EDITORIAL VIEWS

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Complex Pharmacology of Malignant Hyperthermia

Three articles in this issue of ANESTHESIOLOGY concern malignant hyperthermia (MH), providing new insights into the complexity of this disease. First, in a surprising *in vitro* lipid bilayer study of skeletal muscle sarcoplasmic reticulum (SR), a very low (nM) concentration of dantrolene was found to open the isolated Ca^{2+} release channel, whereas a higher (μM) concentration caused the expected blockade of the channel.[1] In a second paper, stimulation of the Ca^{2+} release channel by a common drug preservative (*i.e.*, chlorocresol) caused contractures in intact muscle fibers *in vitro*.[2] Finally, stimulation of the Ca^{2+} release channel by a serotonin agonist, as measured by biopsied muscle *in vitro,* resulted in contracture.[3] These findings help to expand our view of MH myopathies and enhance our understanding of some of the complex drug effects on the Ca^{2+} release channel of skeletal muscle and its associated proteins.

It is useful to review the subcellular basis and molecular machinery responsible for excitation-contraction coupling in skeletal muscle cells (fig. 1).[4] The 10,000-fold inwardly directed Ca^{2+} gradient across the sarcolemma of resting skeletal muscle cells is maintained by a large array of Ca^{2+}-ATPases that sequester the ion into the lumen of the SR. Elevation of cytoplasmic Ca^{2+} is orchestrated by the activation of voltage-operated Ca^{2+} channels of the plasma membrane[5,6] and ryanodine-sensitive Ca^{2+} channels of SR[7,8] that are juxtaposed where the surface membrane transverse tubule meets the junctional SR membrane (the triad).[9,10] The plant alkaloid ryanodine binds to the SR Ca^{2+} release channel with multiple allosterically coupled affinities.[11–13] Ryanodine has been essential in identifying the junctional "foot" structure that spans the transverse tubule/SR junction as the Ca^{2+} release channel protein,[10] now commonly referred to as the ryanodine (Ry) receptor.[8,14] Each Ry receptor is composed of four identical monomers of approximately 5,000 amino acids. Ry receptor monomers are encoded by at least three distinct genes,[4,15] of which Ry_1 receptor is the isoform expressed by skeletal muscle.[16,17,*] When the transverse tubule depolarizes, charge movement within the skeletal muscle dihydropyridine-sensitive L-type Ca^{2+} channel (DHP receptor) activates the Ry_1 receptor to open, and Ca^{2+} is released into the myoplasm, causing contraction. Although the mechanism coupling the DHP receptor and the Ry_1 receptor has yet to be fully defined, the intracellular loop between repeats II and III of the Ca^{2+} channel α_1 subunit (fig. 1) is essential for excitation-contraction coupling.[6,18]

Ry receptor is an enormous protein complex modulated by physiologic ligands including Ca^{2+}, Mg^{2+}, adenine nucleotides, calmodulin, and sphingosine.[18–20] In addition to such intracellular mediators, cellular pathways that result in phosphorylation of Ry_1 receptors may influence the gating and response of the release channel.[21] A wide variety of drugs also can modulate Ry_1 receptor gating and SR Ca^{2+} release.[15] The volatile anesthetics are noteworthy among the drugs that may increase Ry_1 receptor activity and perturb Ca^{2+} regulation,[22] especially in MH-susceptible individuals.[23,24] Normal skeletal muscle may tolerate a modest perturbation of Ca^{2+} homeostasis (*e.g.*, increased Ca^{2+} "leak" from the SR) without evidence of a problem. However, various myopathic skeletal muscle disorders that have a "leaky" membrane due to a variety of causes may be compensated normally until perturbed by an agent that further activates Ca^{2+} release and further strains homeostatic mechanisms. Certain combinations of drug and disease can result in a smoldering reaction that may abate or intensify. The wrong combination of susceptibility and heterogeneous triggers creates a potentially explosive response, so that the sleeping giant of skeletal muscle metabolism overwhelms whole body homeostasis. In addition, triggering of hypermetabolism partly depends on dose of triggering agent, *i.e.*, smaller concentrations trigger more slowly, and larger concentrations are decidedly quicker.[25] Consequently,

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* According to International Union of Pharmacology Committee on Drug Classification and Receptor Nomenclature (December 1995), the ryanodine receptor isoforms from skeletal muscle, heart, and brain are denoted as Ry_1, Ry_2, and Ry_3 receptors, respectively.

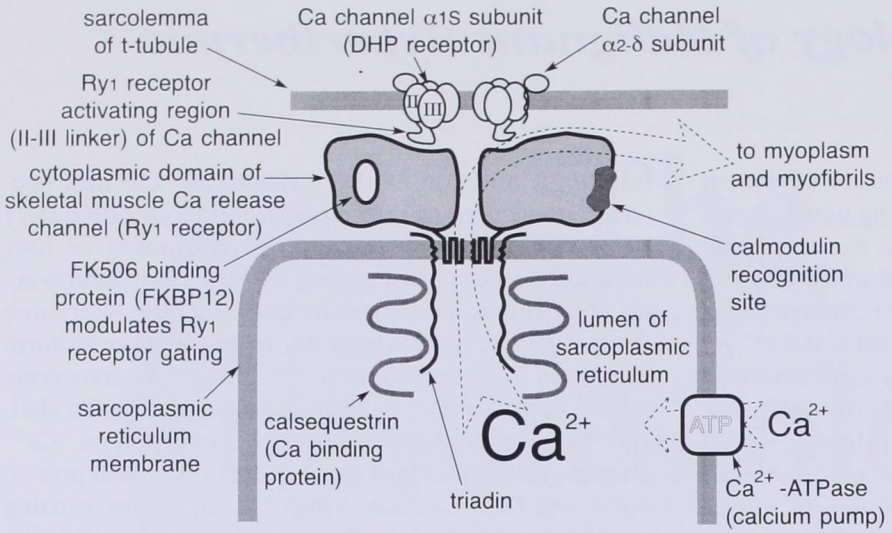

Fig. 1. The major components of the excitation-contraction pathway of skeletal muscle. The major feature is the Ca^{2+} release channel (Ry_1 receptor) of the junctional sarcoplasmic reticulum (JSR); two of the four monomers that compose the Ca^{2+} release channel are shown in cross-section. A voltage-dependent conformational change in the skeletal muscle Ca^{2+} channel (DHP receptor, α_{1S} subunit) is transmitted to the Ry_1 receptor through the protein segment linking motifs II and III, which activates Ry_1 receptor opening. The large cytoplasmic domain ("foot process") has multiple binding sites for ATP, Ca^{2+}, Mg^{2+}, and a variety of drugs, including volatile anesthetics. The FK binding protein (FKBP12) binds to the Ry_1 receptor and modulates its gating. Triadin is a protein closely associated with JSR that appears to regulate Ca^{2+} release from the SR lumen, possibly by transmitting the release signal to calsequestrin, an acidic protein with many low affinity Ca^{2+} binding sites.

the reports that chlorocresol and serotonin agonists can activate Ca^{2+} release are important reminders that pharmacologic or even physiologic regulatory mechanisms can perturb muscle homeostasis. In normal muscle, such additional "leak" caused by drugs or by normally adaptive physiologic regulatory pathways may cause no appreciable change in muscle metabolism. However, in myopathic muscle, either physiologic or pharmacologic mediators of increased release may cause the muscle to become hypermetabolic. In this setting, the MH episode represents the "final common pathway" resulting from the convergence of a number of small abnormalities. Such interventions may be insufficient triggers by themselves, but in combination with other agents, such as volatile anesthetics, their effect may result in excessive Ca^{2+} leak, which triggers an MH episode. It is possible that, in rare instances, sufficient pharmacologic effects and physiologic modulations may lead to triggering of muscle that bears no inherited defect, *i.e.*, acquired MH.

The critical physiologic role of the Ry_1 receptor in controlling Ca^{2+} release is emphasized by a mutation in this protein (Arg^{615}→Cys) that is associated with and almost certainly accounts for porcine MH.[26] Anesthetics or the neuroendocrine changes induced by stress cause loss of Ca^{2+} homeostasis, hypermetabolism, and the catastrophic consequences. A homologous mutation (Arg^{614}→Cys) to that in the pig accounts for perhaps 5% of human MH.[27,28] Central core disease is a rare myopathy characterized by hypotonia, weakness in the lower extremities, and MH susceptibility. This defect, which is associated with Ry_1 receptor mutations,[29,30] probably accounts for a small fraction of MH. Other mutations in Ry_1 receptor (Gly^{248}→Arg; Gly^{341}→Arg; Gly^{2433}→Arg),[31-33] or at least located on chromosome 19q13.1, have been defined and may underlie the etiology of additional forms of MH. However, the gene locus responsible for MH in more than 50% of European MH families appears not to be located within the Ry_1 receptor gene.[34]

Although the Ry_1 receptor is the major component of the Ca^{2+} release pathway, important interactions between Ry_1 receptors and at least five other triadic proteins (DHP receptor α_{1S} subunit, FKBP12, triadin, calsequestrin, and calmodulin) appear to contribute to the function and modulation of the Ca^{2+} release mechanism. An exogenous peptide fragment (KC7) that copurifies with Ry_1 receptor[35] was shown to be identical to the major cytoplasmic immunophilin of human T-cells, FKBP12 (FK-506 binding protein-12kDa).[36] FKBP12 is a 12 kDa protein that belongs to a growing class of cytosolic proteins that regulate signal transduction pathways essential to immune function. Recent evidence suggests that the FKBP12/Ry_1 receptor heterocomplex appears to stabilize the closed conformation of the Ca^{2+} release channels.[37] Mack *et al.* identified a class of macrocyclic alkaloids known as bastadins, which mediate their actions by markedly altering

the interaction between FKBP12 and Ry_1 receptors, resulting in a marked (~50-fold) increase in the time the channel is open but with no change in unitary conductance.[38] Through their actions on FKBP12, bastadins modulate the ratio of ryanodine-insensitive "leak" states to ryanodine-sensitive channel states in the SR membrane† and may define a new site of drug action. Triadin is a 95-kDa protein that closely associates with Ry_1 receptors and appears to play a functional role in excitation-contraction coupling.[39,40] A small number of highly reactive sulfhydryl moieties on both Ry_1 receptors and triadin proteins have been identified and appear necessary for normal Ca^{2+} release channel function.[41] Calsequestrin is a highly acidic protein that resides in the lumen of the SR and has a high capacity for binding Ca^{2+}. Ikemoto *et al.*[42] showed that activation of Ry_1 receptors by ligands elicits a signal in the junctional face membrane which is transmitted to calsequestrin. This signal appears to be important to release of bound Ca^{2+} within the SR lumen. In support, intraluminal SR Ca^{2+} has been shown to be an important factor in Ry_1 receptor function; furthermore, triadin may functionally relate calsequestrin to Ry_1 receptor.[42]

These Ry_1 receptor-associated proteins may be of considerable importance in a number of ways in addition to their physiologic role. First, these proteins represent sites at which mutations might occur that may alter skeletal muscle function, leading to altered Ca^{2+} homeostasis, myopathies, and possibly some of the as yet genetically undefined forms of MH. It is interesting that a mutation in the largely extracellular α_2-δ subunit of the surface Ca channel, which can modulate the α_1 DHP receptor, is associated with MH.[43]

Second, these proteins may be additional sites of drug action. Although limited by the solubility of dantrolene, Parness and Palnitkar[44] identified a specific binding site for [^{3}H]-dantrolene in preparations of porcine skeletal muscle SR having a K_d of 277 nM. In addition, the binding characteristics were highly distinct from ryanodine, an agent that also stimulates release at low concentrations (<10 μM) and blocks at higher concentrations (>100 μM). More recent data from that laboratory suggests that the specific dantrolene binding site resides on a smaller (10–120 kDa) protein,[45] compatible with either triadin or FKBP12. Multiple sites may exist for dantrolene binding to explain its biphasic effects. The observation that Ry_1 receptor may not be a site of action for dantrolene is consistent with the fact that dantrolene can be used to treat human MH, which presumably arises from a variety of defects.

Finally, the presence of these proteins in "native" channels isolated from SR may differ from the behavior of "purified" Ry_1 receptor protein reconstituted into bilayer lipid membranes.[8] The isolation process may alter responses such that the preparation does not accurately reflect *in vivo* responses. When pure Ry_1 receptor channels are expressed from cDNA, their gating is considerably different from channels obtained with fused SR vesicles. The incidence of subconductance states is reduced when purified FKBP12 protein is added to pure Ry_1 receptor (expressed from cDNA), but normal (rapid) gating behavior is not completely restored.[46] Consequently, when examining studies such as that by Nelson *et al.*[1] in this issue, it is important to recognize that the presence and role of the additional regulatory segments are uncertain.

What should we conclude regarding dantrolene? The effect of dantrolene in enhancing Ca^{2+} release might be only a biochemical curiosity, were it not for the MHAUS data suggesting that it may result clinically in mild hypermetabolism after treatment. Unfortunately, we cannot be certain whether the situations between the dantrolene-treated and untreated groups are truly comparable, nor can we specifically determine the basis for the MH episode. Nevertheless, as drug metabolism results in declining blood dantrolene concentrations, recrudescence of hypermetabolism appears to be a risk, although its severity is unclear. It is interesting that abnormal Ry_1 receptor channels were less profoundly activated by dantrolene than were the normal Ry_1 receptor channels. Perhaps individuals with normal Ry_1 receptor but with myopathies and MH susceptibility due to other gene defects are the most susceptible to activation by low concentrations of dantrolene, particularly if combined with other agents (*e.g.*, chlorocresol) or particular clinical settings. Ongoing investigation into the various genetic causes of MH and the pharmacology of dantrolene should provide answers. In any case, dantrolene remains an as yet irreplaceable drug for MH crises and is the lifeline to survival once MH is triggered. No other drug permits dependable reversal of skeletal hypermetabolism. However, careful informed observation is essential during recovery from dantrolene-treated MH episodes.

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