Sealing the leak, healing the heart

Patrick Most & Walter J Koch

Stabilization of the cardiac ryanodine receptor may represent a new concept in treating sudden cardiac death in several heart disorders.

Sudden cardiac death occurs mostly in patients suffering from heart failure, but also strikes apparently healthy individuals without known genetic or acquired cardiac abnormalities. Such sudden deaths account for about half of all fatalities from cardiovascular diseases. Although randomized trials have shown that implantable cardioconverter-defibrillators can improve survival in high-risk patients, these devices have had relatively little effect on the cumulative incidence of sudden cardiac death in the population at large[1]. Filling in our sparse knowledge of the molecular events underlying fatal arrhythmogenic events is necessary to inform more effective therapeutic approaches.

In the 27 June issue of *Cell*, Wehrens *et al.*[2] rise to this challenge, pinpointing a potential new molecular target underlying calcium-triggered arrhythmias in the heart (**Fig. 1**). The authors draw our attention to the major calcium-release channel required for excitation-contraction coupling in cardiac muscle: RyR2, a cardiac calcium-release channel and ryanodine receptor in the sarcoplasmic reticulum. The authors link defective RyR2 function to exercise-induced sudden cardiac death. This catastrophic event was first described in 490 B.C., when Pheidippides, a Greek soldier and conditioned runner, collapsed and died of sudden cardiac death after a marathon run to announce military victory over Persia.

In the sarcoplasmic reticulum of healthy hearts, RyR2-mediated calcium release during systole activates the contractile proteins responsible for cardiac muscle contraction[3]. During diastole, the resting phase of the cardiac cycle, RyR2 must shut tightly (**Fig. 1a**) or calcium will leak uncontrollably into the cytoplasm from the sarcoplasmic reticulum. Alternate opening and closing of the tetrameric channel requires tight regulation by numerous inhibitory and activating accessory factors that form a macromolecular signaling complex with RyR2 (ref. 4).

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One such accesory factor is FK506 binding protein 12.6 (FKBP12.6), originally described as a molecule that can bind immunosuppressant drugs such as FK506. FKBP12.6 has, as yet, no apparent function in immunosuppression. Instead, FKBP12.6 binds tightly to each subunit of the tetrameric RyR channel to prevent subconductance states and aberrant activation of the channel during diastole[4] (**Fig. 1a**). In normal hearts, RyR2 phosphorylation by PKA seems to cause a reversible dissociation of FKBP12.6, resulting in a transient increase in sarcoplasmic reticulum calcium release and enhanced contractility[3].

The authors showed that targeted gene deletion of *Fkbp12.6* in mice caused 'leaky' sarcoplasmic reticulum calcium channels in response to both exercise-induced stress and phosphorylation by cyclic adenosine monophosphate–dependent protein kinase A (PKA; **Fig. 1b**). Both exercise and PKA phosphorylation consistently triggered fatal cardiac ventricular tachyarrhythmias and sudden cardiac death[2]. Thus, diastolic calcium leakiness, as in *Fkbp12.6*$^{-/-}$ mice, can be lethal (**Fig. 1b**)

Wehrens *et al.*[2] went on to implicate leaky RyR2 as the probable cause for sudden cardiac death in patients with catecholaminergic polymorphic ventricular tachycardia (CPVT), a rare inherited disorder associated with mutations in RyR2. RyR2 from these patients showed reduced affinity for FKBP12.6 and had the same defective single-channel properties as RyR2 from *Fkbp12.6*$^{-/-}$ mice[2] (**Fig. 1c**). In an elegant experiment, the authors restored defective gating of recombinant CPVT mutant

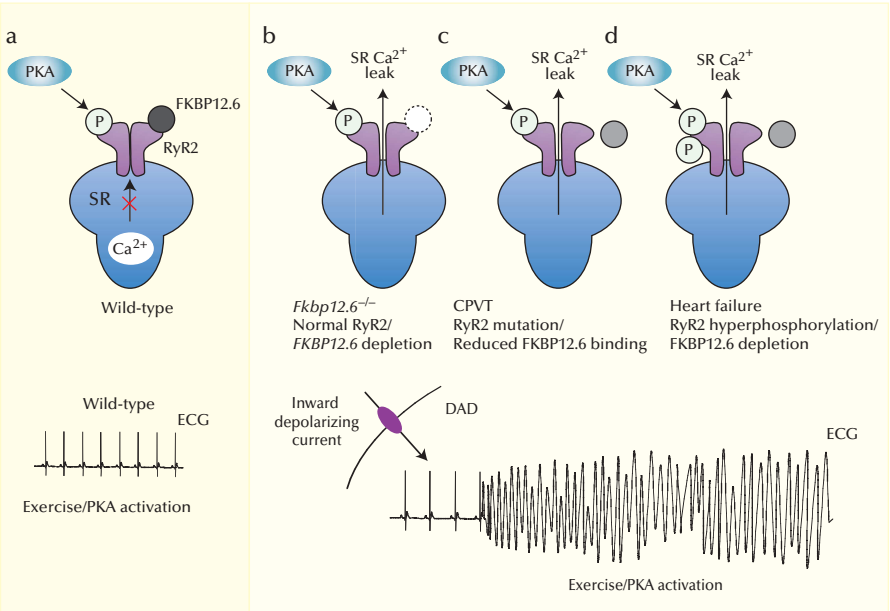

Figure 1 Exercise-induced diastolic calcium leak from the sarcoplasmic reticulum may induce delayed-after depolarizations (DADs) and fatal ventricular tachyarrhythmia in different cardiac disorders. PKA is activated by exercise- or stress-induced activation of the sympathetic nervous system. (**a**) In normal hearts, during the resting phase of the cardiac cycle (diastole), FKBP12.6 prevents aberrant gating of the PKA phosphorylated RyR2 channel and leakage of calcium from the sarcoplasmic reticulum (SR). The electrocardiogram (ECG) shows electrical activity without pathological findings. (**b**–**d**) RyR2, under three different conditions, showing defective gating and calcium leakage from the sarcoplasmic reticulum in response to exercise- or stress-induced activation of PKA during diastole. Leakage occurs in FKBP12.6-deficient mice (**b**; dashed circle), in patients with CPVT (**c**) and when RyR2 is hyperphosphorylated, which commonly occurs in heart failure (**d**). Diastolic sarcoplasmic reticulum calcium leakage caused by leaky RyR2 can activate an inward depolarizing current and DADs, possibly through the sodium/calcium exchanger, initiating fatal ventricular tachyarrhythmias and sudden cardiac death (ECGs in **b**–**d**).

RyR2 in lipid planar bilayers using a using a genetically altered FKBP12.6 protein that can bind CPVT-associated RyR2[2]. Thus, stabilization of mutant RyR2 might be a promising future therapeutic strategy for the treatment of CPVT.

The study also seems to have even broader implications for heart disease. The sustained activation of the sympathetic nervous system in heart failure apparently results in PKA-induced hyperphosphorylation of RyR2, leading to FKBP12.6 dissociation and diastolic leakage of calcium from the sarcoplasmic reticulum[5] (**Fig. 1d**). Thus, stabilization of RyR2 function could be an important target for the treatment of heart failure. Given that approximately 10 million people in the United States, Europe and Japan have heart failure, and that the five-year survival rate of the disease is less than 50%, an urgent question arises: can we put this knowledge to use right now? The answer is yes.

Two recent publications have unequivocally shown that oral administration of β-adrenergic receptor blockers reverses PKA hyperphosphorylation of RyR2, restores the FKBP12.6 stoichiometry of the RyR2 macromolecular complex and normalizes single-channel function in failing hearts[6,7]. These findings suggests that β-blockers reduce RyR2-mediated calcium leakage from the sarcoplasmic reticulum. Thus, stabilization of cardiac RyR by β-blockers in heart failure patients could provide, in part, a molecular basis for the beneficial long-term clinical effects of these drugs: improvement of contractility and reduction of fatal arrhythmogenic events (sudden cardiac death). This explanation is particularly poignant in light of evidence that many heart failure patients do not receive β-blockers, despite overwhelming evidence mandating their use in patients without contraindications.

In addition to β-blockers, the experimental drug JTV519, a 1.4-benzothiazepine derivative, also effectively prevents FKBP12.6 dissociation. The drug inhibits diastolic calcium leakage in the sarcoplasmic reticulum and improves contractile performance in a canine experimental model of heart failure[8]. JTV519 operates through an unclear mechanism, however, and its side effects are unknown, so the compound does not currently represent a valid alternative to β-blockers. These observations lend strong support to the strategy of treating heart failure by sealing the RyR2-mediated calcium leakage in the sarcoplasmic reticulum. And the new data could also renew interest in other modulators of RyR2 function as additional potential therapeutic targets, such as S100A1, a positive inotropic calcium-binding protein that interacts with cardiac RyR2 (ref. 9).

By linking disruptions in the FKBP12.6-RyR2 interaction and leaks in RyR2-mediated calcium release to exercise-induced sudden cardiac death, Wehrens *et al.* have identified the RyR2 macromolecular signaling complex as a promising therapeutic target. A common mechanism, it now seems, underlies calcium-triggered arrhythmias in *Fkbp12.6*$^{-/-}$ mice, patients with CPVT and perhaps also in patients with failing hearts.

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Seizing hold of seizures

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Epilepsy remains a hard-to-treat disorder for millions of patients. Gene therapy to target brain regions with seizure-reducing compounds is one approach that has met with limited experimental success. An innovative form of genetic manipulation now reduces seizure susceptibility and seizure-induced brain injury in rats (pages 1076–1080).

John Hughlings Jackson[1] formulated the modern definition of epilepsy in the late nineteenth century: "an occasional, excessive and disorderly discharge of nerve tissue." His emphasis on the clinical description of a seizure, beginning with the mode of onset, subsequently led to the concept of focal epilepsy. The majority of patients with epilepsy suffer from focal seizures, which begin in one part of the brain and then spread[2].

Most of the 50 million people in the world with epilepsy are treated with antiepileptic drugs. Unfortunately, pharmacological agents are often not effective; more than 30% of patients continue to have epileptic seizures despite receiving optimal treatment[3]. While some of these patients may respond to surgical or dietary therapy, vast numbers of people suffer from uncontrolled seizures. In this issue, Haberman *et al.*[4] have devised a genetic strategy that has considerable promise for the treatment of focal epilepsy.

Normal brain function is characterized by a fine balance between excitatory (depolarizing) or inhibitory (hyperpolarizing) forces (**Fig. 1**). Excessive excitation or reduced inhibition can result in the paroxysmal excessive discharge of neurons that characterize a seizure. Thus, seizures occur when the threshold for firing of the neuronal membrane is reduced so far that intrinsic membrane threshold–stabilizing mechanisms are incapable of preventing firing.

Many antiepileptic drugs work by influencing this excitatory-inhibitory balance. Antiepileptic drugs such as phenobarbital, valproate and tiagabine increase inhibition by modulating the γ-aminobutyric acid

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