental Fig. S7, initially the cells with two different $D$ settings were at equivalent physiological states, because their steady state values had been used as initial data. As a result, the relevant currents and ionic concentrations were comparable except for the diffusion currents (i.e., $−D\bar{v}\partial v/\partial x$ in the monodomain model). Notably, in the case of $D = 0.000766*0.005 cm^2/ms$ (red line), the emergence of DAD-mediated TA beats ($n = 6$) occurred with very small diffusion currents. In contrast, in the case of $D = 0.000766*99 cm^2/ms$ (black line), huge outward diffusion currents depicted as positive deflections prevented DADs ($n = 4$) from becoming DAD-mediated TA beats. These findings implied that the diffusion current was an important contributing factor for the generation of DAD-mediated TA in this strand model study (19, 25, 27, 29).

Secondly, when an $I_{rel,SOICR}$-related event occurs to induce depolarization in a cell, its membrane potential would become higher than that of its neighboring cells. As a result, it would need to incur more outward diffusion current to reduce the spatial gradient of membrane potential, thereby exhibiting a suppressive effect on depolarization. Under this circumstance, the larger is the diffusion coefficient $D$, and the more marked is its suppressing effect on depolarization. Note the membrane potential in the setting of $D = 0.000766*0.005 cm^2/ms$ is higher compared with that of $D = 0.000766*99 cm^2/ms$ during both systolic and diastolic phases because of its having a smaller outward diffusion current as shown in Supplemental Fig. S7. With higher membrane potential, $I_{Ca,L}$ is stronger even in the resting phase (see inset of $I_{Ca,L}$ plot in Supplemental Fig. S7), resulting in higher $[Ca^{2+}]_{ls}$, $[Ca^{2+}]_{int}$, and $I_{UP}$ followed by higher $[Ca^{2+}]_{int}$, thereby promoting $I_{rel,SOICR}$ to generate DADs and DAD-mediated TA. Note there are six $I_{rel,SOICR}$ events in the case of $D = 0.000766*0.005 cm^2/ms$ and 4 in the case of $D = 0.000766*99 cm^2/ms$, and these $I_{rel,SOICR}$ events lead to six DAD-mediated TA beats and four DADs, respectively, due to the difference in the size of diffusion currents, along with its accompanying difference in the level of membrane potential.

To compare the incidence of generation of DAD-mediated TA as a function of the cell type, we conducted single cell simulations after steady state values of isolated cells at 2 Hz under BAS had been obtained. Results are shown in Supplemental Fig. S8. One stimulus captures the cell (arrow) and induced 8, 10, and 3 DAD-mediated TA beats in the Endo (blue line), M (red line), and Epi (black line) cells, respectively. Together with findings of Supplemental Fig. S6, it appeared that the ease with which DAD-mediated TA generation could be generated was in the order of the M cell first and then the Endo cell followed finally by the Epi cell. The mechanism by which the M cell was more susceptible than the other two cell types was due to its having less GKs:GKr, thereby manifesting a longer APD and a higher RMP (see inset of $I_{Ca,L}$ plot in Supplemental Fig. S7). The longer APD coupled with higher RMP was accompanied by having more $Ca^{2+}$ influx through the $I_{Ca,L}$ channel, leading to a cascade of higher $[Ca^{2+}]_{ls}$ and $[Ca^{2+}]_{int}$, followed by enhancement of $I_{UP}$ and an increase in $[Ca^{2+}]_{int}$ during diastole (see inset of $V_m$, $[Ca^{2+}]_{int}$, $[Ca^{2+}]_{ls}$, and $I_{UP}$ plots in Supplemental Fig. S8B), the latter of which then activated $I_{rel,SOICR}$ to generate DADs and DAD-mediated TA.

**Contribution of each individual element of BAS on induction of DAD-mediated TA and potential targeted sites of antiarrhythmic therapy.** After steady state values under BAS at 2 Hz had been used as initial data, pacing for 421 stimuli in the M cell at 2.0 Hz was performed in RyR2R4496C+/− under full effects of BAS (top trace in Fig. 6), and it induced a sustained run of DAD-mediated TA. This was compared with those under BAS with removal of individual element of $I_{Ca,L}$, $I_{K1}$, $I_{Ks}$, and $I_{UP}$. Removal of $I_{K1}$ was totally ineffective and removal of $I_{Ks}$ first accelerated the rate of DAD-mediated TA and then converted it to sustained DADs (3rd and 4th traces in Fig. 6). Notably, removal of $I_{Ca,L}$ totally prevented DADs and DAD-mediated TA from occurrence and removal of $I_{UP}$ slowed the rate of DAD-mediated TA and rendered it to become nonsustained (2nd and bottom traces in Fig. 6).

To identify potential targeted sites for effective pharmacotherapy, we paced the M cell of RyR2R4496C+/− for 422 stimuli at 2.0 Hz after steady state values under BAS at 2 Hz had been used as initial data. Under full BAS, it elicited a sustained run of DAD-mediated TA (top traces of $a$, $b$, $c$, $d$, and $e$).
Reduction of \( I_{\text{Ca,L}} \) or \( I_{\text{UP}} \) to 30% or more was required to totally suppress inducibility of DADs and DAD-mediated TA (Fig. 7A). Reduction of \( I_{\text{rel}} \) (effects of 20% and 30% reduction are shown in Fig. 7B) and up to 50% (not shown) exerted no antiarrhythmic efficacy and reduction of \( I_{\text{Na,Ca}} \) accelerated DAD-mediated TA during pacing and then converted to sustained DADs activity.

The corresponding time course of \([\text{Ca}^{2+}]_{\text{jsr}}, I_{\text{rel}}, \) and \([\text{Ca}^{2+}]_{\text{i}}\) evidently showed that reduction of either \( I_{\text{Ca,L}} \) or \( I_{\text{UP}} \) up to 30% could effectively prevent \([\text{Ca}^{2+}]_{\text{jsr}}\) to attain the threshold value of 5.2, thereby suppressing onset of DADs and DAD-mediated TA in RyR2R4496C−/− under BAS (Fig. 7A). Because enhancement of both \( I_{\text{Ca,L}} \) and \( I_{\text{UP}} \) was crucial underlying BAS-facilitated induction of DADs and DAD-mediated TA (Fig. 6), we examined effect of combined reduction of these two currents. As shown in Fig. 8, reduction of both \( I_{\text{Ca,L}} \) and \( I_{\text{UP}} \) each by 15% exhibited a synergistic effect as it could totally suppress the inducibility of DADs and DAD-mediated TA in RyR2R4496C−/− in the presence of BAS.

**DISCUSSION**

With modeling of the RyR2 R4496C mutation using the LRd ventricular myocyte model (8, 9), we were able to simulate Fig. 5. A: intracellular events underlying responses of the wild-type (RyR2wt) and the heterozygous R4496C mutant (RyR2R4496C+/−) to 1 single stimulus without BAS. After the steady state values of isolated cell simulations without BAS at 2 Hz had been obtained and used as initial data, 1 stimulus (denoted by a vertical arrow) was delivered. It induced neither DADs nor DAD-mediated triggered activity in either RyR2wt (black line) or RyR2R4496C+/− (red line; see text). The corresponding time courses of \( I_{\text{Ca,L}}, I_{\text{Na,Ca}}, I_{\text{rel}} \) (inset for diastole), open probability of RyR2, SOICR, \([\text{Ca}^{2+}]_{\text{jsr}}, [\text{Ca}^{2+}]_{\text{i}}\), and driving force of \( I_{\text{rel}} \) ([\Ca^{2+}]_{\text{jsr}}−[\Ca^{2+}]_{\text{i}}) are shown. Note that similar to findings of the strand model study (Fig. 4), \([\text{Ca}^{2+}]_{\text{jsr}}, [\text{Ca}^{2+}]_{\text{i}}\), and \( I_{\text{rel}} \) of RyR2R4496C+/− are lower than those of RyR2wt. When compared with RyR2wt, RyR2R4496C+/− has larger diastolic \( I_{\text{rel}} \) because of enhanced open probability, but smaller systolic \( I_{\text{rel}} \) due to effect of \Ca^{2+}-dependent inactivation. B: intracellular events underlying responses of the wild-type (RyR2wt) and the heterozygous R4496C mutant (RyR2R4496C+/−) to 1 single stimulus under BAS. After steady state values of isolated cell simulations with BAS at 2 Hz had been obtained and used as initial data, 1 stimulus (denoted by a vertical arrow) was delivered. It elicited a sustained run of DAD-mediated triggered activity (10 beats are shown) in RyR2R4496C+/− but not in RyR2wt (even no DADs; see text). The corresponding time courses of \( I_{\text{Ca,L}}, I_{\text{Na,Ca}}, I_{\text{rel}} \) (inset for diastole), open probability of RyR2, SOICR, \([\text{Ca}^{2+}]_{\text{jsr}}, [\text{Ca}^{2+}]_{\text{i}}\), and the driving force of \( I_{\text{rel}} \) ([\Ca^{2+}]_{\text{jsr}}−[\Ca^{2+}]_{\text{i}}) are shown. Note comparative values of \([\text{Ca}^{2+}]_{\text{jsr}}, [\text{Ca}^{2+}]_{\text{i}}\), and \( I_{\text{rel}} \) between RyR2R4496C+/− and RyR2wt show similar findings as indicated in A.
RyR2 mutant cells that could exhibit cellular functional properties as well as cellular phenotypic manifestations in close similarity to those described experimentally (10, 15, 22), i.e., 1) enhanced sensitivity of RyR2 to luminal Ca²⁺ (Fig. 1, A–C) was comparable with those depicted by Jiang et al. in heterologous HEK293 cell lines (15); this was further confirmed in channel-to-cell scale simulations (Supplemental Figs. S1 and S2); 2) rate- and BAS-dependent emergence of DADs and DAD-mediated triggered activity similar to those illustrated by Liu et al. in a RyR2 R4496C knock-in mouse model (22); and 3) dynamic [Ca²⁺]ᵢ changes underpinning these cellular characteristics (Supplemental Figs. S3 and S4) were in good correlation with [Ca²⁺]ᵢ imaging findings elegantly reported by Fernandez-Velasco et al. in the RyR2 R4496C knock-in mouse model (10).

Fig. 7. A: effects of targeted therapy on BAS-enhanced inducibility of DADs and DAD-mediated triggered activity in heterozygous R4496C mutant (RyR2R4496C/H11001/H11002). Initially (at t = 0) steady state values under BAS were used as initial data in all protocols. In columns a–d, membrane potential (Vₑ) trace (1) shows induction of DAD-mediated triggered activity after pacing for 422 stimuli (last stimulus denoted by a vertical arrow) at 2.0 Hz in RyR2R4496C/H11001/H11002 under BAS. Vₑ trace with corresponding time course of [Ca²⁺]ᵢ, IᵢCa,L, and [Ca²⁺]ᵢ (2) shows effects of reduction of IᵢCa,L (a and b) and IᵢCa,L (c and d) by 20% and 30%, respectively, after pacing for 422 stimuli (last stimulus denoted by a vertical arrow) at 2.0 Hz in RyR2R4496C/H11001/H11002 under BAS (see RESULTS).

B: effects of targeted therapy on BAS-enhanced inducibility of DADs and DAD-mediated triggered activity in heterozygous R4496C mutant (RyR2R4496C/H11001/H11002). Same condition as in A. In columns a–d, Vₑ trace (1) shows induction of DAD-mediated triggered activity after pacing for 422 stimuli (last stimulus denoted by a vertical arrow) at 2.0 Hz in RyR2R4496C/H11001/H11002 under BAS. Vₑ trace with corresponding time course of [Ca²⁺]ᵢ, IᵢCa,L, and [Ca²⁺]ᵢ (2) shows effects of reduction of IᵢCa,L (a and b) and IᵢNaCa (c and d) by 20% and 30%, respectively, after pacing for 422 stimuli (last stimulus denoted by a vertical arrow) at 2.0 Hz in RyR2R4496C/H11001/H11002 under BAS (see RESULTS).
 Furthermore, by applying steady state values of isolated simulation of Endo, M, and Epi cells as initial data for single cell and one-dimensional strand studies, we could demonstrate that 1) DADs and DADs/DAD-mediated TA intermittently emerged under BAS in RyR2R4496C+/− (e.g., Supplemental Figs. S4 and S6), but not in RyR2wt; 2) by having longer APD and higher RMP, the M cell was more prone to develop DADs and DAD-mediated TA compared with Endo and Epi cells under BAS (Supplemental Figs. S6 and S8); 3) a critical range (window) of the gap junction coupling, i.e., 0 ≤ D ≤ 0.000766*98 cm²/ms was needed for generating DAD-mediated TA in RyR2R4496C+/− (Supplemental Figs. S6 and S7); 4) in contrast with the long QT syndrome caused by ion channelopathies involving Ikss, Ikrs, INa, IK1, or ICa-L (23, 33, 34), genesis of DADs and DAD-mediated TA was not associated with QT interval prolongation (Tables 1 and 2 and Fig. 4); and 5) BAS facilitated induction of DAD-mediated TA via enhancing both ICa-L and IUP (Figs. 5 and 6). It is also noteworthy to note that the study demonstrated that 30% or more reduction of either ICa-L or IUP was required to suppress the inducibility of BAS-enhanced arrhythmogenesis and that combined 15% reduction of both ICa-L and IUP could exert a synergistic antiarrhythmic efficacy (Figs. 7 and 8).

Experimentally, electrophysiological mechanisms of arrhythmogenesis in CPVT have been well elucidated over the past several years (2, 3, 10, 12, 16, 22). Relevant to our modeling study, Cerrone et al. (2) showed that RyR2R4496C+/− knock-in mouse mice were prone to bidirectional ventricular tachycardia after exercise followed by epinephrine or caffeine administration and that the tachycardia could degenerate into ventricular fibrillation. Liu et al. (22) demonstrated that in RyR2R4496C+/− knock-in mouse mice, DADs, and/or DAD-mediated TA induced by pacing and/or isoproterenol could be abolished by ryanodine but not by K201 (an agent that enhances binding of FKBP12.6 to RyR2). Using the technique of optical imaging, Fernandez-Velasco et al. (10) showed an increase in Ca²⁺ sensitivity of RyR2 along with a lowered SOICR threshold in the cardiac myocyte isolated from the RyR2R4496C+/− knock-in mouse. Similarly, Kashimura et al. (16) confirmed that the RyR2R4496C+/− cell had lower SR Ca²⁺ content and a lower SOICR threshold compared with the RyR2wt counterpart and that BAS-induced increase in SR Ca²⁺ was sufficient to attain the SOICR threshold resulting in the production of Ca²⁺ waves in the RyR2R4496C+/− but not in the RyR2wt cell. Results of our study are in agreement with observations made in these experimental studies.

That the M cell was more vulnerable than Endo and Epi cells to develop DADs and DAD-mediated TA is in accordance with what have been described in experimental and computer modeling studies (1, 9, 34). However, the observation that the gap junction coupling represented by the diffusion coefficient D was crucial in the generation of DAD-mediated TA in the strand model is intriguing. In general, propagation of an action potential follows the source-sink relationship, depending on active and passive properties of individual elements of a conducting network (19, 27, 29). Among various passive properties, gap junctions are essential for determining how much depolarizing current passes from excited to nonexcited regions of the network, with which the safety factor for conduction is defined as the ratio of charge generated for the fiber by cell excitation to the minimal amount of charge required to cause the excitation (19, 27). Although the safety factor was not measured, findings of our study conform to the source-sink relationship. Specifically, under the setting of 0 ≤ D ≤ 0.000766*98 cm²/ms, a cell could exhibit a higher RMP than in the setting of D > 0.000766*98 cm²/ms due to its having less outward diffusion currents, thereby enabling DADs to attain the excitation threshold to manifest DAD-mediated TA (Supplemental Fig. S7). Given that there was a difference in D between the two settings: Endo-M and M-Epi (the latter of which had a preset 5-fold decrease in the gap junction coupling as described in METHODS), it became easier for DAD-mediated TA to transmit from the M cell to the Endo than to the Epi (Supplemental Fig. S6). Nevertheless, we realize that our study did not contain all the complexity of action potential propagation encountered in experiments such as specific cellular architecture, separate contribution of different ion currents, gap junction resistance, cell dimensions to propagation safety, etc. (19, 27).

With regard to targeted sites for effective pharmacotherapy, recent experimental data and preliminary clinical observations are of interest (18, 26, 28). In CASQ2 mutant mice, verapamil (a Ca²⁺-channel blocker) exhibited a dose-dependent protection against sympathetically stimulated-induced ventricular arrhythmia and its action was markedly enhanced when combined with propranolol (a β-blocker) (18), and clinically, combination therapy by adding verapamil to a β-blocker seemed more effective than β-blocker therapy alone in preventing exercise-induced arrhythmias in a limited number of patients with CPVT (18, 28). However, in a rat chronic heart failure model, SR Ca²⁺-ATPase2a (SERCA2a) gene therapy restored SR Ca²⁺ load and reduced RyR2 phosphorylation, thereby increasing the threshold of SOICR (without affecting the open probability of RyR2) and in turn decreased the SR Ca²⁺ leak; consequently, SERCA2a gene therapy attenuated TA in vitro and catecholamine-sensitive ventricular arrhythmias in vivo in failing hearts (26). Barring the complex action of IUP, its activity depends on [Ca²⁺]i, and a balance between [Ca²⁺]i and the ease with which Ca²⁺ waves propagate determines whether any change in the IUP activity would be antiarrhythmic or proarrhythmic in each specific setting (37). At present, all IUP blocking agents such as thapsigargin remain investigational and most of them exhibit extraneous side effects (5). Because BAS exerts its proarrhythmic effects through enhancement of both ICa-L and IUP activities in RyR2R4496C+/−, it is no surprise to find that combined 15% reduction of both ICa-L and IUP could synergistically suppress BAS-facilitated induction of DADs and DAD-mediated TA (Fig. 8B).

Whereas Ca²⁺ waves are produced by Ca²⁺ release through RyR2, experimentally it is well appreciated that not all ICa,L channel inhibitors (e.g., tetracaine) can suppress Ca²⁺ waves (37, 38). Despite INaCa involvement in the genesis of INaCa (17, 37), serving as a trigger for the genesis of DADs and DAD-mediated TA, our simulation study demonstrated that up to 50% reduction of INaCa failed to prevent arrhythmia from occurrence under BAS (Fig. 7). Finally, although reduction of INaNa was not tested because of slow accommodation of intracellular Na⁺ (25), we anticipated that INaNa reduction would increase cytosolic Na⁺ and thereby elevate [Ca²⁺]; via the reverse mode INaCa elevation of [Ca²⁺], would then lead to an increase in [Ca²⁺]i, thereby causing SR Ca²⁺ overload (17, 30) and triggering IrelSOICR events. Hence, INaNa inhibitors such
as digitalis should be deemed contraindicated in patients with CPVT (30).

Clinical implications. CPVT is a malignant arrhythmogenic disorder that predisposes the young with normal cardiac structure to syncope and cardiac arrest (21, 23). Our study has demonstrated the ease with which BAS facilitates induction of DADs and/or DAD-mediated TA. Although most patients are benefited by taking β-blockers, many of them continue to experience arrhythmic spells and are impervious to therapy with other antiarrhythmic drugs (18, 19, 21, 23, 28). Similar to patients with Timothy syndrome (LQT8) (34), ICD therapy may cause repeated delivery of electrical shocks (electrical storm) due to first shock-induced activation of sympathetic tone. Consequently, it is worthwhile to explore an additional or alternative therapeutic potential. To that end, our study has suggested that a regimen capable of reducing \( I_{Ca,L} \) and/or \( I_{UP} \) might be a useful adjunctive to β-adrenergic blocking agents and/or ICD implantation for suppressing ventricular tachyarhythmias associated with CPVT.

Recently, it has been reported that flecainide inhibits Ca\(^{2+}\) waves by blocking the open state of RyR2 and might thus be useful for suppressing ventricular arrhythmias in CPVT patients (13, 36, 38). Also experimentally in RyR2\(^{R4496C/+}\) knock-in mice, CaMK protein kinase II (CaMKII) overexpression enhances RyR2 phosphorylation and thereby increases the propensity toward triggered ventricular arrhythmias accounting for increased mortality (6). On the other hand, CaMKII inhibition counteracts with effects of BAS and thereby prevents arrhythmias in the ventricular myocyte of RyR2\(^{R4496C/+}\) knock-in mice (24). Thus research endeavors targeting at specific sites in the dynamics of intracellular Ca\(^{2+}\) cycling appear promising for treating patients with CPVT.

**Limitations.** Data obtained in the present study should be interpreted with caution. Besides inherent limitations of the computer modeling, precise correlation cannot always be achieved due to difference in species (e.g., mouse vs. guinea pig) and the study methodology (experiment vs. model simulation). While a common pathway (23) for RyR2 and CASQ2 mutations is yet to be proved, findings described herein might not be applicable to CPVT patients due to CASQ2 mutations, those due to RyR2 mutations caused by dysfunctional FKBP12.6 or defective interdomain interaction (23, 40) and those due to mutations other than RyR2 and CASQ2 that have yet to be determined.

Currently, for testing of a specific pharmacological agent, besides inclusion of ion channels Markov scheme (4), the recently described multiscale modeling linking ion channel molecular dynamics and electrostatics to the cardiac action potential (31) may confer more in-depth systematic information. Nevertheless, theoretical observations made in a simulation study as exemplified herein can provide potential directions for basic experiments and for clinical trials in inherited
arrhythmic disorders (34). Similarly, it can be further anticipated that the field of medical modeling will have an important role in studying various clinical conditions with acquired dysfunction of intracellular Ca\textsuperscript{2+} cycling such as myocardial ischemia and congestive heart failure.

**DISCUSSION**

**REFERENCES**


