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Defective Conformational Regulation of the Ryanodine Receptor as a Key Pathogenic Mechanism of Catecholaminergic Polymorphic Ventricular Tachycardia

Hitoshi Uchinoumi, Masafumi Yano and Masunori Matsuzaki

Department of Medicine and Clinical Science, Division of Cardiology,
Yamaguchi University Graduate School of Medicine, 1-1-1 Minami-Kogushi,
Ube, Yamaguchi 755-8505, Japan
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Abstract Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmogenic disease occurring in patients with a structurally normal heart: the disease is characterized by life-threatening arrhythmias elicited by stress and emotion. CPVT is known to be caused by a mutation-linked channel disorder in the cardiac ryanodine receptor (RyR2). However, the underlying mechanism by which a single mutation in such a large molecule produces drastic effects on channel function remains unresolved. The unique distribution of these mutation sites has led to the concept that interaction among the putative regulatory domains within the RyR has a key role in regulating channel opening. Here, we report that introduction of R2474S CPVT mutation into the central domain of mouse RyR2 interfered with a normal tight interaction between the central domain (aa 2000-2500) and the N-terminal domain (aa 1-600), which reduced the threshold of luminal $[Ca^{2+}]$ for channel activation, sensitized to the protein kinase A-dependent phosphorylation, and in turn led to CPVT.

Key words: ryanodine receptor, calcium, ventricular tachycardia, sarcoplasmic reticulum

Introduction

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmogenic disease occurring in patients with a structurally normal heart: the disease is characterized by life-threatening arrhythmias elicited by stress and emotion. CPVT is caused by a single point mutation in a well-defined region of the cardiac type 2 ryanodine receptor (RyR2). However, the underlying mechanism by which a single mutation in such a large molecule produces drastic effects on channel function remains unresolved. In this review, we focus on the role of the RyR2 in the pathogenesis of CPVT, and on the possibility of developing a new therapeutic strategy by targeting this receptor.

Domain switch hypothesis

More than 100 cardiac ryanodine receptor (RyR2) missense mutations have been so far identified that are linked with 2 inherited forms of sudden cardiac death—CPVT¹ and arrhythmogenic right ventricular cardiomyopathy type 2.¹ These mutations are not randomly distributed, but cluster in 3 well-defined regions of the RyR2 that correspond to malignant hyperthermia or the central core disease mutable regions, designated as the N-terminal domain (aa 1-600), central domain (aa 2000-2500), and the C-terminal transmembrane channel domain of the skeletal muscle-type ryanodine receptor (RyR1).¹ Such unique distribution of missense mutations within the RyR2 suggests that the RyR2 shares a common domain-mediated channel regulation

mechanism with RyR1. Based on the notion that mutations at different positions in each of these domains result in the nearly identical phenotype of channel dysfunctions such as hyperactivation of the Ca^{2+} channel and hypersensitization to agonists, Ikemoto and Yamamoto^{2,3} proposed the so-called “domain switch hypothesis”. Namely, in the resting or non-activated state, the N-terminal domain and the central domain make close contact at several sub-domains (domain zipping). Upon physiological or pharmacological stimulation, these critical inter-domain contacts are weakened, resulting in the loss of conformational constraints (domain unzipping), thus lowering the energy barrier for Ca^{2+} channel opening. Consistent with this hypothesis, single particle analysis of the three-dimensional structure of the RyR2 molecule revealed that the N-terminal and central domains (located in domains 5 and 6 of the so-called clamp region, respectively) are in a close apposition to each other.^{4,5} In failing hearts, we found that defective inter-domain interaction within the RyR2 (aberrant unzipping of the N-terminal/central domain pair and channel activation in an otherwise resting state) causes diastolic Ca^{2+} leakage and contractile dysfunction.⁶ Supporting the domain switch hypothesis, pathological conditions (diastolic Ca^{2+} leakage and contractile dysfunction) were reproduced in the otherwise normal system by adding DPc10, a central domain peptide (Gly²⁴⁶⁰-Pro²⁴⁹⁵) of the RyR2 that interferes with the interaction between the N-terminal and central domains of the RyR2 and causes defective domain unzipping.

Knock-in (KI) mouse model with a human CPVT-associated RyR2 mutation (R2474S)

Although the domain peptide approach provided important information regarding the underlying mechanism of the RyR2 abnormalities during heart failure and lethal arrhythmia, further *in vivo* studies with the mutation-linked disease model are required for a straightforward test of the inter-domain interaction hypothesis. Using the Knock-in (KI) mouse model with a human CPVT-associated RyR2 mutation, R2474S, we investigated the molecular mechanism by which CPVT is induced by a single point mu-

tation within the RyR2.⁷

The R2474S/+ KI mice showed no apparent structural or histological abnormalities in the heart, but they showed clear indications of human CPVT.⁷ Bidirectional or polymorphic VT was induced after exercise on a treadmill.⁷ The interaction between the N-terminal and central domains of the RyR2 was weakened.⁷ Upon protein kinase A (PKA)-mediated phosphorylation of the RyR2, this domain unzipping further increased, resulting in a significant increase in the frequency of spontaneous Ca^{2+} transients.⁷ cAMP-induced aberrant Ca^{2+} release events (Ca^{2+} sparks/waves) occurred at much lower sarcoplasmic reticulum (SR) Ca^{2+} content as compared to the wild-type (WT).⁷ Addition of a domain-unzipping peptide, DPc10, to the WT reproduced the aforementioned abnormalities that are characteristic of the R2474S/+ KI mice.⁷

Defective inter-domain interaction plays a key role in mutation-linked channel disorder

The most important new aspect of our recent study⁷ is the finding that introduction of the R2474S CPVT mutation into the central domain of RyR2 induced a defective interaction between the central domain and the N-terminal domain, as predicted from the “domain switch hypothesis”, and this caused channel dysfunction similar to that of CPVT patients in KI mice. The three lines of evidence are consistent with this. First, DPc10, which contains the mutable R2474 residue and is known to interfere with normal inter-domain interaction between the N-terminal and central domains,⁶ reproduced the abnormal cellular Ca^{2+} events seen in the R2474S/+ KI mice (e.g., increased frequency of Ca^{2+} sparks) in an otherwise normal system (i.e., in cardiomyocytes isolated from WT mice).⁷ However, the addition of DPc10 to the (cAMP-treated) R2474S KI cardiomyocytes produced no further effect, suggesting that the defective inter-domain interaction (aberrant domain unzipping) had already taken place in the KI cardiomyocytes.⁷ Second, The R2474S mutation, introduced into the central domain of RyR2 of KI mice, did produce defective inter-domain interaction between the N-terminal and central domain (aberrant domain unzipping), as evidenced by the accessi-

bility of the fluorescent probe MCA attached to the N-terminal domain to a high molecular weight fluorescence quencher QSY[®]7-BSA was considerably higher in the KI RyR2 than the WT RyR2.⁷ Finally, dantrolene, which corrects aberrant domain unzipping, did suppress aberrant phenomena characteristic of CPVT KI mice, such as reduced threshold of luminal Ca²⁺ for channel activation, spontaneous Ca²⁺ sparks, and delayed afterdepolarization (DAD).⁷ We previously demonstrated that dantrolene corrects defective inter-domain interactions within the RyR2 in failing hearts, inhibits spontaneous Ca²⁺ leakage, and in turn improves cardiomyocyte function in failing hearts.⁸ Dantrolene was indeed equally effective in the CPVT-type mutated RyR2 as in failing hearts, indicating that channel dysfunction in CPVT and heart failure are caused by a common mechanism, that is, defective inter-domain interaction within the RyR2.

PKA-dependent phosphorylation of the RyR2 at Ser2808 facilitates domain unzipping only in the CPVT mutant ryanodine receptor

An interesting new finding of our recent study⁷ is that the threshold of luminal [Ca²⁺] for activation of Ca²⁺ sparks was much lower in R2474S/+ KI mice than in WT mice. In other words, the sensitivity of the RyR2 channel to activation by luminal [Ca²⁺] was increased in R2474S/+ KI mice. More importantly, we could reproduce this sensitized channel gating to luminal [Ca²⁺] that is characteristic of the R2474S/+ KI mice, in WT cardiomyocytes by adding DPc10. This provides further support for the notion that the aberrant channel gating in R2474S/+ KI mice is produced by defective inter-domain interaction between the N-terminal and central domains.

This study also showed that the level of PKA-dependent phosphorylation of the RyR2 at Ser2808 was virtually indistinguishable between KI and WT RyR2s, yet PKA phosphorylation produced a much larger effect in increasing the frequency of Ca²⁺ sparks in the KI cardiomyocytes than the WT myocytes. This suggests that the CPVT mutation also sensitizes the channel to PKA phosphorylation-dependent activation. In the three-

dimensional image of the RyR2, Ser2808, the site of PKA phosphorylation, has been localized in the vicinity of the boundary between the N-terminal and the central domains.⁹ Earlier cryo-electron microscopy single particle study of RyR1¹⁰ also showed that domain 5 (including the N-terminal domain) and domain 6 (including the central domain) at the clamp region are indeed in close apposition to each other in the resting state, whereas these domains become separated in the activated (channel-open) state (e.g., in the presence of cAMP and activating Ca²⁺). Thus, it is tempting to suggest that PKA phosphorylation at Ser2808 accelerates domain unzipping in the KI channel, where domain unzipping has already progressed because of a weakened inter-domain interaction caused by the CPVT mutation.

A new molecular mechanism for CPVT

We propose a new molecular mechanism underlying CPVT (Fig. 1). In the normal channel, domain-domain interaction between the N-terminal and central domains is maintained in a zipped state, and thus, the channel is stabilized, preventing Ca²⁺ leakage and delayed afterdepolarization (DAD) at a physiological range of SR Ca²⁺ contents. In the mutant channel, the stabilized inter-domain interaction is disrupted, causing aberrant domain unzipping; domain unzipping is further aggravated by the PKA-phosphorylation of Ser2808, located at the boundary between the 2 domains at the clamp region.⁹ In turn, the threshold of luminal [Ca²⁺] for channel activation is decreased.¹¹ Together, this results in SR Ca²⁺ leakage, DAD, and lethal arrhythmia. DAD triggered arrhythmia can be induced by intracellular Ca²⁺ overload.

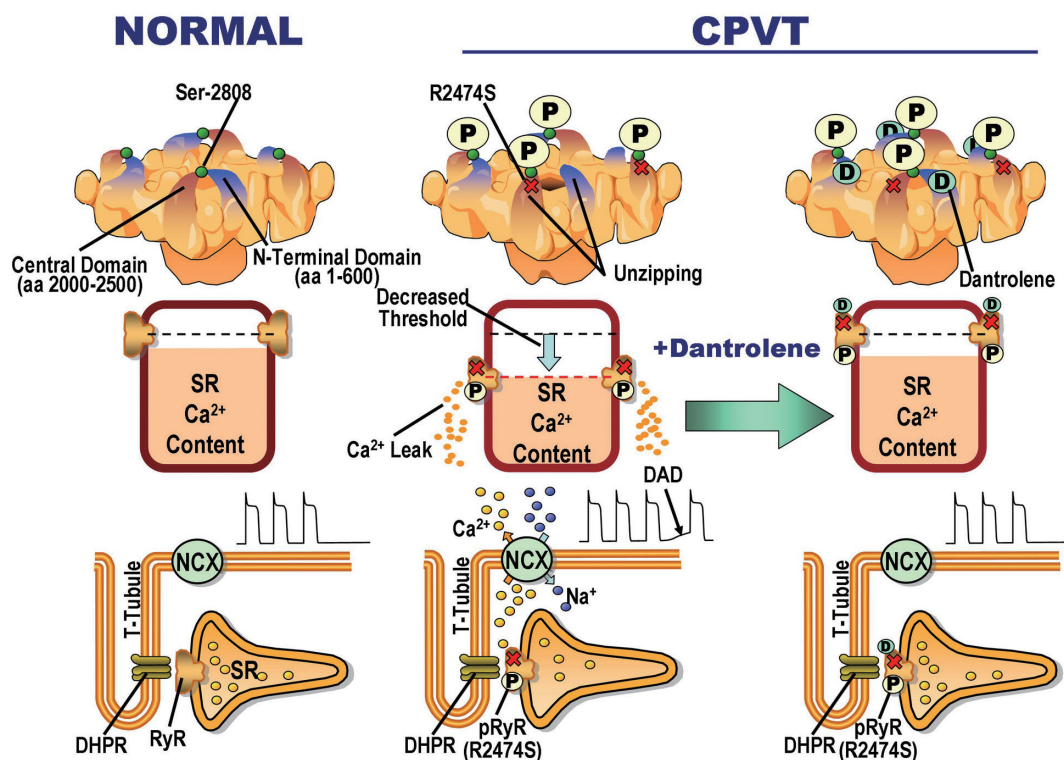


Fig. 1 Proposal of a new molecular mechanism of CPVT. In the normal channel, domain-domain interaction between the N-terminal (aa 1-600) and central (aa 2000-2500) domains is maintained in a zipped state, then the closed state of the channel is stabilized. In the mutant channel, however, evident domain unzipping, which is facilitated by the protein kinase A (PKA)-phosphorylation of Ser2808 located at the boundary between these regulatory domains, occurs in the mutant channel. This induces sequential abnormal events: reduction of the threshold SR Ca^{2+} content for channel activation (or increased sensitivity to the luminal Ca^{2+}), Ca^{2+} leak, and delayed afterdepolarization (DAD) (spontaneous after potential induced by an inward Na^+ current via Na^+ - Ca^{2+} exchange (NCX)). Dantrolene, which corrects defective domain unzipping to a normal zipped state, stops these abnormal events.

Conflict of Interest

The authors state no conflict of interest.

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