COMMENTARY

Mind the magnesium, in dantrolene suppression of malignant hyperthermia

Stephen C. Cannon[a,1]

Malignant hyperthermia (MH) is a pharmacogenetic syndrome wherein exposure to halogenated volatile anesthetics or to depolarizing muscle relaxants during general anesthesia may trigger a life-threatening hypermetabolic state driven by excessive Ca^{2+} release from the sarcoplasmic reticulum (SR) of skeletal muscle (1). Over 25 y ago, the acute administration of dantrolene was shown to be highly effective at aborting an episode of MH (2), but the mechanism and site of action have been incompletely resolved because of conflicting reports on the ability of dantrolene to inhibit the SR Ca^{2+} release channel (RyR1) in different experimental preparations (3, 4). In PNAS, Choi et al. from the Launikonis laboratory in Queensland, Australia, now provide evidence in skinned muscle fibers from rat that inhibition of SR Ca^{2+} release by dantrolene is Mg^{2+}-dependent, and that suppression of halothane or caffeine-induced Ca^{2+} waves in human MH muscle requires an elevation of Mg^{2+} above basal levels (5). These observations offer a mechanism to reconcile conflicting reports on whether dantrolene is able to inhibit RyR1 in reductionist experimental systems, and suggest that a modest degree of metabolic stress, sufficient to reduce local [ATP] and release Mg^{2+}, is required for the drug to suppress an impending episode of MH.

Dantrolene Reduced the Mortality of MH More than 10-Fold

The initial description of MH was in an Australian family with 10 anesthesia-related deaths (6), and so it is fitting that this most recent advance in understanding the basis for dantrolene's remarkable efficacy in preventing loss of life has come from a group in Queensland. MH presents with a rise of end-tidal CO_2, sinus tachycardia, and skeletal muscle rigidity, followed by fever, hyperkalemia, and acidosis. If the signs of an MH crisis are not recognized, the mortality is 70–80% in the absence of intervention. The genetic susceptibility to MH is inherited as an autosomal dominant trait, most often arising from mutations of *RYR1* encoding the Ca^{2+} release channel of skeletal muscle (7, 8). Over 30 causative mutations of *RYR1* have been identified in MH families (https://emhg.org/genetics/mutations-in-ryr1/), many of which represent "private" mutations found in only one kindred. Viewed from the patient's perspective, 70% of MH-susceptible individuals, as identified by in vitro caffeine–halothane contracture testing, will have a mutation of *RYR1*. Mutation of a second MH gene, *CANA1S*, encoding for the voltage-sensing subunit of the skeletal muscle L-type Ca^{2+} channel that is coupled to activation of RyR1, occurs in only about 1% of cases (9). Dantrolene is the only available drug approved for the management of MH, and the clinical impact of dantrolene therapy has been dramatic. Discontinuation of the inciting anesthetic agent reduces mortality from >70–30%, and with administration of dantrolene mortality is <5% (2.5 mg/kg, producing a blood level of ∼5 μM) (10).

Although it has been known for over 40 y that the muscle relaxant properties of dantrolene are caused by uncoupling the excitation–contraction mechanism of skeletal muscle (11), the molecular target and the mechanism of action have remained open questions that are still under investigation. A consistent picture has emerged that dantrolene suppresses the release of Ca^{2+} from the SR, without affecting neuronal excitability, neuromuscular transmission, propagation of action potentials in skeletal muscle, or the intramembranous charge displacement by the voltage-sensors of the L-type Ca^{2+} channel (3, 4, 12). The controversy has been whether dantrolene acts directly on the Ca^{2+} release channel RyR1 or suppresses release through indirect actions on other molecular components of the SR. Evidence in favor of a direct action includes the identification of dantrolene binding sites with nanomolar affinity mapped to RyR1 (13, 14), dantrolene inhibition of Ca^{2+} release from isolated heavy SR vesicles, and dantrolene block of [^{3}H]ryanodine binding to SR vesicles (4). However, dantrolene did not suppress RyR1 channel activity when measured in lipid bilayers incorporating SR vesicles (15) or from patch-clamp recordings on nuclear membranes containing RyR1 (16). One bilayer study using SR vesicles as the source of RyR1 reported enhancement of RyR1 activity with nanomolar dantrolene and inhibition

[a]Department of Physiology, David Geffen School of Medicine at the University of California, Los Angeles, CA 90095
Author contributions: S.C.C. wrote the paper.
The author declares no conflict of interest.
See companion article on page 4811.
[1]Email: sccannon@mednet.ucla.edu.

www.pnas.org/cgi/doi/10.1073/pnas.1704103114

at micromolar concentrations (17). To explore the possibility that the variability of dantrolene responses in the bilayer experiments might be attributed to variations in the molecular composition of SR vesicles, Szentesi et al. incorporated purified RyR1 into lipid bilayers and failed to detect an effect of 50 μM dantrolene on channel activity (3). Taken together, the available data strongly support the notion that dantrolene suppresses voltage-dependent and pharmacologic release of Ca^{2+} from the SR, and the drug binds RyR1 with nanomolar affinity, but the evidence for a direct inhibition of RyR1 channel activity is lacking.

A Mg^{2+} Dependence for Dantrolene Inhibition of SR Ca^{2+} Release Revealed in Skinned Muscle Fibers

Choi et al. revisit the question of dantrolene action on RyR1 by considering the differences in ionic composition among the various muscle, SR, and bilayer preparations, and hypothesize that the exclusion of Mg^{2+} from the bilayer experiments might account for the disparity in observed effects on channel activity (5). Free Mg^{2+} in the cytosol of skeletal muscle is about ∼1 mM (18) and 50% inhibition of RyR1 occurs with ∼100 μM Mg^{2+} (19). This scenario of potent basal inhibition has led to one interpretation of excitation–contraction coupling as a voltage-dependent conformational change in the L-type Ca^{2+} channel that activates RyR1 channels by relieving Mg^{2+} block (20). The Mg^{2+} inhibition of Ca^{2+} release is, in fact, the reason why Mg^{2+} is usually omitted from bilayer experiments. In the present study, Choi et al. (5) used mechanically skinned fibers where the bath solution with a controlled free [Mg^{2+}] serves as the "cytosol," and the spontaneously resealed transverse tubules are electrically excitable and support excitation–contraction coupling. Calcium transients, measured with rhod-2 fluorescence, were not inhibited by 50 μM dantrolene for release events evoked by removal of Mg^{2+} or by electrical stimulation in low Mg^{2+} (0.4 mM). As the free [Mg^{2+}] was increased to ≥1 mM, however, dantrolene inhibited Ca^{2+} release. In 3 mM Mg^{2+}, for example, dantrolene reduced the peak Ca^{2+} transient with an IC50 of 0.4 μM. Viewed another way, dantrolene enhanced the Mg^{2+} block of SR Ca^{2+} release. The discovery that inhibition of SR Ca^{2+} release by dantrolene requires Mg^{2+} nicely resolves the controversy of the conflicting observations on dantrolene inhibition of RyR1 channel activity in Mg^{2+}-free bilayer experiments. The question still remains, however: Does dantrolene directly inhibit RyR1 or does the mechanism involve an indirect pathway acting through other intermediates? Perhaps it would be possible to make this distinction in bilayer experiments incorporating purified RyR1 and a low concentration of Mg^{2+}, sufficient to support dantrolene block but not so high as to suppress basal activity?

Increased Cytosolic Mg^{2+} May Be a Prerequisite for Dantrolene's Efficacy in Managing an MH Crisis

The dantrolene effect is modest; a saturating concentration (∼50 μM) reduces the IC50 for Mg^{2+} block about twofold, but studies on human skinned fibers support the notion that this effect is sufficient to abort an MH crisis. Efficacy was demonstrated by suppression of halothane-evoked Ca^{2+} oscillations in MH fibers with 5 μM dantrolene, but interestingly the inhibition was detectable only for free [Mg^{2+}] ≥1.5 mM (5). This requirement for elevated Mg^{2+} is also consistent with the notion that impaired Mg^{2+} regulation of mutant RyR1 channels may contribute to the genesis of MH susceptibility (21). The requirement for a free [Mg^{2+}] substantially above the resting value in muscle implies that the efficacy of dantrolene is dependent on release of Mg^{2+} from its main cytosolic buffer, ATP. A rise of cytosolic Mg^{2+} to ∼1.5 mM has been observed with fatigue of mammalian skeletal muscle by repetitive stimulation ex vivo, but only after several minutes of activity when the tetanic force began to precipitously decline, most likely because of a critical reduction of ATP (18). The metabolic stress of an MH crisis will likely also produce a decline of ATP sufficient to raise cytosolic Mg^{2+} to 1.5 mM or even greater. But then, why is prophylactic dantrolene so effective in preventing halothane-induced MH for studies of porcine stress syndrome (22) or decreasing the incidence of even mild signs of impending MH in patients (elevated end-tidal CO_2 and masseter muscle rigidity)? Either a substantial reduction of ATP and increase of Mg^{2+} occurs before signs of a hypermetabolic state are detectable, or the dynamics for supporting sustained waves of halothane-triggered Ca^{2+} release are different for intact fibers compared with the skinned fiber preparation with its enormous reservoir of "cytoplasm."

Acknowledgments

This work was supported by the National Institute of Arthritis, Musculoskeletal, and Skin Diseases of the NIH (Grant R01-AR42703).

1 Loke J, MacLennan DH (1998) Malignant hyperthermia and central core disease: Disorders of Ca2+ release channels. *Am J Med* 104:470–486.
2 Kolb ME, Horne ML, Martz R (1982) Dantrolene in human malignant hyperthermia. *Anesthesiology* 56:254–262.
3 Szentesi P, et al. (2001) Effects of dantrolene on steps of excitation-contraction coupling in mammalian skeletal muscle fibers. *J Gen Physiol* 118:355–375.
4 Fruen BR, Mickelson JR, Louis CF (1997) Dantrolene inhibition of sarcoplasmic reticulum Ca2+ release by direct and specific action at skeletal muscle ryanodine receptors. *J Biol Chem* 272:26965–26971.
5 Choi RH, Koenig X, Launikonis BS (2017) Dantrolene requires Mg^{2+} to arrest malignant hyperthermia. *Proc Natl Acad Sci USA* 114:4811–4815.
6 Denborough MA, Forster JF, Lovell RR, Maplestone PA, Villiers JD (1962) Anaesthetic deaths in a family. *Br J Anaesth* 34:395–396.
7 Gillard EF, et al. (1991) A substitution of cysteine for arginine 614 in the ryanodine receptor is potentially causative of human malignant hyperthermia. *Genomics* 11:751–755.
8 Rosenberg H, Sambuughin N, Riazi S, Dirksen RT (2003, updated January 31, 2013) Malignant hyperthermia susceptibility. *GeneReviews*, eds Pagon RA, et al. (University of Washington, Seattle). Available at https://www.ncbi.nlm.nih.gov/books/NBK1146/. Accessed March 30, 2017.
9 Monnier N, Procaccio V, Stieglitz P, Lunardi J (1997) Malignant-hyperthermia susceptibility is associated with a mutation of the alpha 1-subunit of the human dihydropyridine-sensitive L-type voltage-dependent calcium-channel receptor in skeletal muscle. *Am J Hum Genet* 60:1316–1325.
10 Litman RS, Rosenberg H (2005) Malignant hyperthermia: Update on susceptibility testing. *JAMA* 293:2918–2924.
11 Ellis KO, Bryant SH (1972) Excitation-contraction uncoupling in skeletal muscle by dantrolene sodium. *Naunyn Schmiedebergs Arch Pharmacol* 274:107–109.
12 Ellis KO, Carpenter JF (1974) Mechanism of control of skeletal-muscle contraction by dantrolene sodium. *Arch Phys Med Rehabil* 55:362–369.
13 Paul-Pletzer K, Palnitkar SS, Jimenez LS, Morimoto H, Parness J (2001) The skeletal muscle ryanodine receptor identified as a molecular target of [3H]azidodantrolene by photoaffinity labeling. *Biochemistry* 40:531–542.
14 Paul-Pletzer K, et al. (2002) Identification of a dantrolene-binding sequence on the skeletal muscle ryanodine receptor. *J Biol Chem* 277:34918–34923.
15 Diaz-Sylvester PL, Porta M, Copello JA (2008) Halothane modulation of skeletal muscle ryanodine receptors: Dependence on Ca2+, Mg2+, and ATP. *Am J Physiol Cell Physiol* 294:C1103–C1112.
16 Wagner LE, 2nd, Groom LA, Dirksen RT, Yule DI (2014) Characterization of ryanodine receptor type 1 single channel activity using "on-nucleus" patch clamp. *Cell Calcium* 56:96–107.

17 Nelson TE, Lin M, Zapata-Sudo G, Sudo RT (1996) Dantrolene sodium can increase or attenuate activity of skeletal muscle ryanodine receptor calcium release channel. Clinical implications. *Anesthesiology* 84:1368–1379.
18 Westerblad H, Allen DG (1992) Myoplasmic free Mg2+ concentration during repetitive stimulation of single fibres from mouse skeletal muscle. *J Physiol* 453:413–434.
19 Laver DR, et al. (1997) Reduced inhibitory effect of Mg2+ on ryanodine receptor-Ca2+ release channels in malignant hyperthermia. *Biophys J* 73:1913–1924.
20 Lamb GD, Stephenson DG (1994) Effects of intracellular pH and [Mg2+] on excitation-contraction coupling in skeletal muscle fibres of the rat. *J Physiol* 478:331–339.
21 Steele DS, Duke AM (2007) Defective Mg2+ regulation of RyR1 as a causal factor in malignant hyperthermia. *Arch Biochem Biophys* 458:57–64.
22 Harrison GG (1975) Control of the malignant hyperpyrexic syndrome in MHS swine by dantrolene sodium. *Br J Anaesth* 47:62–65.