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An invited review following *the Soujinkai Award*: Ryanodine Receptor Bound Calmodulin Is Essential to Protect against Catecholaminergic Polymorphic Ventricular Tachycardia

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Abstract Catecholaminergic polymorphic ventricular tachycardia (CPVT) is caused by a single point mutation in the cardiac type 2 ryanodine receptor (RyR2). Using knock-in mouse (KI) model (R2474S/+), we previously reported that a single point mutation within the RyR2 sensitized the channel to agonists, primarily mediated by defective inter-domain interaction within the RyR2 and subsequent dissociation of calmodulin (CaM) from the RyR2. Here, we examined whether CPVT can be genetically rescued by enhancing the binding affinity of CaM to the RyR2. We first determined whether there was a possible amino-acid substitution within the CaM-binding domain in the RyR2 (3584-3603) that can enhance its binding affinity to CaM, and found that V3599K substitution showed the highest binding affinity of CaM to CaM-binding domain. Hence, we generated a heterozygous KI mouse model (V3599K/+) with a single amino acid substitution in the CaM-binding domain of the RyR2, and crossbred it with the heterozygous CPVT –associated R2474S/+ KI mouse to obtain a double heterozygous R2474S/V3599K KI mouse model. The CPVT phenotypes, bidirectional or polymorphic ventricular tachycardia, were inhibited in the R2474S/V3599K mice. Thus, enhancement of the CaM binding affinity of the RyR2 is essential to prevent CPVT-associated arrhythmogenesis.

Key words: catecholaminergic polymorphic ventricular tachycardia, ryanodine receptor, calmodulin Ca^{2+}

Introduction

The cardiac type 2 ryanodine receptor (RyR2) is a huge tetrameric protein in the sarcoplasmic reticulum (SR) through which a large amount of Ca^{2+} is released from the SR, triggered by a small amount of Ca^{2+} entering the L-type Ca^{2+} channel.[1,2] To date, more than 150 mutations within the RyR2 have been reported to be linked with catecholaminergic polymorphic ventricular tachycardia (CPVT).[3] The point mutations observed in CPVT are not randomly distributed but clustered into 3 hot domains: the N-terminal (1-600 residues), the central (2000-2500 residues), and the C-terminal.[4] Based on the "domain switch hypothesis" Ikemoto and colleagues proposed,[5] we demonstrated that the single

point mutation caused unzipping between the N-terminal and the central domains, which gave rise to a Ca^{2+} leak in the CPVT-associated R2474S/+ -knockin (R2474S/+-KI) mouse[6] and also in a canine model of tachycardia-induced heart failure.[7-9] These findings suggest that domain unzipping between the N-terminal and the central domains plays a critical role in the pathogenesis of lethal arrhythmia and heart failure as well. Recently, we found that domain unzipping displaced calmodulin (CaM) from the RyR2, which otherwise played an inhibitory role in channel opening, causing Ca^{2+} leak in CPVT[10] and heart failure.[11] These notions support the idea that the specific binding of CaM to the RyR2 plays a critical role in the pathogenesis of CPVT and heart failure. We hypothesized that the enhancement of CaM-binding affinity toward the defective RyR2 under stressful conditions prevented spontaneous Ca^{2+} release, thereby preventing CPVT.

Generation of V3599K KI mouse

We first determined whether there was a possible amino acid substitution within the CaM-binding domain in the RyR2 (3584-3603 residues) that can enhance its binding affinity.[12] For this purpose, we assessed the binding of CaM to the RyR2 using a photoreactive cross-linker, sulfosuccinimidyl-6-(4′-azido-2′-nitrophenylamino) hexanoate (sulfo-SANPAH) to evaluate the CaM-binding affinity of 20-mer CaM-binding peptides (CaMBPs) harboring the same amino acid sequence as the CaM-binding domain (3584-3603 residues) except 1 amino acid residue. Of the 25 peptides, peptide 24, in which a V3599K substitution was done, showed the highest inhibition of CaM-SANPAH binding to the RyR2, indicating a higher binding affinity of CaM to the 24 CaMBP than to the RyR2 (Fig. 1). We then generated a heterozygous V3599K/+-KI mouse model in which the binding affinity of CaM to the RyR2 was markedly enhanced by a single amino acid mutation (V3599K) at the CaM-binding domain within the RyR2, and then we crossbred the V3599K/+-KI mouse with the R2474S/+-KI mouse to obtain a heterozygous R2474S/V3599K-KI mouse model.

Effect of enhancement CaM binding affinity to RyR in CPVT model mouse

In this study, we examined whether arrhythmia was observed in R2474S/V3599K-KI mice using 24-hour electrocardiography (ECG) monitoring and examined the effect of epinephrine or exercise on ventricular arrhythmia. Ventricular tachycardia (VT) was easily induced by epinephrine or exercise in all R2474S/+ mice but not in R2474S/V3599K-KI mice as well as WT and V3599K/+-KI mice (Fig. 2, A and B). Under 24-hour ECG monitoring, both VT and ventricular bigeminy were frequently observed even under a small amount of stress in R2474S/+ mice but not mainly in R2474S/V3599K-KI mice (Fig. 2, C and D).

Hypothesis by which V3599K substitution in RyR2 can stabilize the channel

One of the major hypotheses to explain the RyR2 dysfunction in heart failure and lethal arrhythmias, such as CPVT, is that defective interaction between the N-terminal (N: 1-600) and the central (C: 2000-2500) domains4,5 causes an instability of the channel. On the basis of the above-mentioned structural background regarding the pathogenic mechanism of RyR2-associated CPVT, we propose a hypothetical model to explain why CaM was not displaced from the R2474S-mutated subunits by a single amino acid substitution (V3599K) applied against the subunits not harboring R2474S mutation (Fig. 3). In R2474S/+-KI hearts the defective intersubunit interaction, namely domain unzipping, that occurred in the R2474S-mutated subunits may allosterically decrease the binding affinity of CaM to the R2474S-mutated subunits and in turn displace approximately half of the bound CaM from the RyR2. On the other hand, in R2474S/V3599-KI hearts the defective intersubunit interaction would be minimized owing to the strong binding of CaM to the V3599K-mutated subunits, thereby preventing the decrease in the binding affinity of CaM to the remaining R2474S-mutated subunits and subsequent displacement of CaM from the RyR2. This postulated model is supported by the previous finding

obtained by fluorescence resonance energy transfer method that the domain switch and CaM-binding regions are distinct but interact allosterically (rather than orthosterically).

Conclusion

Enhancement of the CaM-binding affinity of the RyR2 is essential to prevent CPVT-associated arrhythmogenesis. Thus, CaM may act as an intrinsic natural stabilizer for the RyR2 channel function, and hence increasing the affinity of CaM to the RyR2 could be a potent therapeutic target for heart failure and lethal arrhythmia.

Conflict of Interest

The authors declare no conflict of interest.

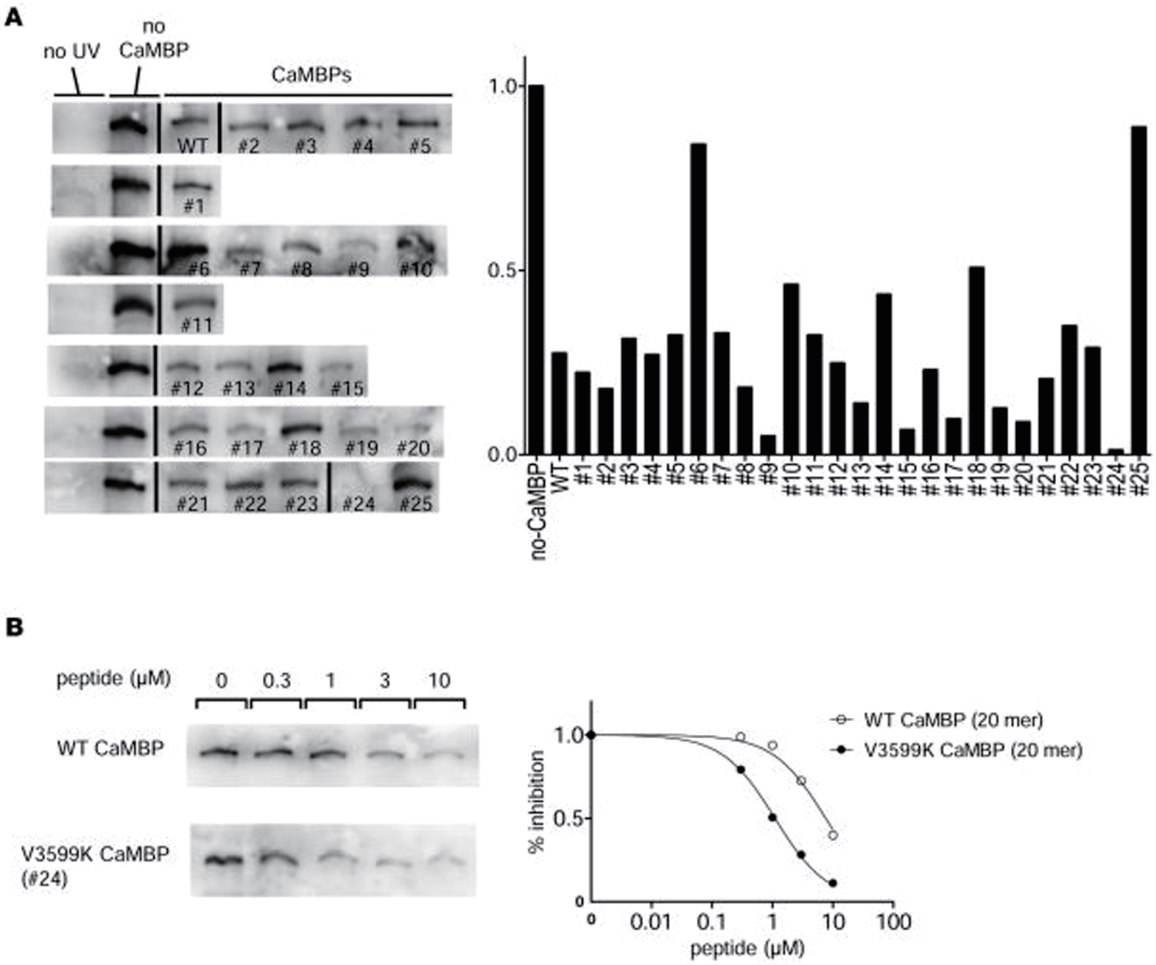

Fig. 1 Determination of a single amino acid substitution within the CaM-binding domain (3584-3603 residues) in the RyR2 by which CaM-binding affinity is highly increased. (A) Evaluation of the binding affinity of 20-mer CaM-binding peptides (CaMBPs) harboring the same amino acid sequence as the CaM-binding domain (3584-3603 residues) except 1 amino acid residue by CaM-SANPAH cross-linking method. Of 25 peptides, peptide 24, in which V3599K substitution was done, showed the highest inhibition of CaM-SANPAH binding to the RyR2, indicating a higher binding affinity of CaM to the 24 CaMBP than to the RyR2. The y axis represents the ratio of the density to no CaMBP. (B) Concentration-dependent inhibitory effect of either WT CaMBP or V3599K CaMBP on CaM-SANPAH binding to the RyR2 V3599K CaMBP showed a strong inhibition compared with WT CaMBP, indicating a higher binding affinity of V3599K CaMBP to CaM than of WT CaMBP

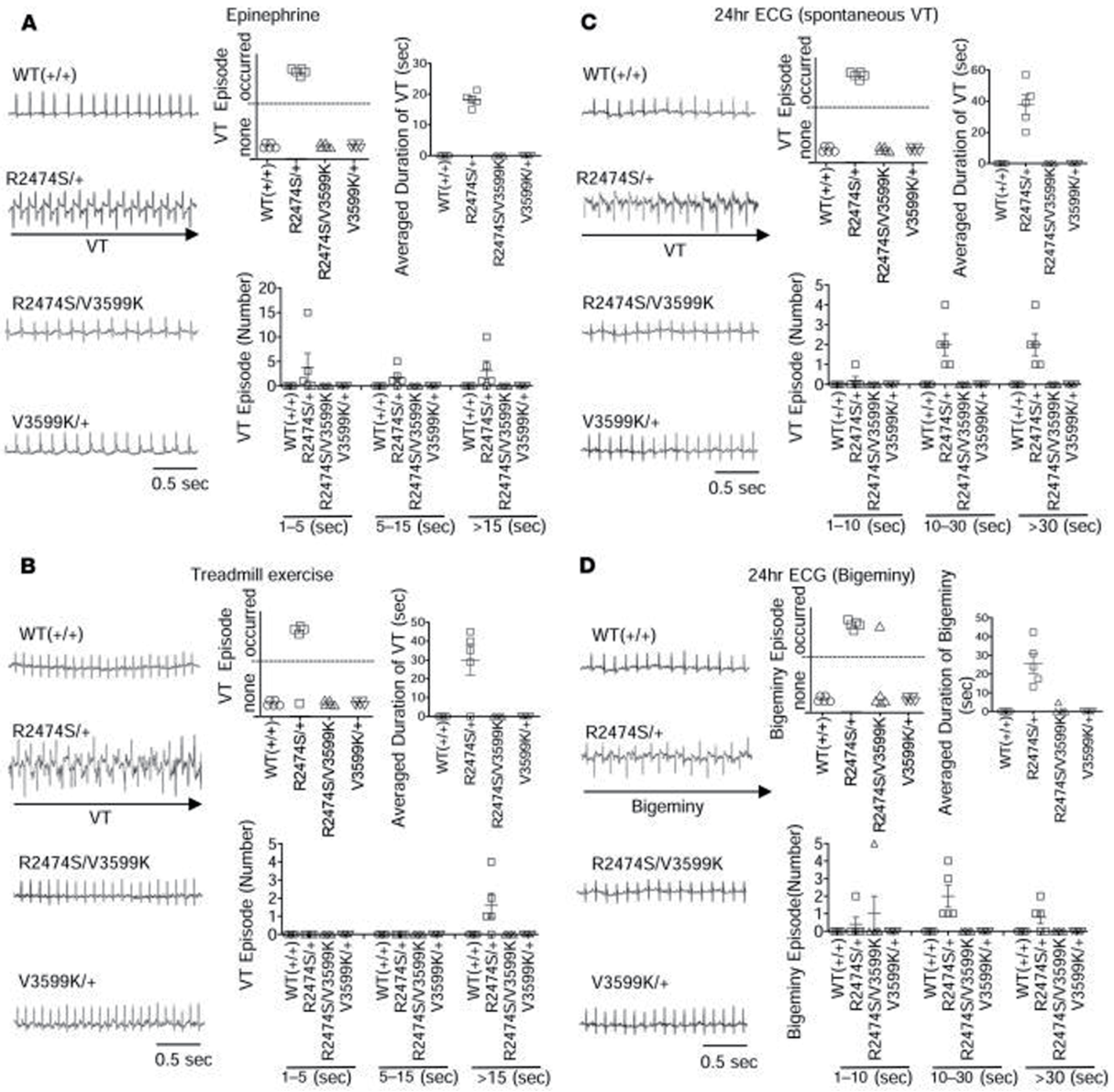

Fig. 2 Inhibition of ventricular arrhythmia by V3599K mutation in mice. Representative ECG recordings (left) and the summarized data for VT induction (right) after (A) administration of epinephrine (1 mg/kg of body weight, i.p.) or (B) treadmill exercise in WT (+/+) (n = 5), R2474S/+-KI (n = 5), V3599K/+-KI (n = 5), and R2474S/V3599K-KI mice (n = 5). Representative 24-hour ECG recordings (left) and the summarized data (right) on (C) spontaneous VT or (D) ventricular bigeminy in WT (n = 5), R2474S/+-KI (n = 5), V3599K/+-KI (n = 5), and R2474S/V3599K-KI mice (n = 5).

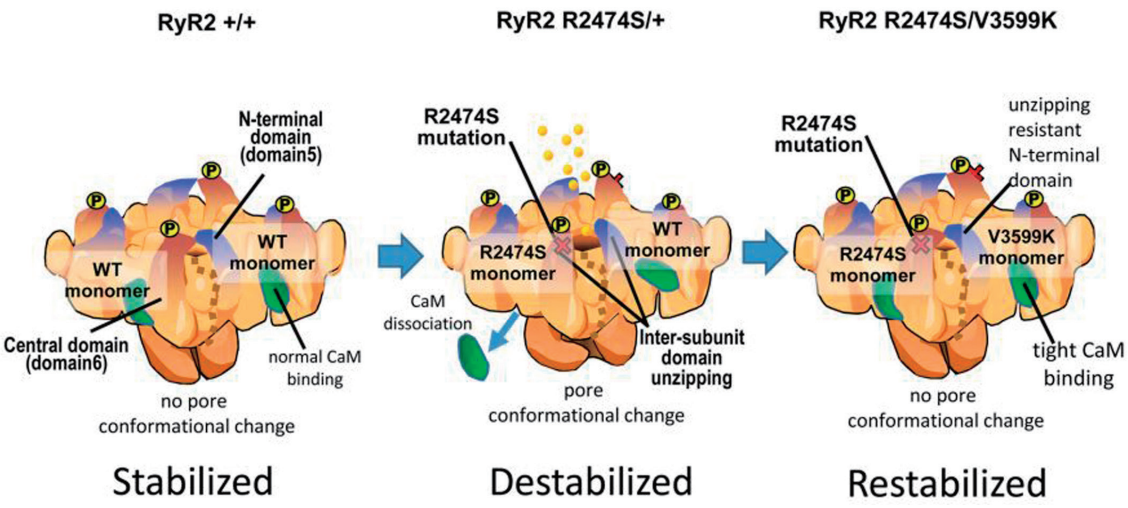

Fig. 3 Schematic illustration of the mechanism by which V3599K substitution in RyR2 can stabilize the channel and therefore protect against Ca^{2+} leak. The domain unzipping in the R2474S-mutated subunits may induce a global intersubunit interaction, allosterically decreasing the binding affinity of CaM to the R2474S-mutated subunits and in turn displacing approximately half of the bound CaM from the RyR2, whereas in R2474S/V3599-KI hearts, the postulated global intersubunit interaction followed by domain unzipping would be minimized owing to the stability of the V3599K-mutated subunits, thereby preventing the decrease in the binding affinity of CaM to the remaining R2474S-mutated subunits and subsequent displacement of CaM from the RyR2.

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