Sudden Death in Familial Polymorphic Ventricular Tachycardia Associated With Calcium Release Channel (Ryanodine Receptor) Leak

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Background—Familial polymorphic ventricular tachycardia (FPVT) is characterized by exercise-induced arrhythmias and sudden cardiac death due to missense mutations in the cardiac ryanodine receptor (RyR2), an intracellular Ca^{2+} release channel required for excitation-contraction coupling in the heart.

Methods and Results—Three RyR2 missense mutations, P2328S, Q4201R, and V4653F, which occur in Finnish families, result in similar mortality rates of ≈33% by age 35 years and a threshold heart rate of 130 bpm, above which exercise induces ventricular arrhythmias. Exercise activates the sympathetic nervous system, increasing cardiac performance as part of the fight-or-flight stress response. We simulated the effects of exercise on mutant RyR2 channels using protein kinase A (PKA) phosphorylation. All 3 RyR2 mutations exhibited decreased binding of calstabin2 (FKBP12.6), a subunit that stabilizes the closed state of the channel. After PKA phosphorylation, FPVT-mutant RyR2 channels showed a significant gain-of-function defect consistent with leaky Ca^{2+} release channels and a significant rightward shift in the half-maximal inhibitory Mg^{2+} concentration (IC_{50}). Treatment with the experimental drug JTV519 enhanced binding of calstabin2 to RyR2 and normalized channel function.

Conclusions—Sympathetic activation during exercise induces ventricular arrhythmias above a threshold heart rate in RyR2 mutation carriers. Simulating the downstream effects of the sympathetic activation by PKA phosphorylation of RyR2 channels containing these FPVT missense mutations produced a consistent gain-of-function defect. RyR2 function and calstabin2 depletion were rescued by JTV519, suggesting stabilization of the RyR2 channel complex may represent a molecular target for the treatment and prevention of exercise-induced arrhythmias and sudden death in these patients. **(*Circulation*. 2004;109:3208-3214.)**

Key Words: calcium ■ death, sudden ■ arrhythmia ■ sarcoplasmic reticulum ■ drugs

Sudden cardiac death (SCD) due to ventricular arrhythmias is a leading cause of mortality in patients with heart disease. The mechanisms that trigger fatal arrhythmias, however, are incompletely understood, and treatment and prevention remain largely ineffective. Familial polymorphic ventricular tachycardia (FPVT) is an autosomal-dominant disease associated with highly reproducible ventricular tachycardias during physical or emotional stress and SCD in the absence of structural heart disease.[1] FPVT was initially linked to 3 missense mutations (P2328S, Q4201R, and V4653F) in the cardiac ryanodine receptor (RyR2) gene in Finnish families (Figure 1A).[2] Distinct RyR2 missense mutations were reported in patients with catecholaminergic polymorphic ventricular tachycardia (CPVT)[3] and in patients with arrhythmogenic right ventricular dysplasia and exercise-induced arrhythmias.[4]

The RyR2 gene encodes the major intracellular Ca^{2+} release channel on the sarcoplasmic reticulum (SR) of cardiomyocytes. During excitation-contraction coupling, activation of a voltage-dependent L-type Ca^{2+} channel triggers a several-times-larger intracellular SR Ca^{2+} release via RyR2 that activates cardiac muscle contraction via Ca^{2+}-induced Ca^{2+} release.[5] RyR2 is a tetrameric channel complex comprised of 4 RyR2 monomers, each associated with 1 calstabin2 protein.[6,7] Calstabin2, also known as the FK506-binding protein (FKBP12.6), stabilizes the RyR2 channel in the

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The Data Supplement, which contains additional data and information about Methods, is available online at http://www.circulationaha.org.

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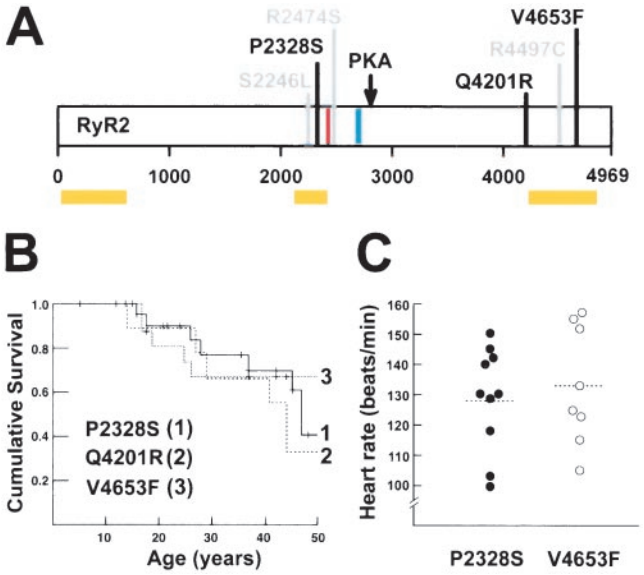

Figure 1. Relationship between RyR2 missense mutations, mortality, and exercise-induced arrhythmias. A, Schematic presentation of the human RyR2 protein with FPVT (black) and CPVT (gray) mutation sites, the calstabin2 binding-site (red), PKA phosphorylation site RyR2-Ser^{2809} (arrow), and a proposed calmodulin binding region (blue). RyR2 mutations correspond to cluster regions (yellow) in the highly homologous RyR1 channel from skeletal muscle containing missense mutations, increasing susceptibility to malignant hyperthermia. B, Kaplan-Meyer analysis of cumulative mortality rates for P2328S, Q4201R, and V4653F in RyR2 mutation carriers. At 35 years of age, mortality reaches 30% to 33% for all mutations. C, Threshold heart rates at which ventricular arrhythmias were initiated during exercise testing in P2328S or V4653F carriers (Q4101R not included because of small carrier number). Average thresholds as indicated by dashed lines are not significantly different ($P=0.58$) and approximate a heart rate of 130 bpm.

closed conformational state.[8,9] Cardiac output is increased by stimulation of β-adrenergic receptors, which activate RyR2 via cAMP-dependent protein kinase A (PKA) phosphorylation of 1 through 4 of the RyR2-Ser^{2809} phosphorylation sites present in the tetrameric RyR2 channel.[9–11] During sympathetic activation, PKA phosphorylation partially dissociates calstabin2 from the tetrameric RyR2 complex,[9,12] resulting in increased sensitivity to Ca^{2+}-dependent activation and higher RyR2 open probability (P_o). As a net result, β-adrenergic receptor signaling increases the gain of excitation-contraction coupling as part of an evolutionarily highly conserved stress pathway known as the fight-or-flight response.[10]

In the present study, we sought to delineate the clinical and biophysical mechanisms of exercise-induced SCD in FPVT patients and to determine if these overlap with CPVT patients who have similar symptoms but distinct mutations in the *RyR2* gene.[12] In addition, we wanted to investigate the potential therapeutic efficacy of Mg^{2+} and an experimental drug, a 1,4-benzothiazepine derivative (JTV519) that has been shown to decrease intracellular Ca^{2+} leak via RyR2 and to prevent heart failure.[13] Therefore, we examined the effects of Mg^{2+} and JTV519 on FPVT-mutant RyR2 channels.

Methods

The Ethics Review Committee of the Department of Medicine, University of Helsinki, approved this study. Informed consent was obtained from all patients.

Clinical Analysis

A detailed description of the clinical and electrocardiographic characteristics of FPVT mutation carriers was recently reported.[1,2] For the present study, additional carriers of the RyR2 mutations P2328S, Q4201R, and V4653F were identified, increasing the total number to 29. A Kaplan-Meyer analysis included 19 family members who had died suddenly under the age of 50 years. Carriers of different mutations underwent bicycle exercise testing using standard workload increments as described previously.[1] The threshold heart rate at which frequent premature complexes (PVCs) and nonsustained episodes of ventricular tachycardias (NSVTs) (3 or more premature ventricular complexes) first appeared was determined. All mutation carriers received β-adrenergic blocker therapy after completion of diagnostic testing combined with implantation of automatic implantable cardioverter-defibrillators in cases of incomplete protection.

Ryanodine Receptor Expression and Purification

Chameleon site-directed mutagenesis (Stratagene) was used to generate missense mutations of the hRyR2 gene in the pBS-SK$^-$ vector. HEK293 cells grown in MEM supplemented with 10% (vol/vol) FBS (Invitrogen), penicillin (100 U/mL), streptomycin (100 μg/mL), and L-glutamine (2 mmol/L) were cotransfected with 20 μg of wild-type or different FPVT-mutant cDNA and 2.5 μg of calstabin2 cDNA by Ca^{2+} phosphate precipitation for the expression of homotetrameric channels (RyR2-WT, RyR2-P2328S, RyR2-Q4201R, and RyR2-V4653F). In a separate experiment, RyR2-WT and RyR2-P2328S subunits were coexpressed at a 1:1 ratio (10 μg cDNA each) with 2.5 μg of calstabin2 cDNA (RyR2-WT×P2328S for heterotetrameric channels).[12,14]

Phosphorylation of Ryanodine Receptors and Single-Channel Recordings

Microsomes containing recombinant RyR2 isolated from transfected HEK293 cells were in vitro phosphorylated by the PKA catalytic subunit as described.[9] PKA phosphorylation of RyR2 aimed at maximal phosphorylation of all subunits, because FPVT patients develop syncope and SCD only during intense stress and because we found similar degrees of RyR2 phosphorylation during maximal exercise in vivo.[12] RyR2 single-channel recordings were performed under voltage-clamp conditions in lipid bilayers at variable concentration of *cis* (cytoplasmic) Ca^{2+} or Mg^{2+} as described previously.[12,14] The single-channel currents were filtered at 1 kHz with an 8-pole Bessel filter (Warner Instruments) and digitized at 4 kHz. Data are expressed as mean±SEM. Statistical analysis was performed with unpaired Student's *t* test, and $P<0.05$ was considered significant. Please refer to the online Data Supplement for details.

Results

Genotype-Phenotype Correlation in RyR2 Mutation Carriers

The P2328S missense mutation was found in 17 family members. Exercise bicycle testing induced ventricular arrhythmias in 10 of 12 tested P2328S mutation carriers (Data Supplement Table). In the second family, 3 subjects were positive for the Q4201R mutation, and 1 obligate carrier had died suddenly at age 27 years. Exercise-induced ventricular arrhythmias occurred in 2 Q4201R carriers. The V4653F mutation occurred in 9 family members, and 8 carriers displayed ventricular arrhythmias during exercise. A DNA sample from 1 family member, who had died suddenly at the

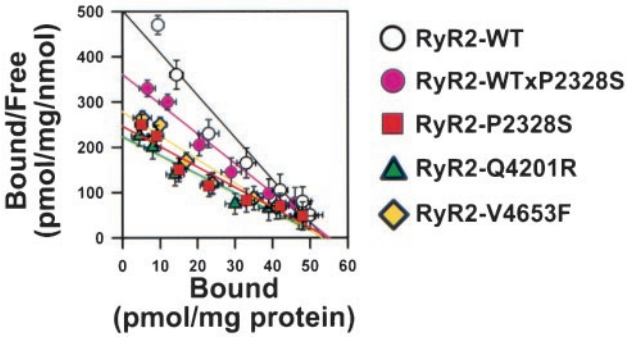

Figure 2. Calstabin2 binding is decreased in FPVT-mutant RyR2 channels. Scatchard analysis of ^{35}S-labeled calstabin2 binding to RyR2-WT, RyR2-WT×P2328S, RyR2-P2328S, RyR2-V4653F, or RyR2-Q4201R channels as indicated. All FPVT-mutant RyR2 channels showed significantly decreased calstabin2 binding affinities.

age of 50 years in the absence of any diagnostic findings during autopsy, demonstrated a positive V4653F carrier status, and 5 members had a history of SCD. On average, 91% of the RyR2 mutation carriers showed exercise-induced ventricular arrhythmias, which is similar to previously reported results from 8 unrelated carriers with distinct RyR2 missense mutations.[3] All FPVT mutation carriers had a normal QTc interval at rest and during exercise, and epsilon waves or negative T waves in leads V_1 through V_3 were excluded. Kaplan-Meyer analysis revealed similar mortality rates of 30% to 33% by the age of 35 years for the P2328S, Q4201R, and V4653F mutation carriers (Figure 1B). The threshold heart rates at which ventricular arrhythmias occurred during exercise testing were not significantly different between P2328S (129±17 bpm) and V4653F (133±20 bpm) mutation carriers (P=0.58; Figure 1C; 2 Q4201R mutation carriers not included). None of the mutation carriers had structural cardiac abnormalities or heart failure, as evidenced by echocardiography or ventriculography excluding ARDV2.[2,4] The characteristic pattern of arrhythmia induction in RyR2 mutation carriers suggests that 3 unrelated RyR2 mutations result in a highly reproducible phenotype of exercise-induced ventricular arrhythmias in the absence of structural heart disease.

RyR2 Missense Mutations Cause a Gain-of-Function Defect

To determine the functional effects of the RyR2 mutations, we coexpressed homotetrameric (RyR2-WT, RyR2-P2328S, RyR2-Q4201R, and RyR2-V4653F) and heterotetrameric (RyR2-WT×P2328S) channels with calstabin2 (Supplementary Figure, A). All FPVT-mutant channels were phosphorylated to the same degree as RyR2-WT by PKA, as evidenced by immunoblotting using the RyR2-Ser^{2809} phospho-epitope antibody. Specificity of PKA phosphorylation and dissociation of calstabin2 was shown in the presence or absence of the PKA inhibitor PKI_{5-24} (Supplementary Figure, B and C).[12] FPVT-mutant RyR2 channels showed significantly decreased binding affinity of ^{35}S-labeled calstabin2 (Figure 2). The dissociation constants (K_d) of homotetrameric channels (RyR2-P2328S, 223±23 nmol/L; RyR2-Q4201R, 242±38 nmol/L; and RyR2-V4653F, 191±29 nmol/L) and heterotetrameric channels (RyR2-WT×P2328S, 153±21 nmol/L) were significantly increased compared with control (P<0.001 versus RyR2-WT 107±19 nmol/L) at similar B_{max} values (53.3 to 55.1 pmol/mg per nmol). The significant increase in K_d as determined from Scatchard analysis (n=3) indicates decreased calstabin2 binding affinities of FPVT mutant RyR2.

Unphosphorylated RyR2-WT Ca^{2+} release channels exhibited low P_o (0.2±0.1%) at 150 nmol/L cytosolic Ca^{2+} in the presence of 1 mmol/L Mg^{2+} as expected, because under diastolic conditions, RyR2 channels have to be tightly closed to allow for relaxation of the heart muscle. Accordingly, the low P_o of homotetrameric FPVT-mutant channels (RyR2-P2328S, RyR2-Q4201R, and RyR2-V4653F) was not significantly different from RyR2-WT (Figures 3A and 3B). Because arrhythmias in FPVT patients are characteristically triggered by exercise, we simulated the effects of sympathetic activation on RyR2 by in vitro PKA phosphorylation of the channels, as confirmed by a phosphospecific antibody against RyR2-Ser^{2809} (Supplementary Figure, B). PKA phosphorylation significantly increased the P_o of all FPVT-mutant RyR2 channels, consistent with a gain-of-function defect (Figures 3C and 3D). Accordingly, the distribution of opening events was shifted to higher current amplitudes, as shown in respective histograms. Mutagenesis studies of the PKA phosphorylation site RyR2-Ser^{2809} in the full-length channel have excluded additional PKA phosphorylation sites.[11] The unchanged activity of nonphosphorylated FPVT mutant channels and the gain-of-function defect consistently observed in all phosphorylated FPVT mutant channels support the concept that PKA phosphorylation of Ser^{2809} is a necessary event to activate the functional defect.

FPVT occurs as an autosomal-dominant trait, where both mutant and WT subunits are thought to be present in heterotetrameric RyR2 complexes.[2] To investigate the phenotype of heterotetrameric FPVT-mutant RyR2 channels, we coexpressed RyR2-WT and RyR2-P2328S mutant subunits at a 1:1 ratio and characterized the resulting heterotetrameric channel (RyR2-WT×P2328S). In the presence of the PKA inhibitor PKI_{5-24}, RyR2-WT×P2328S resembled the low P_o seen in the homotetrameric channels (Figures 3A and 3B). Typical recordings of PKA phosphorylated heterotetrameric RyR2-WT×P2328S channels resemble the gain-of-function defects seen in homotetrameric RyR2-P2328S channels (Figures 3C and 3D).

FPVT-Mutant RyR2 Are Resistant to Mg^{2+} Inhibition

Phosphorylated homotetrameric mutant RyR2 channels showed resistance to inhibition by millimolar Mg^{2+} (Figure 4A). Whereas RyR2-WT channel activity was significantly inhibited (P_o <1.0%) at Mg^{2+} concentrations ≥2.0 mmol/L, the P_o of mutant RyR2-P2328S channels remained significantly increased. Inhibition of average P_o ≈1.0% required 10 mmol/L Mg^{2+} in homotetrameric FPVT-mutant RyR2 channels (Figure 4B). Similarly, heterotetrameric RyR2-WT×P2328S channels showed a significant resistance to inhibition by Mg^{2+} (Figure 4B). Thus, after PKA phosphorylation, heterotetrameric and homotetrameric FPVT-mutant

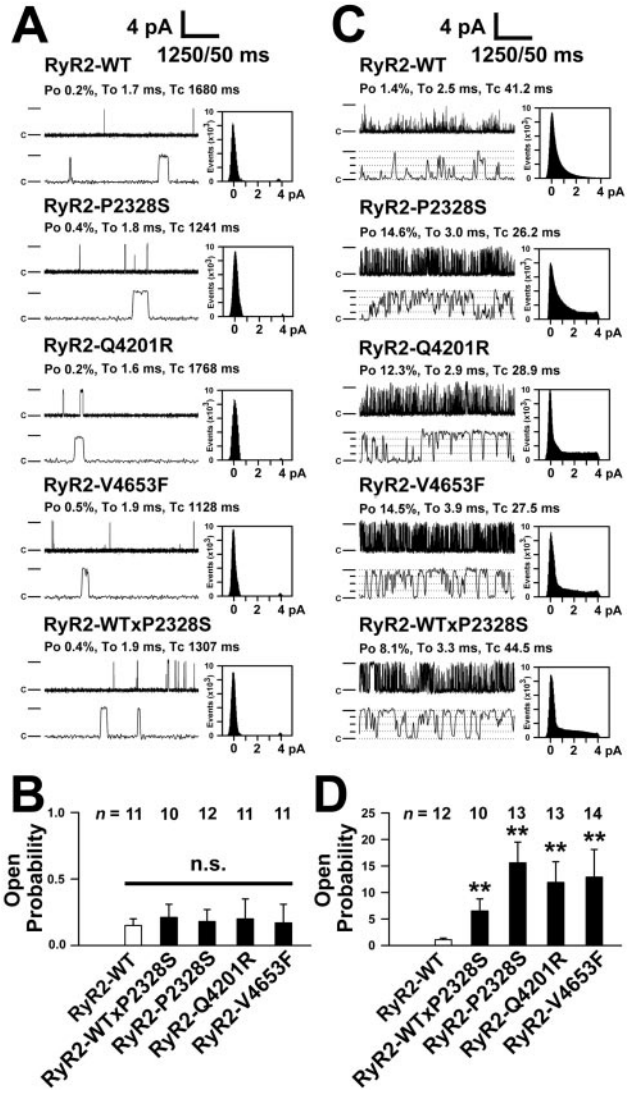

Figure 3. PKA phosphorylation unmasks a gain-of-function defect in FPVT-mutant RyR2 channels. A, Left column shows representative single-channel experiments from RyR2 channels treated with PKA and the PKA inhibitor PKI_{5-24}. Similar to RyR2-WT (control), FPVT-mutant RyR2 activity at *cis* (cytoplasmic) $[Ca^{2+}]$ of 150 nmol/L and $[Mg^{2+}]$ of 1.0 mmol/L representing diastolic conditions was low, and the closed state appeared dominant in the amplitude histograms. Channel openings are upward deflections; the difference between horizontal bars indicates 4-pA current amplitude between open and closed states (c), and 1pA subconductance levels are indicated for phosphorylated channels. Examples of upper and lower current traces represent 5000 ms or 200 ms throughout, as indicated by dimension bars, respectively. To, average open time; Tc, average closed time. B, Bar graph summarizes P_o (%) of unphosphorylated RyR2 channels. C, On PKA phosphorylation, which simulates sympathetic activation during exercise, FPVT-mutant RyR2 channels, but not RyR2-WT, display significantly increased activities and long-lasting open states. D, Summary bar graph shows significantly increased P_o (%) in phosphorylated homotetrameric and heterotetrameric RyR2 channels. $^{**}P<0.001$. For display purposes, B and D have different dimensions.

RyR2 channels displayed a significant gain-of-function abnormality that was not inhibited within the physiological range of Mg^{2+} concentrations. The resistance of FPVT-mutant RyR2 to Mg^{2+} inhibition involved a significant

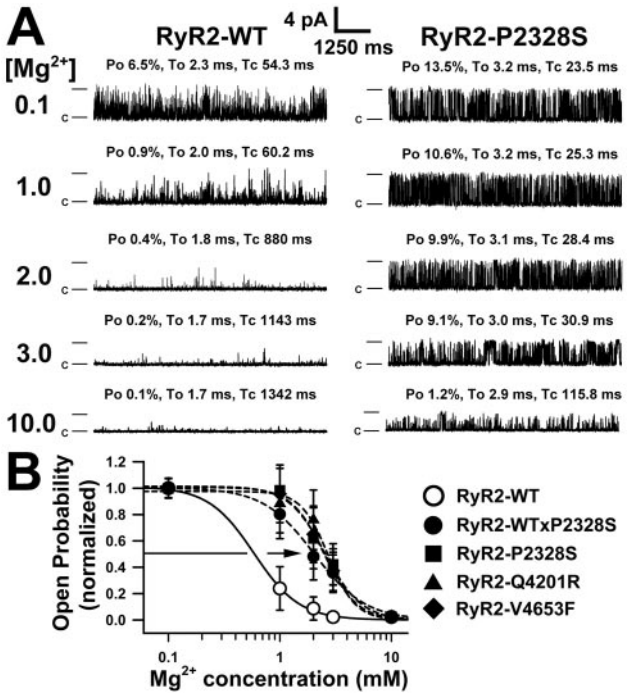

Figure 4. Mutant RyR2 channels are resistant to inhibition by millimolar Mg^{2+} concentrations. A, Representative current amplitude traces of PKA-phosphorylated RyR2-WT (control) and RyR2-P2328S channels at increasing millimolar Mg^{2+} concentrations indicate a resistance to Mg^{2+} inhibition in the mutant channel. Channel openings are represented as upward deflections; difference between horizontal bars indicate 4-pA level between open and closed state (c). Temporal resolution is 5000 ms for all traces, $[Ca^{2+}]_{cis}$ 150 nmol/L. At higher Mg^{2+} concentrations, current amplitude becomes progressively decreased. B, Regression analysis of normalized P_o of homotetrameric and heterotetrameric mutant RyR2 channels reveals a significant rightward shift of the half-maximal inhibitory Mg^{2+} concentration EC_{50}, as indicated by arrow.

rightward shift of the half-maximal inhibitory Mg^{2+} concentration (IC_{50} in mmol/L: RyR2-P2328S, 2.89±0.08; RyR2-WT×P2328S, 2.49±0.03; RyR2-Q4201R, 2.72±0.12; RyR2-V4653F, 3.53±0.12; $P<0.01$ versus RyR2-WT 1.53±0.03). These data suggest that the FPVT-associated RyR2 mutations render these channels partially resistant to inhibition by an important endogenous channel modulator, Mg^{2+}.

JTV519 Rescues FPVT-Mutant Channel Function by Rebinding of Calstabin2

Recently, the 1,4-benzothiazepine derivative JTV519 was shown to inhibit progression of canine heart failure, possibly by increasing the binding of calstabin2 to RyR2.[13,15] Therefore, we examined whether JTV519 affects the activity of mutant RyR2-P2328S channels (Figure 5, A and B). PKA-phosphorylated RyR2-P2328S channels showed a significant leftward shift to half-maximal activation by increasing *cis* (cytosolic) Ca^{2+} concentrations (EC_{50} shift in nmol/L from 389.8±44.5 to 204.3±21.7; $P<0.001$ versus unphosphorylated RyR2-P2328S, each n=11), which was significantly more pronounced than in phosphorylated RyR2-WT (EC_{50}, 308.6±36.2 nmol/L; $P<0.005$ vs RyR2-P2328S+PKA,

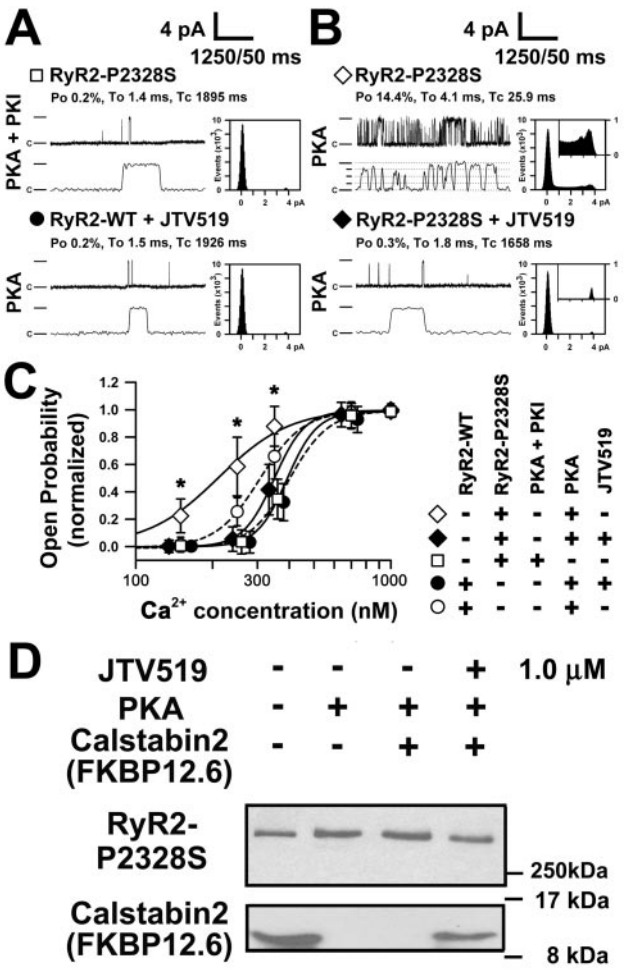

Figure 5. JTV519 normalizes RyR2-P2328S channel function and structure. A, Representative single-channel current traces of unphosphorylated RyR2-P2328S and PKA-phosphorylated RyR2-WT treated with 1.0 µmol/L JTV519. B, PKA-phosphorylated RyR2-P2328S activity becomes normalized by 1.0 µmol/L JTV519. Insets show channel openings >1 pA at a higher resolution. C, Regression analysis of P_o from PKA-phosphorylated, unphosphorylated, and JTV519-treated RyR2-P2328S and RyR2-WT at increasing Ca^{2+} concentrations shows a significant leftward shift compared with unphosphorylated RyR2-P2328S and phosphorylated RyR2-WT treated with 1.0 µmol/L JTV519. JTV519 normalized the hypersensitivity of phosphorylated RyR2-P2328S to Ca^{2+}-dependent activation. RyR2-P2328S, continuous lines; RyR2-WT, dashed lines. D, Immunoblot analysis of calstabin2 binding of RyR2-P2328S in the presence or absence of PKA and 1.0 µmol/L JTV519, as indicated. RyR2-P2328S was immunoprecipitated and in vitro PKA was phosphorylated as described in the online Methods section.

n=10, Figure 5C). The increased Ca^{2+} sensitivity of PKA-phosphorylated RyR2-P2328S channels under diastolic conditions of 150 nmol/L Ca^{2+} and 1.0 mmol/L Mg^{2+} suggests that the mutant channels could be activated inappropriately during diastole.

Next, we treated PKA-phosphorylated RyR2-P2328S and RyR2-WT channels with 1.0 µmol/L of JTV519. Channel activity was normalized by JTV519, and half-maximal activation by *cis* (cytosolic) Ca^{2+} was not significantly different from unphosphorylated RyR2-P2328S (Figure 5B; EC_{50}, 345.3±33.4 nmol/L; n=12), and Ca^{2+} sensitivity was similar to PKA-phosphorylated RyR2-WT treated with JTV519 (Figure 5, A and C; EC_{50}, 399.8±11.9 nmol/L; n=10). Treatment of RyR2-P2328S with 1.0 µmol/L JTV519 resulted in rebinding of calstabin2 to unphosphorylated levels in the channel complex, confirming the effects of our single-channel experiments (Figure 5C). These results indicate that the experimental drug JTV519 may rescue the gain-of-function defect in RyR2-P2328S channels via increased binding of the stabilizing calstabin2 subunit to the channel complex.

Discussion

RyR2 missense mutations have been identified in some familial forms of exercise-induced ventricular tachycardias and sudden death.[2,3] All mutations resulted in high mortality rates in early adulthood, and no phenotypic differences were observed in the appearance of ventricular arrhythmias. Arrhythmogenic activity correlated closely with the degree of exercise increasing from premature ventricular contractions to nonsustained ventricular tachycardias. These data suggest that structural changes and sympathetic activation of FPVT-mutant channels combine to trigger arrhythmias in RyR2 mutation carriers.

Recombinant RyR2 channels containing the missense mutations found in mutation carriers (RyR2-P2328S, RyR2-Q4201R, and RyR2-V4653F) showed a significant gain-of-function defect. The ≈10-times increased P_o of FPVT-mutant RyR2 channels occurred specifically during PKA phosphorylation, which is a downstream effector of sympathetic activation. When PKA phosphorylation was specifically inhibited with the peptide PKI_{5-24}, FPVT-mutant RyR2 channels were not different from RyR2-WT. Observed differences in channel activity were tested in at least 3 different preparations from parallel PKA reactions to exclude experimental variability, and equal phosphorylation levels were confirmed in immunoblots (Supplementary Figure, B). In accordance with our results in homotetrameric FPVT-mutant RyR2, we found similar functional and structural defects related to PKA phosphorylation in heterotetrameric RyR2-WT×P2328S channels. These findings exclude the possibility that the RyR2-WT subunit in the tetrameric channel may rescue the functional phenotype. Although we used a constant molar ratio for mutant and wild-type subunit expression, we were not able to determine the exact subunit composition on an individual channel basis. However, the fact that the degree of the gain-of-function defect increased from heterotetrameric RyR2-WT×P2328S to homotetrameric RyR2-P2328S channels is consistent with a heterotetrameric subunit composition in the former. Unmasking the gain-of-function defect by PKA phosphorylation closely reflects the disease phenotype where ventricular arrhythmias are triggered only above heart rates of 130 bpm and SCD or arrhythmias do not occur in the absence of sympathetic activation.[2,3]

We have recently linked decreased calstabin2 levels in the RyR2 channel complex to delayed afterdepolarizations, exercise-induced arrhythmias, and SCD and proposed this as a disease mechanism in patients with CPVT.[12] Now we find significantly decreased calstabin2 binding affinities and a gain-of-function defect in distinct FPVT-mutant RyR2 channels. The gain-of-function defect during maximal PKA phos-

phorylation of FPVT-mutant RyR2 channels paralleled the effects of PKA phosphorylation on RyR2 channel function in calstabin2$^{-/-}$ mice, which was sufficient to induce arrhythmias and sudden death.[12] We propose that the common final pathway of all RyR2 missense mutations may be reduced calstabin2 levels in the RyR2 channel complex during PKA phosphorylation and increased Ca^{2+} leak, which in turn would activate inwardly depolarizing membrane currents, delayed afterdepolarizations, and triggered arrhythmias.[12,16,17]

We observed a significantly increased resistance to inhibition by Mg^{2+} in all PKA-phosphorylated FPVT-mutant RyR2 channels. In cardiac muscle, free intracellular Mg^{2+} concentrations of approximately 1 mmol/L have been reported.[18,19] Millimolar Mg^{2+} concentrations stabilize the closed state of RyR2-WT channels under diastolic conditions[20,21] and inhibit rapid Ca^{2+} release from SR vesicles.[22] During exercise, sympathetic stimulation decreases Mg^{2+} in heart muscle cells by up to 20%, which may additionally increase the propensity of arrhythmias in RyR2 mutation carriers.[23] Decreased plasma levels of Mg^{2+} were shown to increase the propensity for ventricular arrhythmias and sudden cardiac death,[24–26] whereas interventions that increase Mg^{2+} plasma levels were shown to decrease the incidence of fatal arrhythmias in heart failure, ischemic heart disease, and other conditions with an increased propensity for SCD.[27–29] Therefore, significantly decreased RyR2 sensitivity to inhibition by Mg^{2+} may represent an additional mechanism that contributes to SCD in RyR2 mutation carriers.

Previously, we and others have shown that PKA hyperphosphorylation of RyR2 channels in failing human hearts significantly increases RyR2 activity by depletion of calstabin2 from the channel complex, resulting in increased Ca^{2+} leak.[9,30] In performing experiments to examine the affects of PKA phosphorylation of RyR2 or the FPVT mutations on the binding of calstabin2 to the channel, we are careful to maintain physiological ratios of calstabin2 to RyR2, as addressed in a recent article.[17] Overexpression of calstabin2 outside the physiological range may counteract the shift in K_d induced by PKA phosphorylation and explain different findings reported by other groups.[31,32] Treatment with β-adrenergic receptor blockers reverses PKA hyperphosphorylation and calstabin2 depletion in heart failure, and the beneficial effects of β-blocker treatment in patients with exercise-induced arrhythmias may be related to prevention of RyR2-mediated SR Ca^{2+} leak.[33]

Recently it was reported that the 1,4-benzothiazepine derivative JTV519 inhibits FK506-induced intracellular Ca^{2+} leak in the heart and may normalize leaky RyR2 in failing hearts.[13,15] Treatment of phosphorylated RyR2-P2328S channels with 1.0 μmol/L JTV519 completely normalized the gain-of-function defect, and the significant leftward shift of Ca^{2+} sensitivity was rescued (Figure 5). In contrast to Mg^{2+} (data not shown), JTV519 treatment resulted in significantly increased calstabin2 levels in RyR2-P2328S channels. These studies demonstrate a molecular mechanism whereby JTV519 may prevent diastolic Ca^{2+} leak through the FPVT-mutant RyR2 channels. Thus, the present studies not only provide a basis for treating exercise-induced cardiac arrhythmias that cause SCD but also indicate that JTV519 could be beneficial in the treatment of heart failure, which is associated with aberrant SR Ca^{2+} leak via calstabin2-depleted RyR2 channels.[9,33] Furthermore, these studies confirm results with a high-affinity calstabin2-D37S mutant that normalized the channel function of constitutively PKA-phosphorylated RyR2 channels.[12]

In summary, genotype-phenotype studies in RyR2 mutation carriers showed high mortality rates and a reproducible threshold heart rate above which ventricular arrhythmias occur. The electrophysiological phenotype of FPVT mutation carriers is characterized by exercise-induced polymorphic ventricular arrhythmias above a heart rate threshold of approximately 130 bpm and incomplete suppression of arrhythmias and sudden death by β-blockers. Three structurally unrelated RyR2 missense mutations exhibited a significant gain-of-function defect at the single-channel level with a resistance to inhibition by Mg^{2+}. This defect was specific to conditions that simulate adrenergic activation, in agreement with the clinical phenotype. The experimental drug JTV519 normalized FPVT-mutant RyR2 channel function by rebinding of calstabin2 to the channel complex. Therefore, stabilization of the closed state of mutant RyR2 channels by increased calstabin2 binding may represent a novel pharmacological principle to prevent arrhythmias and sudden death in this population and may have broader implications, ranging from genetic forms of arrhythmias to complex diseases associated with a high incidence of SCD, such as heart failure.

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