Arrhythmogenesis in Catecholaminergic Polymorphic Ventricular Tachycardia
Insights From a RyR2 R4496C Knock-In Mouse Model

Nian Liu, Barbara Colombi, Mirella Memmi, Spyros Zissimopoulos, Nicoletta Rizzi, Sara Negri, Marcello Imbriani, Carlo Napolitano, F. Anthony Lai, Silvia G. Priori

Abstract—Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited disease characterized by life threatening arrhythmias and mutations in the gene encoding the ryanodine receptor (RyR2). Disagreement exists on whether (1) RyR2 mutations induce abnormal calcium transients in the absence of adrenergic stimulation; (2) decreased affinity of mutant RyR2 for FKBP12.6 causes CPVT; (3) K201 prevent arrhythmias by normalizing the FKBP12.6-RyR2 binding. We studied ventricular myocytes isolated from wild-type (WT) and knock-in mice harboring the R4496C mutation (RyR2 R4496C/+/−). Pacing protocols did not elicit delayed afterdepolarizations (DADs) (n=20) in WT but induced DADs in 21 of 33 (63%) RyR2 R4496C/+/− myocytes (P=0.001). Superfusion with isoproterenol (30 nmol/L) induced small DADs (45%) and no triggered activity in WT myocytes, whereas it elicited DADs in 87% and triggered activity in 60% of RyR2 R4496C/+/− myocytes (P=0.001). DADs and triggered activity were abolished by ryanodine (10 μmol/L) but not by K201 (1 μmol/L or 10 μmol/L). In vivo administration of K201 failed to prevent induction of polymorphic ventricular tachycardia (VT) in RyR2 R4496C/+/− mice. Measurement of the FKBP12.6/RyR2 ratio in the heavy sarcoplasmic reticulum membrane showed normal RyR2–FKBP12.6 interaction both in WT and RyR2 R4496C/+/− either before and after treatment with caffeine and epinephrine. We suggest that (1) triggered activity is the likely arrhythmogenic mechanism of CPVT; (2) K201 fails to prevent DADs in RyR2 R4496C/+/− myocytes and ventricular arrhythmias in RyR2 R4496C/+/− mice; and (3) RyR2–FKBP12.6 interaction in RyR2 R4496C/+/− is identical to that of WT both before and after epinephrine and caffeine, thus suggesting that it is unlikely that the R4496C mutation interferes with the RyR2/FKBP12.6 complex. (Circ Res. 2006;99:292-298.)

Key Words: cardiac electrophysiology • ryanodine receptor • sudden death • transgenic mice • ventricular tachycardia

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmogenic disease characterized by adrenergically mediated bidirectional or polymorphic ventricular tachycardia leading to syncpe and/or sudden cardiac death in individuals without structural heart disease.1,2

In 2001, we reported that the autosomal dominant form of CPVT is caused by mutations in the ryanodine receptor gene (RyR2).3 Based on the evidence that the morphology of ventricular tachycardia observed in CPVT resembles that of digitalis induced ventricular tachycardia (VT), it had been suggested that arrhythmogenesis in CPVT could be mediated by delayed afterdepolarizations (DADs) and triggered activity. Although the discovery that CPVT is caused by mutations in the ryanodine receptor has substantiated this hypothesis, up to now no conclusive demonstration that DADs cause CPVT is available.

Furthermore, although several authors have characterized in vitro the functional consequences of RyR2 mutations,4–6 the molecular and electrophysiological derangements leading to arrhythmias in CPVT patients are still unclear.7 In particular, 2 critical aspects are debated. The first is whether the ryanodine receptor is dysfunctional at rest4 or only on exposure to caffeine or to adrenergic stimulation.5 The second controversy concerns the hypothesis of Wehrens and colleagues8,9 that RyR2 mutations alter the interaction between ryanodine receptor and ventricular sarcoplasmic reticulum calcium leak and to the development of DADs. This hypothesis has been debated,5,10,11 and the issue is still open.

Given the importance of these unresolved issues for the understanding of the mechanism of CPVT and, possibly, for the development of novel therapeutic approaches, we attempted to clarify the electrophysiological and molecular mechanisms of RyR2 mutations leading to CPVT in our
conditional knock-in mouse model carrier of the R496C mutation of the RYR2 gene. The results of the investigations described here provide novel insights in the pathophysiology of CPVT.

## Materials and Methods

### Isolation of Adult Mice Ventricular Myocytes

Ventricular myocytes were isolated using an established enzymatic digestion protocol from knock-in mice harboring the CPVT-related R496C mutation (RyR2R496C/+) and wild-type (WT) mice (2 to 3 months) of either sex.

### Electrophysiological Recordings in Isolated Ventricular Myocytes

Laminin-coated dishes containing isolated ventricular myocytes were mounted on the stage of an inverted microscope. The myocytes were bathed with the solution containing (in mmol/L): 140 NaCl, 4 KCl, 2 CaCl2, 1 MgCl2, 10 HEPES, and 5 glucose, pH 7.4, with NaOH. A thermostatically controlled heating ring surrounding the dish was used to maintain the bath solution at 35°C. Transmembrane potentials and currents were recorded in whole cell current mode using a MultiClamp 700B amplifier (Axon Instruments). Patch electrodes were pulled from borosilicate glass on a P-97 horizontal puller (Sutter Instruments). The electrodes had a resistance of 2 to 3 MΩ when filled with patch electrode solutions containing (in mmol/L): 120 potassium aspartate, 20 KCl, 1 MgCl2, 4 Na2ATP, 0.1 GTP, 10 HEPES, 10 glucose, pH 7.2, with NaOH. All signals were acquired at 5 kHz (Digidata 1322A, Axon Instruments) and analyzed with the use of personal computer running pCLAMP version 9.2 software (Axon Instruments).

Only quiescent, calcium-tolerant, rod-shaped cells with clear cross striations and a resting potential of less than or equal to −80 mV were used for electrophysiological recordings. Myocytes were electrically stimulated by intracellular current injection through patch electrodes using depolarizing pulses with duration of 3 ms and a resting potential of less than or equal to −80 mV.

### Preparation of Cardiac Heavy Sarcoplasmic Reticulum Vesicles

Heavy sarcoplasmic reticulum (SR) vesicles from WT and RyR2R496C/+ mouse hearts were prepared as follows: 8 mouse hearts (∼1 g) were homogenized in 4 volumes of homogenization buffer (10 mmol/L, 1,4-piperazinediethanesulfonic acid [Pipes], 0.3 mol/L sucrose, pH 7.4, supplemented with Complete protease inhibitors [Roche]) with the use of a hand-held blender (Ultra-Turrax T25), and the homogenate was centrifuged at ∼8000 g for 10 minutes at 4°C (12 000 rpm; Beckman TL100.4 rotor). The pellet was resuspended in gradient buffer (10 mmol/L Pipes, 10% sucrose, 0.4 mol/L KCl, 0.5 mmol/L MgCl2, 0.5 mmol/L CaCl2, 0.5 mmol/L EGTA, pH 7.0) and layered on a discontinuous sucrose gradient consisting of 3 layers of 40%, 30%, and 20% sucrose (dissolved in gradient buffer). The gradients were centrifuged at ∼70 000 g (30 000 rpm; Beckman TLS55 rotor) for 14 hours at 4°C and the 30% to 40% interface containing the heavy SR vesicles was collected and diluted with an equal volume of 10 mmol/L Pipes (pH 7.4). Cardiac heavy SR vesicles were pelleted at ∼100 000 g for 1 hour at 4°C, resuspended in homogenization buffer, and stored at −80°C.

### Western Blot Analysis

Protein samples were resuspended in SDS-PAGE loading buffer (60 mmol/L Tris, 2% SDS, 10% glycerol, 5 mmol/L EDTA, 2% β-mercaptoethanol, 0.05% bromophenol blue, pH 6.8) and heated at 85°C for 5 minutes, and proteins were separated in a 4% SDS-PAGE gel strengthened with 0.5% agarose (for RyR) or a 15% SDS-PAGE gel (for FKBP). Proteins from 4% gels were electrophoretically transferred to a polyvinylidene difluoride membrane (Immobilon-P, Millipore) using a semidyndury transfer system (Trans-Blot SD, Bio-Rad) in buffer (48 mmol/L Tris, 39 mmol/L glycine, 0.0375% SDS) at 22 V for 4 hours. Proteins from 15% gels were transferred to PVDF membrane using a Mini-Wet blot transfer system (Bio-Rad) in buffer (26 mmol/L Tris, 192 mmol/L glycine, 20% methanol) at 200 mA for 1 hour. The membrane was blocked with either 5% nonfat milk protein in TBS-T buffer (20 mmol/L Tris, 137 mmol/L NaCl, 0.1% Tween-20, pH 7.4). Primary antibodies were applied overnight at 4°C: FKBP antibody at 1:200 dilution (rabbit polyclonal, Affinity Bioreagents). RyR2 antibody at 1:1000 dilution (Ab909, rabbit polyclonal antibodies raised to RyR2 residues 4454 to 4474). Immunoreactive protein bands were visualized by enhanced chemiluminescence detection (ECL, Amersham Biosciences). Densitometry analysis was performed using a GS-700 scanner (Bio-Rad) and Quantity One software (Bio-Rad).

### Chemicals

K201 (formerly JTV519) was kindly provided by Aetas Pharma Co Inc (Japan). 2,3-butanedione monoxime, Aventin, isoproterenol, and ryanodine were purchased from Sigma-Aldrich. All the chemicals used were of the highest grade available. K201 and ryanodine were dissolved in dimethyl sulfoxide (DMSO) as a stock solution of 10 mmol/L for in vitro study. The final concentration of DMSO in bath solution was less than 0.1%.
Data Analysis and Definitions
Data were expressed as mean±SEM. Statistical analysis was performed using the SPSS statistical package (version 13.0). Parametric tests were used to compare normally distributed variables (paired and unpaired t test and ANOVA for multiple comparisons). Cross-tabulations with χ² were used as appropriate for event incidences. Differences with P<0.05 were considered statistically significant.

Action potential duration (APD) was measured at 90% of repolarization (APD₉₀). We defined DADs as positive deflections of the membrane potential following an action potential. Triggered activity was defined as an action potential developing from a DAD rather than from an external stimulus. The incidence of DADs and triggered activity is reported for the faster pacing rate (5Hz).

Arrhythmias were defined as previously described.¹²

Results

Unstimulated WT and RyR²R₄⁴⁹⁶C⁺⁻ Myocytes
The first series of experiments compared the action potentials of WT and RyR²R₄⁴⁹⁶C⁺⁻ myocytes stimulated at 1 to 5 Hz at 35°C. Neither action potential amplitude nor resting potential differed significantly between WT myocytes (n=20) and RyR²R₄⁴⁹⁶C⁺⁻ myocytes (n=33) (amplitude, 120.4±1.3 versus 120.2±1.4 mV, P=NS; resting potential, 87.9±0.6 versus 86.7±1.0 mV, P=NS).

No spontaneous DADs were observed in WT myocytes at any pacing frequency, whereas DADs were observed in 63% of RyR²R₄⁴⁹⁶C⁺⁻ myocytes (21/33; P=0.001 versus WT) and 12% (4/33) of RyR²R₄⁴⁹⁶C⁺⁻ myocytes also presented triggered activity (Figure 1).

Response of WT and RyR²R₄⁴⁹⁶C⁺⁻ Myocytes to Isoproterenol
In the presence of 30 nmol/L isoproterenol, 45% of WT myocytes showed small DADs (9/20) but no triggered activity, whereas the incidence of DADs in RyR²R₄⁴⁹⁶C⁺⁻ myocytes on isoproterenol administration increased to 87% (29/33) of cells (P=0.002 versus WT). The majority of the RyR²R₄⁴⁹⁶C⁺⁻ myocytes also presented triggered activity (20/33, 60%; P=0.001 versus WT) (Figure 2). Spontaneous termination of the triggered rhythm occurred either with a progressive lengthening of the cycle length or abruptly.

Pharmacological Modulation of Triggered Activity in RyR²R₄⁴⁹⁶C⁺⁻ Myocytes

Protocol 1
When DADs, triggered activity, and Iₜ were induced in RyR²R₄⁴⁹⁶C⁺⁻ myocytes by 30 nmol/L isoproterenol, we added to the perfusion either 10⁻⁶ mol/L ryanodine (n=5) or K⁺ at 1 of the 2 dosages: 1⁻³ mol/L (n=5) or 10⁻³ mol/L K⁺ (n=5). Ryanodine completely abolished DADs, triggered activity (Figure 3) in all cells, whereas K⁺ failed to abolish DADs, triggered activity, and Iₜ (Figure 4A and Figure 5) in any cell at either dosage tested. Intriguingly, in 2 cells in which K⁺ failed to abolish DADs and Iₜ, subsequent administration of 10⁻⁶ mol/L ryanodine promptly abolished the DADs, triggered activity, and Iₜ (Figure 5).

Protocol 2
In the myocytes isolated from the hearts of mice (n=5) chronically treated with K⁺ (7 days), all cells developed DADs and triggered activity; thus no protective effect of

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Figure 1. A, Action potentials recorded from a RyR²R₄⁴⁹⁶C⁺⁻ myocytes stimulated at 1 to 5 Hz are shown: DADs develop when pacing is interrupted. B through F, The last 5 driven action potentials at each pacing frequency and DADs and triggered activity developing when pacing is discontinued are shown. Note that DAD amplitude increases and DAD coupling interval decreases at faster pacing frequencies. At 5 Hz, 2 triggered beats develop (F).

Figure 2. Action potential recording from a WT myocyte (A) and a RyR²R₄⁴⁹⁶C⁺⁻ myocyte (B) in the absence (top) and in the presence (bottom) of isoproterenol (30 nmol/L). Arrows indicate the last 5 paced action potentials.
FKBP Content in Heavy SR of WT and CPVT Mutant Mouse Hearts

The RyR2–FKBP12.6 association was indirectly assessed by measuring the ratio of FKBP12.6 to RyR2 detected in the heavy SR membrane subcellular fraction. The FKBP12.6, normally a soluble cytosolic protein, cosediments with the SR membranes of skeletal and cardiac muscle through its interaction with the ryanodine receptor, and because the RyR2 is the only known FKBP–binding protein in the SR, this “SR pull-down” assay is routinely used by a number of investigators as an index of RyR2–FKBP12.6 association.5,14–16

Western blot analysis indicated that the relative amounts of FKBP12.6 to RyR2 found in heavy SR of the stimulated heart was similar to the unstimulated control, for both WT and RyR2R4496C mice (Figure 7A). This result was demonstrated clearly by expressing the immunoblot densitometry data as a ratio of FKBP12.6 to RyR2 and normalizing against the unstimulated condition of WT mice (Table). These observations indicate normal RyR2–FKBP12.6 interaction in the heart from both WT and RyR2R4496C animals, which is not altered following treatment with caffeine and epinephrine. Interestingly, the FKBP12.6–RyR2 ratio in the RyR2R4496C mice was higher compared with WT, suggesting there may be a higher FKBP12.6–binding affinity for RyR2R4496C compared with WT RyR2. Alternatively, in the mutant mice, there were altered cellular FKBP12.6 levels and consequently SR levels, which could be part of a compensatory mechanism for a dysregulated, mutant RyR2 channel.

Discussion

The cardiac ryanodine receptor is a tetrameric intracellular Ca2+-release channel required for cardiac excitation–contraction coupling. Under normal conditions, RyR2 channels are activated by Ca2+ entering the cell through the voltage-dependent L-type Ca2+ channels, causing the release of Ca2+ from the SR into the cytosol, a mechanism known as Ca2+-induced Ca2+ release (CICR). Increased cytosolic Ca2+ levels activate the contractile apparatus. Ca2+ release is terminated when SR luminal Ca2+ levels falls below a threshold level, causing a decline in RyR2 activity via a mechanism called luminal Ca2+ dependent deactivation. The gating of RyR2 and multiple accessory proteins epigenetically control the precise procedure of calcium turnover in myocytes.

In 2001, we demonstrated that mutations in the gene encoding for the cardiac ryanodine receptor cause CPVT; subsequently, different groups characterized in vitro the functional properties of RyR2 mutations identified in patients in the attempt to define the molecular and the electrophysiological abnormalities leading to the development of bidirectional ventricular tachycardia and ventricular fibrillation in CPVT.

Triggered Activity Is the Electrophysiological Mechanism for CPVT

The first RyR2 mutation to be functionally characterized was the mouse homolog (R4496C) of the human mutation (R4497C) identified in the large Italian family in which we discovered that RyR2 is the gene for CPVT.3 Jiang et al4 first
investigated the R4496C-RyR2 mutation by expression in HEK 293 cells and showed that the mutant RyR2 presents enhanced resting activity, increased single-channel open probability, and accentuated sensitivity to caffeine activation leading to Ca^{2+} overload. Recently, this group further confirmed their observations in HL-1 cells.11 At variance with what suggested by Jiang et al, 4 2 independent groups5,8 challenged the view that the RyR2 mutation R4496C enhances Ca^{2+} release in the unstimulated conditions, although they fully confirmed the evidence that after exposure to caffeine or to β-adrenergic stimulation, RyR2 R4496C channels show increased calcium release as compared with WT. Nonetheless, all of the investigators4–6 agree with the view that the abnormal calcium handling observed in R4496C-RyR2 is likely to promote the development of DADs and triggered arrhythmias. However, there is still no direct evidence that DADs occur in response to RyR2 mutations identified in patients with CPVT.

Here we studied ventricular myocytes isolated from the heart of our RyR2^{R4496C+/−} knock in mouse model of CPVT12 to define (1) whether DAD and triggered activity can be elicited on β-adrenergic stimulation in RyR2^{R4496C+/−} myocytes and (2) whether RyR2^{R4496C+/−} myocytes develop DADs also in the absence of adrenergic stimulation as expected according to Jiang et al.4

Our results demonstrated that DADs are spontaneously present in unstimulated RyR2^{R4496C+/−} but not in WT myocytes. On exposure to β-adrenergic stimulation, we observed further enhancement of triggered action potentials arising

Figure 5. A, Protocol used for recording I_{\text{ti}}. B, I-V relationship of I_{\text{ti}} in RyR2^{R4496C+/−} myocyte in the absence and in the presence of K201 (n=5). C, I_{\text{ti}} recording in a RyR2^{R4496C+/−} myocyte exposed to isoproterenol 30 nmol/L (left); 1 μmol/L K201 does not decrease I_{\text{ti}} in the same RyR2^{R4496C+/−} myocyte in the presence of 30 nmol/L isoproterenol (middle), whereas subsequent addition of 10 μmol/L ryanodine to the same cell abolishes I_{\text{ti}} (right).

Figure 6. Bidirectional ventricular tachycardia induced by administration of epinephrine and caffeine in a RyR2^{R4496C+/−} mouse chronically treated with K201 (see text for details).

Figure 7. Western blot analysis of heavy SR vesicles prepared from WT and heterozygote (RyR2^{R4496C+/−}) mouse hearts after treatment (Epi+Caff) or without treatment (Control) with caffeine and epinephrine. SDS-PAGE gel lanes loaded with either 100 μg of homogenate (A and B) or 100 μg of SR (A) or 20 μg of SR protein (B) were separated through 15% (A) or 4% (B) gels, then transferred onto a membrane and probed with Ab^{FKBP} (A) and Ab^{1093} (B).
FKBP12.6 and RyR2 Expression Levels

<table>
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<th>WT Not Treated</th>
<th>WT Treated</th>
<th>RyR2&lt;sup&gt;R4496C&lt;/sup&gt;−/− Not Treated</th>
<th>RyR2&lt;sup&gt;R4496C&lt;/sup&gt;−/− Treated</th>
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<tr>
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<td>96±23.2</td>
<td>112.9±23.2</td>
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Western blot analysis was carried out as described in the legend to Figure 7, followed by immunoblot densitometry analysis of proteins corresponding to FKBP12.6 and RyR2. The cumulative data presented are from 4 such blots and are illustrated after normalization against the WT, untreated sample. Not treated indicates no treatment; treated, treatment with caffeine and epinephrine.

from DADs. The evidence of the development of DADs and triggered activity in the absence of β-adrenergic stimulation suggests that β-adrenergic stimulation worsen a preexisting abnormality of the mutant ryanodine receptor and is consistent with the clinical<sup>19</sup> and experimental<sup>12</sup> evidence that β-blockers attenuate, but do not abolish, ventricular tachycardia in CPVT patients.

Abnormal FKBP12.6–RyR2 Interaction Is Not Critical for the Pathogenesis of CPVT Caused by R4496C RyR2 Mutation

Despite intensive investigations, the molecular mechanisms by which RyR2 mutations alter the physiological properties of RyR2 in CPVT remain highly controversial. Wehrens and colleagues<sup>8,9</sup> first proposed that RyR2 mutants reduce the binding affinity of RyR2 for the regulatory protein FKBP12.6. The authors suggested that this defective interaction is already present in the resting state and it is further aggravated when PKA phosphorylates RyR2 in response to adrenergic stimulation causing further dissociation of FKBP12.6 and promoting Ca<sup>2+</sup> leakage from the SR. In support of their hypothesis, the same group performed a very elegant experiment and showed that K201, a derivative of 1,4-benzoisothiazepine formerly called JTV 519,<sup>20</sup> by enhancing the binding of FKBP12.6 to RyR2 is able to prevent adrenergically induced arrhythmias and sudden death in FKBP12.6<sup>R4496C</sup>−/− mice.<sup>9</sup> These results were interpreted by the authors as compelling evidence that FKBP12.6/RyR2 interaction is critical in the pathogenesis of CPVT. Other investigators challenged this hypothesis. George et al. found that mutant channels had a normal RyR2/FKBP12.6 interaction but an abnormally augmented response to isoproterenol and forskolin.<sup>8</sup> Chen and colleagues revealed that phosphorylation of RyR2 by PKA did not dissociate FKBP12.6 from RyR2.<sup>11</sup> This debate has remained until now unresolved.<sup>7</sup>

We speculated that based on the data by Wehrens et al.,<sup>8,9</sup> it is expected that K201 would prevent/inhibit arrhythmias in RyR2<sup>R4496C</sup>−/− mice. We therefore investigated whether K201, by enhancing FKBP12.6 binding to RyR2, is able to prevent arrhythmias elicited in vivo by caffeine and epinephrine in the RyR2<sup>R4496C</sup>−/− mice. We also compared the ability of K201 and ryanodine to prevent development of DADs in isolated RyR2<sup>R4496C</sup>−/− myocytes. Our results showed that K201 neither abolish nor prevents DADs and triggered activity, and it was unable to prevent the bidirectional ventricular arrhythmia induced by caffeine and epinephrine in RyR2<sup>R4496C</sup>−/− mice.

Furthermore, we assessed the RyR2–FKBP12.6 association in WT and RyR2<sup>R4496C</sup>−/− mice at rest and following treatment with caffeine and epinephrine ie, the protocol used to induce arrhythmias in the in vivo animal model.<sup>12</sup> Our results suggest (1) that in mouse hearts the RyR2 R4496C mutation does not disrupt FKBP12.6 interaction and (2) there is normal RyR2–FKBP12.6 association in the stimulated heart of both WT and RyR2<sup>R4496C</sup>−/− animals. These biochemical findings, together with the observation that K201 neither abolish nor prevent DADs and triggered activity, do not support the hypothesis that impaired FKBP12.6-dependent stabilization of the RyR2 channel is the molecular mechanism leading to arrhythmias in individuals with CPVT attributable to RyR2 R4496C mutation.

On the contrary, our data support the view that the molecular mechanism leading to abnormal calcium control in CPVT is related to FKBP12.6-independent regulation of the ryanodine receptor. RyR2 is a macromolecular complex that binds several proteins, such as calmodulin (CaM), CaM kinase II, FKBP12.6, sorcin, and phosphatases PP1 and PP2A.<sup>21</sup> Accordingly, it is likely that none of these proteins is the sole mediator of the functional consequences of RyR2 mutations. Because mutations linked to the CPVT phenotype are located in physically and functionally distinct regions of RyR2, it is likely that the position of each mutation is the mechanistic determinant of RyR2 dysfunction.<sup>22</sup> It can therefore be hypothesized that by acting on different targets, all mutations lead to the final common pathway of promoting diastolic calcium leak from RyR2.

Conclusions

In summary, our data demonstrate that DADs mediated triggered activity is the electrophysiological mechanism underlying CPVT. Furthermore, FKBP12.6-dependent channel stabilization mediated by K201 neither abolishes nor prevents triggered activity in RyR2<sup>R4496C</sup>−/− myocytes and ventricular arrhythmias in RyR2<sup>R4496C</sup>−/− mice, thus suggesting that at variance with what observed in other expression systems, the R4496C mutation is unlikely to interfere with the RyR2/FKBP12.6 complex in ventricular myocytes. These findings provide novel insights in the pathogenesis of CPVT and contribute to answer an unsolved dispute in this field about the role of the RyR2/FKBP12.6 complex in arrhythmogenesis in CPVT.

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Disclosures

None.

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