Dantrolene: From Better Bacon to a Treatment for Ventricular Fibrillation

Running title: Roden et al.; Dantrolene and VF

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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 challenge with halothane, which results in striking elevation in body temperature. This scenario parallels the clinical entity of familial malignant hyperthermia upon exposure to general anesthetics, which was 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 RyR channels in skeletal muscle and heart

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 couples to contraction differs somewhat in the two 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 1) and there are different genes encoding the channels, RYR1 in skeletal muscle and RYR2 in ventricular 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 last 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 PKA or CaM kinase II increases calcium release, and RyR2 “hyper-phosphorylation” has been implicated as an exacerbating mechanism in these clinical settings. While 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 since 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 due 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 (DADs);\textsuperscript{17} in mice with this mutation, dantrolene stabilized the channels and was antiarrhythmic.\textsuperscript{16} Studies using myocytes derived from induced pluripotent stem cells of a subject with a different CPVT mutation\textsuperscript{18} as well as myocytes from rabbits with heart failure\textsuperscript{19} similarly demonstrated that dantrolene decreased calcium leak through abnormal RyR2 channels and increased 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, while effective in failing rabbit myocytes, dantrolene had no effect on SR calcium release in healthy rabbit myocytes,\textsuperscript{19} and in healthy pigs, dantrolene only inhibited SR calcium release in skeletal but not in cardiac muscle.\textsuperscript{20} 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.\textsuperscript{21} Calmodulin binding is reduced either in heart failure\textsuperscript{22} or by CPVT mutations,\textsuperscript{23} rendering RyR2 channels hyperactive (\textbf{Figure 1}). Defective calmodulin binding can be restored by dantrolene (\textbf{Figure 1}),\textsuperscript{22} providing a possible explanation as to why dantrolene apparently affects SR calcium release in diseased but not in healthy hearts.

\textbf{Use of dantrolene in VF – the present study}

Zamiri and colleagues\textsuperscript{24} report in this issue of \textit{Circulation} that dantrolene, administered after
initiation of ventricular fibrillation in pigs (ironically enough) exerted dramatic beneficial effects on a range of indices of recovery of normal function after CPR and defibrillation: these included a dramatic decrease in the time to return to spontaneous circulation, decreases in the number of shock resistant ventricular fibrillation episodes, and a decrease in re-fibrillation. Dantrolene pre-treatment in isolated perfused rabbit hearts reduced the ability to induce ventricular fibrillation and reduced calcium leak. Interestingly, modeling dantrolene effects in ventricular muscle and in the Purkinje network suggested that while VF or very rapid stimulation promotes calcium-dependent DADs in both cell types, only DADs arising in the Purkinje network propagate to other sites to cause the VF; the extent to which abnormal calcium control and DADs in ventricular muscle serve to create a VF-prone substrate is not addressed. The finding is in keeping with studies suggesting that ablation of the Purkinje network renders ventricular fibrillation much more difficult to elicit and maintain in isolated perfused dog hearts. The demonstration of dantrolene efficacy when administered only minutes after the initiation of ventricular fibrillation provides evidence that disordered RyR2 function plays a critical role in determining the lethality of ventricular fibrillation within minutes of its onset. This, of course, makes the assumption that dantrolene lacks effects on other important electrogenic pathways such as ion channels, exchangers, or other signaling pathways affecting cardiac electrogenesis. While studies to date have not been comprehensive, there is no evidence that dantrolene exerts such effects.

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, is
antiarrhythmic in both mouse models of CPVT as well as in humans with the disease,\textsuperscript{26,27} and a randomized clinical trial comparing flecainide to placebo in patients with CPVT and implanted defibrillators is underway. In addition, new compounds have been reported that target RyR2 channels\textsuperscript{28,29} 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.\textsuperscript{14}

Anesthesiologists 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 ventricular fibrillation. Developing the drug for this indication would be challenging since it is long off patent for arrhythmias and finding a sponsor would be problematic. Nevertheless, the compound could serve as a lead to others with similar actions. The long road from the search for more tender pork to reversal of ventricular fibrillation 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:**


**Figure Legend:**

**Figure 1.** During physiological excitation-contraction coupling (left and top), ryanodine receptor (RyR2) calcium release channels are activated by calcium influx via voltage-gated Cav1.2 channels during the cardiac action potential. In pathological conditions such as heart failure, RyR2 mutations, or ventricular fibrillation (as in the present study), RyR2 channels become hyperactive and can open independent of an action potential (right and bottom); this pathological calcium release 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.
Pathological calcium release (VF, RyR2 mutations, HF) leads to Ventricular Arrhythmia.

- Physiological calcium release acts on the Contractile myofilaments.
- Sarcoplasmic Reticulum 
- RyR2 and CaM interact with Ca^{2+}.
- Dantrolene inhibits RyR2 activity.
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