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Mutation-linked Defective Inter-domain Interactions within Ryanodine Receptor as a Pathogenic Mechanism of Catecholaminergic Polymorphic Ventricular Tachycardia

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Abstract Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited disease characterized by stress- or exercise- induced ventricular tachycardia, frequently leading to sudden cardiac death. A considerable body of evidence accumulated over recent years suggests that mutation-linked cardiac ryanodine receptor (RyR2) defects cause Ca^{2+} leak from sarcoplasmic reticulum, which triggers delayed afterdepolarization and leads to CPVT. However, the underlying mechanism, by which a single mutation in such a large molecule causes drastic effects on the channel function, remains elusive. Here we report that introduction of a human CPVT mutation S2246L into the mouse RyR2 induces aberrant activation of channel gating by forming abnormally tight domain-domain interaction between the S2246L mutable domain and the K201-binding domain. This produces more global conformational change in the RyR2: namely, an aberrant domain unzipping between the N-terminal (a.a. 1-600) domain and the central (a.a. 2000-2500) domain owing to the allosteric conformational coupling mechanism. Pharmacological correction of the defective inter-domain interactions can stop the aberrant Ca^{2+} release and lethal arrhythmia. These results provide a new pathogenic mechanism of CPVT and a novel therapeutic strategy against CPVT.

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

Introduction

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited disease characterized by stress- or exercise- induced ventricular tachycardia, frequently leading to sudden cardiac death. A considerable body of evidence accumulated over recent years suggests that mutation-linked cardiac ryanodine receptor (RyR2) defects cause Ca^{2+} leak from sarcoplasmic reticulum, which triggers delayed afterdepolarization and leads to CPVT. However, the underlying mechanism, by which a single mutation in such a large molecule causes drastic effects on the channel function, remains elusive. In this review, we focus on the RyR2 in the pathogenesis of CPVT, and on the possibility of developing a new therapeutic strategy by targeting this receptor.

Ryanodine receptor (RyR2)

Ryanodine receptor (RyR2), the Ca^{2+} release channel in the cardiac sarcoplasmic reticulum (SR), plays a key role in cardiac excitation-contraction coupling.[1] A considerable body of evidence shows that RyR2 function is defective in failing hearts, causing spontaneous Ca^{2+} leak.[2] The Ca^{2+} leak leads to contractile dysfunction by reducing the SR Ca^{2+} content, and also induces delayed after-depolarization (DAD), which ultimately leads to lethal

arrhythmia.[2] More than 120 RyR2 mutations have been identified in patients with catecholaminergic polymorphic ventricular tachycardia (CPVT) or arrhythmogenic right ventricular cardiomyopathy (ARVC) type 2.[3] RyR2 mutations are not randomly distributed, but they cluster into 3 definable regions. These domains are designated as the N-terminal domain (a.a.1-600), the central domain (a.a. 2000-2500), and the C-terminal channel-forming domain. Since a single point mutation in any of these domains has a severe impact on the channel function, these domains must play a key role in regulating the channel function of both RyR2 and RyR1.

Domain switch hypothesis as a therapeutic target of CPVT

Based on the conformational probe approach, Ikemoto and colleagues[4-6] proposed that the N-terminal domain and the central domain interact with each other to act as the implicit on/off switch that opens and closes the channel, and the interacting domain pair was designated as a "domain switch." Parallel assays of the conformational state of RyR2 and the function of Ca^{2+} channel have shown that zipping of the interacting domains closes the channel, and unzipping opens it. A mutation in either domain weakens the inter-domain interaction and causes domain unzipping in an otherwise resting state, which results in an erroneous activation of the channel and diastolic Ca^{2+} leak.[4-6] We have previously shown that in failing hearts defective interaction between the N-terminal domain and the central domain of RyR2 induces Ca^{2+} leak even under the conditions of reduced SR Ca^{2+} load, leading to contractile dysfunction.[7] Domain unzipping destabilizes the closed state of the channel, resulting in diastolic Ca^{2+} leak. We have also shown that correcting the unzipped configuration to the normal zipped state by the treatment with either K201 (JTV519)[7,8] or dantrolene.[9] Moreover, we recently demonstrated, using a transgenic mouse model in which CPVT-type R2474S mutation is knocked in the central domain of RyR2, that this mutation caused aberrant unzipping of the domain switch regions, lowering the threshold of luminal [Ca^{2+}] for channel activation, sensitizing the channel to PKA-dependent phosphorylation, and led to CPVT.[10] Dantrolene treatment that corrects the defective inter-domain interaction prevented aberrant Ca^{2+} release, thereby preventing DAD and CPVT.[10,11] These findings indicate that the defective inter-domain interaction between the N-terminal and central domains, caused by single point mutation, oxidative stress and/or PKA hyperphosphorylation, destabilizes the channel, leading to lethal arrhythmia and heart failure.

Sub-domain interaction

In our previous study of canine RyR2, we found that the domain switch is conformationally coupled with the interaction of sub-domains (the 2114-2149 region and the 2234-2750 region) within the central domain, playing an important role in the regulation of channel function: namely, their tight interaction (sub-domain zipping) opens the Ca^{2+} channel and their dissociation (sub-domain unzipping) closes the channel.[8] We then demonstrated several important features of the interaction: (a) diastolic SR Ca^{2+} leak of failing heart happens owing to erroneous zipping of these sub-domains and channel activation in an otherwise resting state, (b) specific binding of K201 to RyR2 takes place at the 2114-2149 region, indicating that this is the K201 binding domain, and (c) the binding of K201 to this domain interferes with its interaction with the 2234-2750 region, thereby correcting aberrant domain zipping and diastolic Ca^{2+} leak in the failing RyR2.[8]

Knock-in (KI) mouse model with a human CPVT-associated RyR2 mutation (S2246L)

The above findings suggest the hypothesis that the S2246L (Serine to Leucine mutation at residue 2246) CPVT mutation in the 2234-2750 region (which we call '2246 domain') causes an abnormal zipping between the 2246 domain and the K201 binding domain. To test this hypothesis, we used S2246L knock-in mice.

There were no appreciable abnormalities in structural and functional characteristics of S2246L/+KI mice at the resting state, but they showed clear indications of human CPVT. All KI mice produced VT after exercise on a treadmill. cAMP-dependent increase in

the frequency of Ca^{2+} sparks was more pronounced in saponin-permeabilized KI cardiomyocytes than in WT cardiomyocytes, even though SR Ca^{2+} content was significantly lower in the KI cardiomyocytes. Site-directed fluorescent labeling and quartz microbalance assays of the specific binding of DP2246 (a peptide corresponding to the 2232-2266 region: the 2246 domain) showed that DP2246 binds with the K201-binding sequence of RyR2. Introduction of S2246L mutation into the DP2246 increased the affinity of peptide binding. Fluorescence quench assays of inter-domain interactions within RyR2 showed that tight interaction of the 2246 domain/K201-binding domain is coupled with domain unzipping of the N-terminal (1-600)/central (2000-2500) domain pair in an allosteric manner. Dantrolene corrected the mutation-caused domain unzipping of the domain switch, and stopped the exercise-induced ventricular tachycardia.

Two sub-domains located in the central domain

The important new concept deduced from the present study is that the S2246L mutation introduced into the region of RyR2 different from that of the aforementioned R2474S mutation causes an abnormally tight interaction of a new interacting domain pair, namely two sub-domains [the 2246-domain / the K201-binding domain (2114-2149)] located in the central domain, and that this inter-subdomain interaction is coupled with unzipping of the domain switch in an allosteric manner. Thus, the S2246L mutation ends up with aberrant unzipping of the domain switch, then produced the same type of channel dysfunction and CPVT phenotypes as those produced by the R2474S mutation.

Defective inter-domain interaction of the domain switch is a source mechanism underlying CPVT

Another important aspects in the present study is the finding that dantrolene corrected defective unzipped state of the domain switch, and then inhibited exercise-induced ventricular tachycardia in the S2246L/+ KI mice. Since the dantrolene binding site is localized in the N-terminal domain of RyR2,[9] this indicates that dantrolene binding to its binding site corrected defective unzipping of the interacting N-terminal domain/central domain pair that had been produced by the S2246L mutation in the 2246 domain. Conversely, dantrolene restores aberrant unzipped configuration of the domain switch to a normal stabilized state, even though the drug produced virtually no effect on the subdomain interaction. We have previously shown in the canine heart failure model that dantrolene corrects defective unzipping between the N-terminal domain and the central domain to a normal zipped state, and prevents the development of heart failure inducible by ventricular pacing.[9] Thus, it appears that defective inter-domain interaction of the domain switch is a source mechanism underlying CPVT and heart failure.

K201 showed no appreciable corrective effect on both domain switch unzipping and channel activation

Although the R2474S and S2246L mutations produce basically identical impacts on the structure and function of the RyR2, there are delicate differences in the two aspects: (a) the response to PKA phosphorylation, and (b) pharmacological effect of K201. (a) We previously reported that the R2474S mutation caused a partial unzipping of the domain switch, and upon PKA phosphorylation at Ser2808 of RyR2, the domain switch was unzipped to a 'full' extent.[10] The S2246L/+ KI RyR2, on the other hand, showed a nearly maximal extent of domain unzipping under baseline conditions, accompanied with increased Ca^{2+} spark frequency with reduced SR Ca^{2+} content; consequently, PKA phosphorylation produced no further domain unzipping and hence no further effect on Ca^{2+} spark frequency. (b) As we demonstrated previously using canine heart failure model,[8] K201 binding to its binding domain interfered with the interaction between the K201-binding domain and the 2246 domain, and corrected channel dysfunctions of RyR2 of the failing heart. In the R2474S/+ KI mouse model, K201 as well as dantrolene suppressed PKA phosphorylation-dependent channel activation (Uchinoumi et al., unpublished data). In the S2246L/+ KI mouse model, however, K201 showed no ap-

preciable corrective effect on both domain switch unzipping and channel activation although dantrolene produced the expected effects. These differences may be accounted for by the fact that the S2246L mutation causes an excessively tight interaction of the K201 binding domain/2246 domain pair, which results in (a) facilitated unzipping of domain switch by the conformational coupling between the two regions, and results in (b) the inaccessibility of the K201 binding domain for the drug binding owing to its tight interaction with the 2246 domain.

Taken together, conformational coupling of different regions of RyR2 may explain the fact that mutations in different areas of the receptor produce a similar phenotype. However, in view of non-identical effects of the two types of potential therapeutic agents described here, differences in the mode of interdomain interaction in those different areas, to which dantrolene and K201 bind, may result in the observed differences in their therapeutic efficacy.

A new therapeutic strategy of CPVT

Introduction of a human CPVT mutation S2246L into the mouse RyR2 induces aberrant activation of channel gating by forming abnormally tight domain-domain interaction between the two sub-domains located in the central domain. This produces a defective domain unzipping between the N-terminal domain and the central domain owing to the allosteric conformational coupling between the two sets of interacting domain pairs. The coupled conformational changes in these regions trigger diastolic Ca^{2+} release and lethal arrhythmia. Dantrolene treatment corrected the defective inter-domain interaction and prevented aberrant Ca^{2+} release and CPVT, indicating that correction of conformational disorder of the RyR2 is a new therapeutic strategy of CPVT.

Conflict of Interest

The authors state no conflict of interest.

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