The porcine stress syndrome is a major cause of poor meat quality and death in the pork industry. It is known to be more prevalent in some pig strains than others, and susceptible animals can be identified by a challenge with halothane, which results in striking elevation in body temperature. This scenario parallels the clinical entity of familial malignant hyperthermia, one of the earliest recognized human pharmacogenetic syndromes. We now know that affected pigs and people share the same molecular mechanism, mutations in the sarcoplasmic reticulum (SR) calcium release channel of skeletal muscle encoded by RYR1. In the pig world, selective breeding programs have been used to develop strains resistant to malignant hyperthermia. In humans, malignant hyperthermia is an anesthetic emergency and is treated by immediate intravenous administration of dantrolene, which is effective and thought to be safe. Chronic oral dantrolene is also approved to treat severe muscle spasticity, and, in this setting, the limiting toxicity is hepatitis, which can be fulminant and fatal in up to 1% of exposed subjects.

**Leaky Ryanodine Receptor Channels in Skeletal Muscle and Heart**

A abrupt membrane depolarization (excitation) in skeletal or in cardiac muscle results in calcium release from SR stores via ryanodine receptor (RyR) calcium release channels, and the ensuing rise in intracellular calcium then activates the contractile apparatus. The details of the way in which excitation–contraction coupling to contraction differs somewhat in the 2 types of muscle (in the heart, but not in skeletal muscle, calcium influx via voltage-gated Cav1.2 channels is required to activate RyR channels; Figure), and there are different genes encoding the channels, RYR1 in skeletal muscle and RYR2 in cardiac muscle. Early studies of the mechanism of action of dantrolene highlighted its ability to decouple excitation from contraction in skeletal muscle, and we now know that, in malignant hyperthermia, mutant RyR1 channels become leaky on exposure to drugs like halothane, and dantrolene is thought to act by preventing this leak. A body of evidence over the past decade has shown that, in heart failure models, RyR2 channels display a baseline leak that is thought to contribute to arrhythmia susceptibility and may also exacerbate contractile dysfunction. Further, phosphorylation of the cardiac channel by protein kinase A or calmodulin kinase II increases calcium release, and RyR2 hyperphosphorylation has been implicated as an exacerbating mechanism in these clinical settings. Although the extent to which RyR2 phosphorylation maintains or exacerbates contractile dysfunction and arrhythmias in human heart failure is controversial, molecular genetic studies have left no doubt that leaky RyR2 channels cause arrhythmias, because mutations in RYR2 or CASQ2 (encoding the major SR calcium-buffering protein) are the predominant cause of the syndrome of catecholaminergic polymorphic ventricular tachycardia (CPVT), a rare disease first described by Coumel and colleagues in the 1970s.

**Dantrolene as an Antiarrhythmic**

The striking mechanistic parallels between RYR1 mutations causing malignant hyperthermia and RYR2 mutations causing CPVT, both attributable to leaky ryanodine release channels, raises the question of whether dantrolene could be effective in CPVT or other settings in which defective RyR2 function leads to arrhythmias. In fact, dantrolene’s effects on cardiac rhythm were first investigated in the 1980s, with somewhat mixed results. Dantrolene significantly reduced the frequency and duration of episodes of ventricular fibrillation after coronary artery ligation in rats, but another early study suggested that pretreatment with dantrolene actually increased the frequency of ventricular fibrillation induced by coronary artery occlusion in dogs. More recent studies demonstrated that dantrolene prevents abnormal calcium leak in both malignant hyperthermia RyR1 and CPVT RyR2 channels. In vitro, a CPVT mutant channel (R2474S) was shown to decrease the threshold at which luminal calcium elicited RyR2 channel opening, and thereby induced calcium sparks and delayed afterdepolarizations; in mice with this mutation, dantrolene stabilized the channels and was antiarrhythmic. Studies using myocytes derived from induced pluripotent stem cells of a subject with a different CPVT mutation, and myocytes from rabbits with heart failure similarly demonstrated that dantrolene decreased calcium leak through abnormal RyR2 channels and increased the threshold for spontaneous calcium release, both effects predicted to normalize pathophysiologic RyR2 function and thus be antiarrhythmic without altering, or perhaps even improving, contractile dysfunction.

Interestingly, although effective in failing rabbit myocytes, dantrolene had no effect on SR calcium release in healthy rabbit myocytes, and, in healthy pigs, dantrolene only inhibited SR calcium release.
calcium release in skeletal but not in cardiac muscle. These findings beget the question of how dantrolene acts on RyR channels. Recent work has demonstrated that dantrolene binds to the Leu590-Cys609 region of RyR1 and stabilizes interdomain interactions within the RyR1 channel, which are thought to be disrupted by mutations that cause malignant hyperthermia. The dantrolene binding site has not yet been identified in cardiac RyR2, but dantrolene action in the heart may require altered calmodulin binding to RyR2: calmodulin physiologically bound to RyR2 reduces channel activity. Calmodulin binding is reduced either in heart failure or by CPVT mutations, rendering RyR2 channels hyperactive (Figure). Defective calmodulin binding can be restored by dantrolene (Figure), providing a possible explanation as to why dantrolene apparently affects SR calcium release in diseased but not in healthy hearts.

Use of Dantrolene in Ventricular Fibrillation – The Present Study

Zamiri and colleagues report in this issue of Circulation that dantrolene, administered after initiation of ventricular fibrillation (VF) in pigs (ironically enough) exerted dramatic beneficial effects on a range of indices of recovery of normal function after cardiopulmonary resuscitation and defibrillation; these included a dramatic decrease in the time to return to spontaneous circulation, decreases in the number of shock-resistant VF episodes, and a decrease in refibrillation. Dantrolene pretreatment in isolated perfused rabbit hearts reduced the ability to induce VF and reduced calcium leak. Interestingly, modeling dantrolene effects in ventricular muscle and in the Purkinje network suggested that, whereas VF or very rapid stimulation promotes calcium-dependent delayed afterdepolarizations in both cell types, only delayed afterdepolarizations arising in the Purkinje network can depolarize the cell membrane and trigger ventricular arrhythmias. Dantrolene interrupts this arrhythmogenic chain of events by inhibiting RyR2 channels, likely by stabilizing calmodulin (CaM) binding to abnormal RyR2 channels. HF indicates heart failure; and VF, ventricular fibrillation.

Where to Next?

In the present study, an old drug with an increasingly well-understood mechanism of action was used as a probe to define the contribution of perturbed RyR2 function early in VF. We have demonstrated that the sodium channel blocker flecainide also inhibits RyR2 channels; it is antiarrhythmic in both mouse models of CPVT, and in humans with the disease, as well; and a randomized clinical trial comparing flecainide with placebo in patients with CPVT and implanted defibrillators is underway. In addition, new compounds have been reported that target RyR2 channels and may therefore find a clinical niche. Whether chronic therapy would be feasible would...
require a lot more work: in the Zamiri experiment, pretreatment was beneficial in isolated rabbit hearts, but pretreatment also exacerbated arrhythmias in earlier dog studies. Anesthetologists have little compunction in reaching for intravenous dantrolene in the occasional patient with malignant hyperthermia. It is therefore possible to envision a similar use of intravenous dantrolene in the setting of VF. Developing the drug for this indication would be challenging, because it is long off a patent for arrhythmias and finding a sponsor would be problematic. Nevertheless, dantrolene could serve as a lead to other compounds with similar actions. The long road from the search for more tender pork to reversal of VF once again illustrates the importance that understanding underlying mechanisms can contribute to the development or deployment of rational drug therapies.

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References

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