The couplonopathies: A comparative approach to a class of diseases of skeletal and cardiac muscle

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A novel category of diseases of striated muscle is proposed, the couplonopathies, as those that affect components of the couplon and thereby alter its operation. Couplons are the functional units of intracellular calcium release in excitation–contraction coupling. They comprise dihydropyridine receptors, ryanodine receptors (Ca^{2+} release channels), and a growing list of ancillary proteins whose alteration may lead to disease. Within a generally similar plan, the couplons of skeletal and cardiac muscle show, in a few places, marked structural divergence associated with critical differences in the mechanisms whereby they fulfill their signaling role. Most important among these are the presence of a mechanical or allosteric communication between voltage sensors and Ca^{2+} release channels, exclusive to the skeletal couplon, and the smaller capacity of the Ca stores in cardiac muscle, which results in greater swings of store concentration during physiological function. Consideration of these structural and functional differences affords insights into the pathogenesis of several couplonopathies. The exclusive mechanical connection of the skeletal couplon explains differences in pathogenesis between malignant hyperthermia (MH) and catecholaminergic polymorphic ventricular tachycardia (CPVT), conditions most commonly caused by mutations in homologous regions of the skeletal and cardiac Ca^{2+} release channels. Based on mechanistic considerations applicable to both couplons, we identify the plasmalemma as a site of secondary modifications, typically an increase in store-operated calcium entry, that are relevant in MH pathogenesis. Similar considerations help explain the different consequences that mutations in triadin and calsequestrin have in these two tissues. As more information is gathered on the composition of cardiac and skeletal couplons, this comparative and mechanistic approach to couplonopathies should be useful to understand pathogenesis, clarify diagnosis, and propose tissue-specific drug development.

The contraction–relaxation cycle of skeletal and cardiac muscle is controlled by temporary increases in the concentration of free cytosolic Ca^{2+} ions. Failures of this excitation–contraction (EC) coupling function constitute either the central feature or a salient aspect of multiple diseases of diverse etiologies.

In an attempt to organize and classify these diseases, Dowling et al. (2014) recently reviewed the "triadopathies" of skeletal muscle, among which they included all diseases that originated at or substantially involved the triad. The triad consists of two terminal cisternae of the main Ca storage organelle, the SR, and one transverse tubule, a plasma membrane invagination which brings the action potential to the depths of the cell and converts it into a signal for Ca^{2+} release from the SR. Because of the large number of proteins present in triads (Treves et al., 2009; Rebbeck et al., 2014), essential for electrical propagation and long-term homeostasis and housekeeping, triadopathies include numerous diseases.

In a work of similar scope for cardiac muscle, Venetucci et al. (2012) proposed "calcium channelopathies" as a category to group related diseases. Again, this covered a large number of conditions, comprising failures of the process of intracellular calcium release as well as arrhythmogenic syndromes resulting from primary failures of action potential propagation.

Here we test a third approach. We narrow the focus to only include diseases that affect components of the couplon and thereby alter the operation of these functional units. However, we expand the definition to include diseases of skeletal and cardiac couplons, an extension which affords a comparative approach. The couplon is a structural and functional entity, originally defined in skeletal muscle as the set of calcium release channels and associated voltage sensors on one side of a triad junction (Stern et al., 1997) and later in cardiac muscle as the corresponding set of release channels and L-type Ca^{2+} channels (dihydropyridine receptors [DHPRs]) in a dyad (Stern et al., 1999). The couplons of skeletal and cardiac muscle are similar, with a few marked

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Abbreviations used in this paper: CCD, central core disease; CICR, Ca^{2+}-induced Ca^{2+} release; CPVT, catecholaminergic polymorphic ventricular tachycardia; DAD, delayed afterdepolarization; DHPR, dihydropyridine receptor; EAD, early afterdepolarization; EC, excitation–contraction; MH, malignant hyperthermia; MHS, MH susceptibility; RyR, ryanodine receptor; SOICR, store overload-induced Ca^{2+} release.

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differences in structure, mechanism, and functional output.[1] Underlying the definition of couplonopathies is the expectation that their comparative examination in skeletal and cardiac muscle will yield two kinds of insights. For diseases caused by alterations in homologous components of the two couplons, mechanisms and pathogenesis of one disease, and perhaps aspects of diagnosis and therapeutics, are expected to apply to the other disease. Conversely, the pointed differences in some structural and functional aspects of the two couplons should be predictors of differences in pathogenic mechanism. Here, we examine some couplonopathies, in search of the insights promised by these two notions. Foremost among the differences is the mechanical communication between voltage sensor and Ca^{2+} release channel present in skeletal but not cardiac muscle. Hence mechanical (or allosteric) interactions will be central to our analysis.

The focus of the article is narrow: we discuss only diseases where the comparative approach appears useful, especially those where allosteric interactions may be relevant. Comprehensive descriptions of these diseases can be found in Venetucci et al. (2012) and Dowling et al. (2014) and in reviews by Durham et al. (2007), MacLennan and Zvaritch (2011), and Priori and Chen (2011). We start by reviewing the couplon concept and its functional underpinnings.

The couplons of skeletal and cardiac muscle

Ca2+ release occurs via channel clusters. The Ca transients of EC coupling occur when, in response to action potentials traveling down the plasma membrane and transverse (T) tubules, channels of the SR open and let Ca^{2+} ions flow from the lumen of the organelle to the cytosol. A major difference between the SR Ca^{2+} release channels, the ryanodine receptors (RyRs), and most channels of the plasma membrane is that RyRs are clustered, whereas Na^+, K^+, and Cl^- channels of the plasmalemma may either be clustered or work independently (e.g., Sigworth, 1994). This difference is consistent with the involvement of most plasmalemmal channels in "electrical signaling" and reflects a radical advantage in speed of propagation of the electric field versus chemical messengers, including Ca^{2+} ions, which move by diffusion.[2]

RyR clustering confers at least five advantages: (1) the summation of individual channel currents contributes to a large Ca^{2+} concentration gradient and faster flux, (2) the grouping of sources facilitates efficient local signaling, (3) the interchannel proximity enables synchronized control by sensors of the action potential, and (4) the mutual proximity of RyRs enables synchronization of their opening because of their sensitivity to Ca^{2+} as a stimulus for channel opening, Ca^{2+}-induced Ca^{2+} release (CICR; Endo, 2009). Channel proximity may also enable a mutual allosteric interaction that reinforces collective opening and closing (e.g., Stern et al., 1999), but firm evidence for such interactions is lacking. (5) Finally, clustering enhances Ca^{2+}-dependent inactivation (CDI), which promotes channel closure and termination of Ca^{2+} transients (Laver, 2007).

The presence of efficient positive feedback processes results in a potentially unstable system. This instability becomes real in the paradigmatic couplonopathies malignant hyperthermia (MH) and catecholaminergic polymorphic ventricular tachycardia (CPVT), respectively, in skeletal and cardiac muscle.

The couplon was defined by Stern et al. (1997) as a logical emergent of a mathematical simulation that attempted to explain the main features of calcium transients, both local and cell wide.

In the simulation, initially developed for frog skeletal muscle, Ca^{2+} release is initiated by action potential–induced movements in a T tubule channel protein, the DHPR. These movements translate the action potential into a signal that stimulates opening of RyRs of the facing SR membrane. The simulation found that channel opening spread within a cluster via CICR after one or two RyRs opened, producing a local Ca^{2+} transient that mimicked calcium sparks independent of the electrical stimulus (Cheng et al., 1993; Tsugorka et al., 1995).

Within the range of model parameters investigated (Stern et al., 1997), this propagation never progressed from the channels on one side of the triad (that is, on one of its terminal cisternae) to the other. Therefore, we defined the couplon as "the set of release channels on one face of one junctional strip, together with its associated voltage sensors and other triadic proteins." This definition limits the couplon to elements in one "junctional strip," reflecting that junctional regions of transverse tubules are discrete and never much longer than 1 µm (Franzini-Armstrong et al., 1999). The couplon definition was extended in 1999 to the junctions of cardiac muscle (Stern et al., 1999).

Multiple proteins are found in the triads of skeletal muscle and in the dyads of cardiac muscle. Of these, only DHPRs and RyRs are essential to function, but the absence or modification of the others usually causes functional and/or structural abnormalities. Three of these proteins are considered major because of their abundance (Treves et al., 2009): triadin and junctin, which are single-span SR membrane proteins, and calsequestrin, an intra-SR protein linked to the RyR via triadin and junctin (Zhang et al., 1997). At least seven

[1]Sites of close interaction between plasmalemmal and Ca storage organelles, with structural and functional similarities to couplons, have been described in other tissues, including smooth muscle (Collier et al., 2000) and neurons (De Crescenzo et al., 2004).

[2]The difference is, in fact, more fundamental. Electrical forces act at a distance and do not depend on the nature of the ion that produces the underlying charge imbalance. For Ca signaling, instead, ions must bind to their target. Paraphrasing a well-worn construct: for propagation of the action potential, the medium (Na, Ca, K, and Cl ions) is not the message (depolarization). For muscle contraction instead, the medium (Ca) is also the message.

other junctional protein components have been identified (Treves et al., 2009; Rebbeck et al., 2014). Among them, JP-45 and junctate are also SR membrane spanning; JP-45 is notable for interacting with both the main subunit of the DHPR ($Ca_V1.1$) and calsequestrin. Consistent with the original definition, we include in the couplon every protein that is in mechanical interaction, directly or indirectly, with either the DHPR (a heteromer of four or five subunits, depending on the tissue) or the RyR. At last count these include triadin, junctin, calsequestrin, JP-45, two small proteins bound to the RyR (FKBP 12 and FKBP 12.6 [Timerman et al., 1994]), and histidine-rich Ca^{2+}-binding protein (HRC), an SR-luminal component which interacts with triadin in cardiac myocytes (Pritchard and Kranias, 2009). Various widely expressed small proteins also interact, transiently or stably, with the cytosolic aspect of the RyRs. These include calmodulin (Meissner and Henderson, 1987), S100A1 (Prosser et al., 2011), homer, sorcin (Farrell et al., 2003), CHERP, selenoprotein (reviewed by Treves et al. [2009]), protein kinase A (Timerman et al., 1994), calcium/calmodulin-dependent kinase II (Wehrens et al., 2004), phosphodiesterase 4D3 (Lehnart et al., 2005), and protein phosphatases 1 and 2A (Hwang et al., 2012). Although actually or potentially linked to disease, they will not be considered further here.

Skeletal and cardiac couplons are different. Similarities and differences in couplon structure are reflected in the skeletal and cardiac couplonopathies (see Fig. 1). Terminal cisternae of skeletal muscle are roughly tubular structures ∼100 nm in diameter, with a length close to the width of a myofibril (∼1 µm). As indicated in Fig. 2 A, skeletal muscle junctions accommodate RyRs in double rows. DHPRs form tetrads in the junctional T tubule membrane, which connect mechanically, one-to-one, with the four protomers of an opposing RyR. Typically, a skeletal couplon has 20–60 RyRs (Franzini-Armstrong et al., 1999).

In contrast, the cardiac cisternae are shallower, spanning ∼30 nm in the direction perpendicular to the junctional surface (Fig. 1 C). They are also shorter in the direction of the T tubule axis and wider than the skeletal cisternae, which gives them a "pancake" shape. Within this pancake, the channels can be arranged in multiple rows (as depicted in Fig. 2 B), a feature which initially led to overestimation of their numbers per couplon. These estimates have been reduced in work that elucidated the two-dimensional arrangement of cardiac RyR channels in groups, now viewed as small clusters of irregular shape and orientation assembled into clusters of higher order (Baddeley et al., 2009; Scriven et al., 2013).

A second fundamental difference between skeletal and cardiac couplons (Fig. 2) is the absence in the latter of direct, stoichiometric contact between DHPRs and RyRs, reflecting the different mechanisms by which the two tissues translate membrane depolarization to Ca^{2+} release. Additionally, there is much more calsequestrin in skeletal than cardiac muscle. Calsequestrin 1, the skeletal isoform, fills the large skeletal cisternae with its dense ramified polymeric network (Perni et al., 2013), whereas cardiac calsequestrin is concentrated in tight clumps. In spite of the different appearance of the cisternae and calsequestrin networks, there are few differences in the topology of protein contacts, which is represented identically for both couplons in Fig. 2. Junctin and triadin anchor the calsequestrin network to the junction (Tijskens et al., 2003; Franzini-Armstrong et al., 2005) and establish a mechanical link between the RyR and calsequestrin (Zhang et al., 1997). The large difference in volume of skeletal and cardiac muscle cisternae is believed to depend on the amount of protein, specifically calsequestrin, as indicated by the large increase in cisternal volume observed in cardiac muscle overexpressing calsequestrin 2 (Fig. 1 D, modified from Jones et al. [1998]).

Although they derive from a single gene, the cardiac and skeletal isoforms of triadin differ (Marty et al., 2009). There are two predominant forms in skeletal muscle, Trisk 95 and Trisk 51, that differ in the length of the cytosolic C-terminal segment; both are restricted to the triad and linked to the RyR; both are therefore

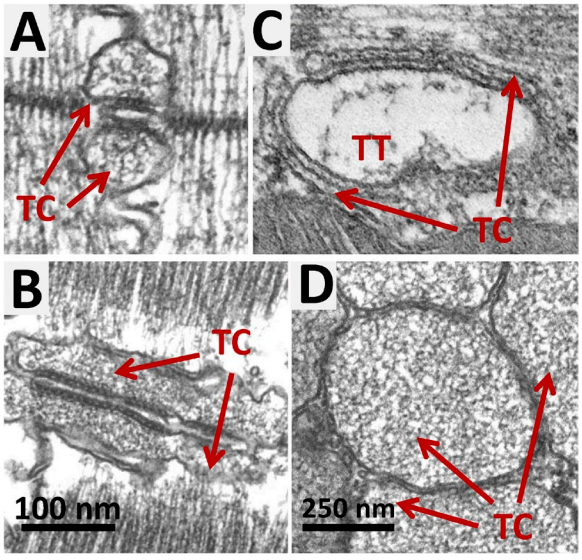

Figure 1. Sites of Ca^{2+} release in skeletal and cardiac muscle. (A and B) Transversal and longitudinal sections of a skeletal muscle triad junction. TC, terminal cisternae. (C) Transversal section of junctions in a cardiac myocyte, between a larger transverse tubule (TT) and very narrow SR cisternae. (D) Protein-filled vesicles (presumably dilated cisternae) in cardiomyocytes of mouse overexpressing calsequestrin 2, to demonstrate that the volume differences are largely determined by the quantity of calsequestrin. A and B show unpublished images by C. Franzini-Armstrong. C and D show images modified from Jones et al. (1998) with permission from The American Society for Clinical Investigation, Inc.

bona fide components of the couplon. The main heart triadin, named CT1, is identical to a further truncated skeletal isoform, Trisk 32, which can be found in the skeletal triad as well as in the longitudinal SR. All isoforms have calsequestrin interaction sites, but the longer isoforms appear to have more of them.

Allosteric connections are instrumental to the skeletal couplon. The skeletal couplon therefore has mechanical connections in the "transversal" direction (i.e., between RyRs along their double row in the junctional membrane) and "longitudinally," starting at the DHPRs, via junctin and triadin all the way to calsequestrin (Fig. 2, arrows). There is also a parallel link from DHPR to calsequestrin that bypasses the RyR, mediated singly by JP-45 (Fig. 2; Anderson et al., 2003).

The cardiac couplons, which lack the DHPR–RyR contact, may have a greater number of RyR-to-RyR connections in their wider junctions. As stated above, their calsequestrin network is smaller and tightly anchored to the junctional membrane by junctin and shorter triadins. There is no JP-45 in cardiac muscle (Zorzato, F., personal communication).

The couplon concept has been linked since its inception to that of allosteric action. This term, meaning literally "another site," was introduced to describe the interaction between conformational change ("allosteric transition") and ligand binding in hemoglobin (Monod et al., 1965). The concept was rapidly extended to interpret regulation of multiple polymeric enzymes (see Whitehead [1970] for an early review). The concerted model of allostery, also known as the "MWC" model, was first applied by Marks and Jones (1992) to ion channels to describe the effect of dihydropyridine binding on activation of L-type Ca^{2+} channels. We (Ríos et al., 1993) realized that this concept was especially apt to describe the activation of RyR channel opening in skeletal muscle, where a moving voltage sensor in the T tubule membrane acts on cytosolic domains of the RyR to open the permeation path in its transmembrane region, located >10 nm away. As Marks and Jones (1992) had done previously to describe effects of dihydropyridines,

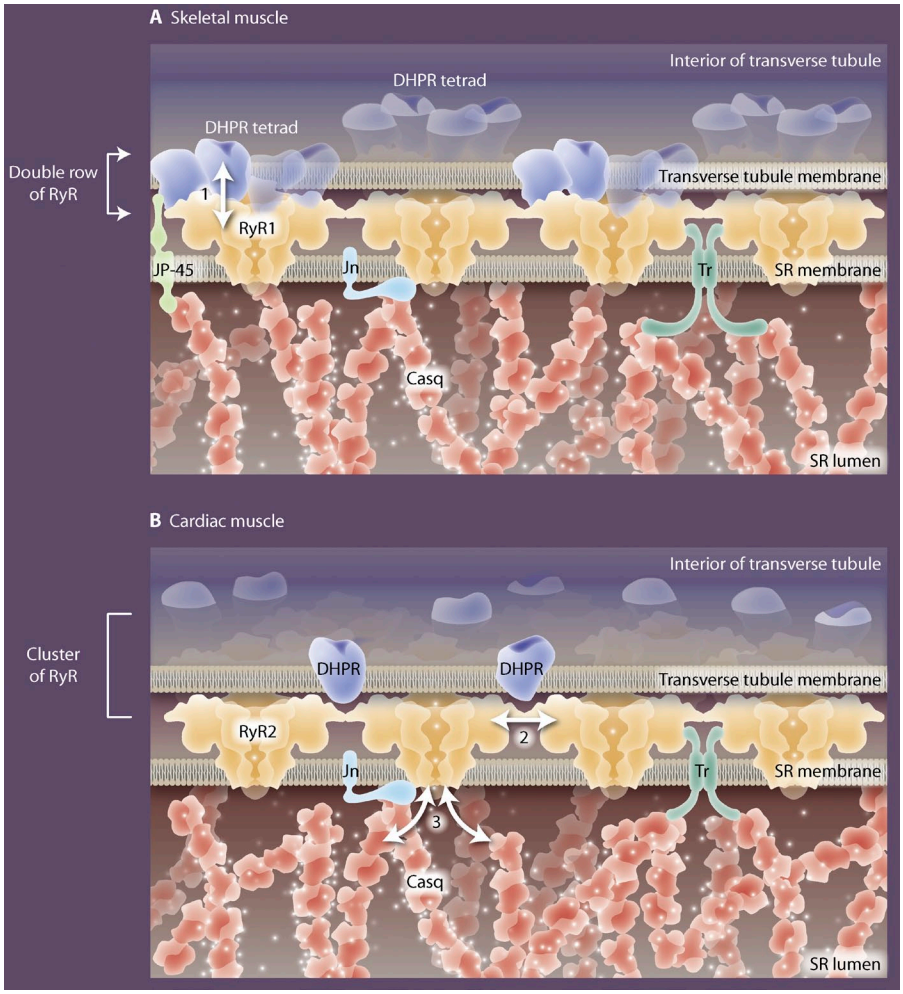

Figure 2. Diagrammatic view of couplons. (A) In skeletal muscle, a double row of RyR1 channels in SR membrane faces tetrads of DHPRs in transverse tubule membrane in a pattern consistent with mechanical contact between DHPRs and alternate RyRs in each row. RyRs are bound to triadin (Tr) and junctin (Jn), transmembrane proteins which link to calsequestrin inside the SR (Casq). Formation of linear ramified polymers of Casq is promoted by Ca^{2+} (dots). The SR protein JP-45 links DHPRs to Casq, bypassing the RyR. (B) In cardiac muscle, the DHPRs of plasmalemma and transverse tubules neither form tetrads nor connect mechanically with RyRs. These are placed in multiple rows, which form clusters of variable size and shape. Linkage of RyR to calsequestrin by Tr and Jn is depicted identically in both tissues, but the cytosolic segment of cardiac Tr is shorter. The cartoon also reflects the absence of JP-45 in cardiac muscle. Other proteins that bind to RyR and Casq are omitted. Allosteric interactions, verified or putative, are represented by arrows. In A, double arrow 1 represents the two-way "longitudinal" interaction between DHPRs and RyRs present in skeletal muscle. The arrows in B represent actions proposed for both tissues: the "transversal" interaction between RyRs is indicated by arrow 2, whereas arrows 3 depict possible $[Ca^{2+}]_{SR}$-dependent conformational effects of calsequestrin on RyR channels. See additional details in section "Skeletal and cardiac couplons are different."

we showed how, with little change, the MWC mathematical formalism could be adapted to describe quantitatively the relationship between measurable movement of electrical charge at the DHPR and gating of the RyR.

The allosteric interaction concept involves several ingredients: mechanical contact, action at a distance mediated by conformational changes in a protein, and the concerted movement of multiple subunits, which is imposed by symmetries in the moving moieties and is especially suited to describe gating of multimeric channels. In the case of DHPRs and RyRs of skeletal muscle, allosteric transitions affect change in the longitudinal direction (the movement at DHPRs results in RyR channel gating, and reciprocally, RyRs affect the operation of DHPRs as channels, as shown by Nakai et al. [1996]). RyRs may also establish communication in the transversal direction in the form of cooperative gating interactions between neighboring channels. In cardiac couplons, where the DHPR–RyR communication is not allosteric, allosteric interactions between cardiac RyRs (RyR2–RyR2) remain possible, but the evidence for them is scant (Diaz-Sylvester et al. 2014. 58th Annual Meeting of the Biophysical Society. Abstr. #576).

A third allosteric mechanism has been proposed for both skeletal and cardiac couplons, whereby Ca^{2+}-dependent conformational changes in calsequestrin, which may involve changes in the state of polymerization, are transmitted to the RyR, via triadin or junctin, as a way for the SR content to modulate RyR gating and Ca^{2+} release. Whether and how $[Ca^{2+}]_{SR}$ modulates Ca^{2+} release remains controversial in skeletal (Sztretye et al., 2011; Fénelon et al., 2012) as well as cardiac muscle (Györke et al., 2009; MacLennan and Chen, 2009; Chen et al., 2014).

Couplon diseases

We define couplon diseases or couplonopathies as disorders that affect one or more proteins of the couplon, to significantly perturb its Ca^{2+} release function. The alterations can be primary or secondary to other, more systemic, conditions. Our definition includes diseases of skeletal and cardiac muscle.

Some couplonopathies are listed in Table 1. Diseases that involve different couplon proteins are listed in different rows, with corresponding components in the two couplons shown in the same rows. We will examine these diseases, while comparing corresponding entities in the two couplons. Fuller lists and descriptions of the diseases may be found in references provided in Table 1. Junctin (reviewed by Dulhunty et al. [2009] and Pritchard and Kranias [2009]) is included in the table as a potential locus for disease, but no junctin polymorphisms have been associated with cardiomyopathies or skeletal muscle diseases. Junctate (a product of the same gene that cannot be considered part of the couplon) appears to have a Ca^{2+}-sensing role to implement store-operated Ca^{2+} entry in T cells (Srikanth et al., 2012). Junctin and junctate will not be discussed further here.

MH and CPVT1. MH is diagnosed (reviewed by MacLennan and Zvaritch [2011] and Dowling et al. [2014]) upon occurrence of the MH reaction, a hypermetabolic, often hyperpyrexic state, with muscle contractures and other activity resulting from uncontrolled Ca^{2+} release, in reaction to certain anesthetics or other stimuli. In the absence of such stimuli, the phenotype is typically mild or nonexistent; therefore, current laboratory tests are said to only diagnose MH susceptibility (MHS).

The primary defect in MH is an excessive proclivity of the RyR to open (elevated p_{open}), which may become manifest at rest or with physiological stimulation. In 60% of the cases, the defect is caused by a mutation in the RyR1 gene. A similar MH phenotype results from a mutation in the gene encoding $Ca_V1.1$, the main subunit of the skeletal DHPR. It is surprising to find disease phenotypes with almost identical features, caused by mutations in separate molecules. This genetic heterogeneity attests to the unity of the couplon as a functional device and prompts the hypothesis that the mechanical connection is essential to this mode of pathogenesis.

Turning to the cardiac couplon, we note the striking correspondence of MH with CPVT (Priori and Chen, 2011). As with MH, CPVT manifests suddenly, usually in situations of stress or intense exercise, in young individuals in apparently good health. Again, the disease is caused by an increase in the propensity of the RyR, in this case RyR2, to open. The link to arrhythmia is well understood (Venetucci et al., 2012): spontaneous channel openings may cause neighbors in the cluster to open via CICR, and the local Ca^{2+} transient may grow to evoke, via the electrogenic plasmalemmal Na–Ca exchanger, membrane potential changes (known as delayed afterdepolarizations [DADs]) that may lead to ectopic action potentials and cardiac arrhythmia. The parallels between MH and CPVT include the primary cause, in most cases mutations at largely similar loci of RyR1 and RyR2. Causative MH and CPVT mutations are reviewed in detail by Durham et al. (2007), MacLennan and Zvaritch (2011), and Priori and Chen (2011).

The comparison between these two tissues is useful to test our hypothesis that the genetic heterogeneity of MH, which may be caused by either DHPR or RyR mutations, relies on mechanical contact between DHPR and RyR. If this hypothesis is correct and given the main difference between the two couplons, CPVT should not show the same genetic heterogeneity. Indeed, CPVT has not been linked to a mutation in the cardiac DHPR. Alterations in its main subunit $Ca_V1.2$ may cause gain or loss of function, which results in various conditions that have in common alterations in the action potential and its propagation. These disease phenotypes are often polygenic and are difficult to distinguish from those

caused by mutations in other ion channels involved in generation and propagation of the action potential (Venetucci et al., 2012). Even mutations that increase the L-type Ca^{2+} channel's propensity to open do not result in the same type of alteration of RyR p_{open} caused by the MH mutations of $Ca_V1.1$.

As was the case for MH, CPVT is genetically heterogeneous. Indeed, CPVT may be caused by mutations in at least four genes. Although the dominant form (CPVT1) is caused by mutations in the RyR, another form (CPVT2) is linked to mutations in the gene encoding cardiac calsequestrin (Faggioni and Knollmann, 2012). A tempting inference is that the mechanical connection between RyR and calsequestrin is implicated in determining the disease phenotype (see below, where we discuss CPVT2).

The mechanical connection between skeletal muscle DHPR and RyR suggests a second hypothesis, which addresses a long-standing question in therapeutics. Dantrolene is the single drug approved by the US FDA for the treatment of MH and is used to treat the acute events of this disease. However, the mechanism of dantrolene action remains elusive. A dantrolene-binding stretch was identified on the primary sequence of RyR1 (Paul-Pletzer et al., 2002), but an almost identical stretch in RyR2 does not confer dantrolene sensitivity to these channels studied in bilayers (Diaz-Sylvester et al., 2008). Dantrolene has inhibitory effects on cardiac muscle (Maxwell et al., 2012; Zamiri et al., 2014), but they are substantially more modest than those in skeletal muscle (Zapata-Sudo et al. 2002. 3rd Annual Meeting of the American Society of Anesthesiologists. Abstr. A-71; Krause et al., 2004). Apparently RyR1 in a complex with DHPR and other physically connected proteins constitutes a better target for dantrolene than do isolated RyRs of any isoform. Just as MH is a "couplon disease," dantrolene may be a "couplon antagonist." This notion is supported by a comparison of the effects of dantrolene in mammalian muscle fibers and on isolated RyR1 (Szentesi et al., 2001).

The idea of dantrolene as a couplon antagonist mirrors our earlier description of the actions of perchlorate, an agonist of both Ca^{2+} release (Lüttgau et al., 1983) and movement of the intramembranous charge of the DHPR voltage sensor (Csernoch et al., 1987). Perchlorate is more effective on the couplon than on its

TABLE 1
Partial list of couplonopathies

Protein	Skeletal muscle			Cardiac muscle		
	Gene	Disease	References	Gene	Disease	References
DHPR	*CACNA1S*	MHS5	Monnier et al., 1997, 2002; Pirone et al., 2010; Toppin et al., 2010	*CACNA1C*	Conduction and other diseases	Venetucci et al., 2012
RyR	*RYR1*	MHS1	Durham et al., 2007; MacLennan and Zvaritch, 2011	*RYR2*	CPVT1, conventional	Durham et al., 2007; Priori and Chen, 2011; Venetucci et al., 2012
		EHS and ER	Capacchione and Muldoon, 2009		CPVT1, unconventional[e]	Zhao et al., 2015
		CCD and related CMs	Durham et al., 2007; Dowling et al., 2014			
Calsequestrin	*CASQ1*	CAM	Rossi et al., 2014	*CASQ2*	CPVT2	Faggioni and Knollmann, 2012
		Unknown[a]	Kraeva et al., 2013a			
		Muscle weakness, MH-like EHS[b]	Paolini et al., 2007; Protasi et al., 2009; Canato et al., 2010; Olojo et al., 2011			
Triadin	*TRDN*	Muscle weakness[b]	Shen et al., 2007; Oddoux et al., 2009	*TRDN*	CPVT5	Roux-Buisson et al., 2012
Junctin and Junctate	*ASPH*	-[c]	Divet et al., 2007	*ASPH*	-[b,c]	Yuan et al., 2007; Hong et al., 2008
JP-45	*JSRP1*	Muscle weakness[b,f]	Gouadon et al., 2006; Yasuda et al., 2013	-[d]		

Corresponding proteins of skeletal and cardiac muscle are listed on the same row. CAM, calsequestrin aggregate myopathy; CMs, congenital myopathies related to CCD (multiminicore disease, congenital myopathies with cores and rods, central nuclear myopathy, and congenital fiber type disproportion); EHS, exertional environmental heat stroke; ER, exertional rhabdomyolysis.
[a]A Calsequestrin 1 variant is predicted to reduce SR Ca store but showed no disease phenotype in carriers.
[b]Disease phenotype observed in null or knockout animals.
[c]Not associated with a disease phenotype in humans. See references for studies on animal models.
[d]JP-45 is not present in cardiac muscle (Zorzato, F., personal communication).
[e]Unconventional for being accompanied by EADs instead of DADs.
[f]JP-45 variants found in the Swiss MH population.

separate molecular parts (compare González and Ríos [1993] with Ma et al. [1993]; see also Gallant et al. [1993] and Percival et al. [1994]). Fig. 3, a collage from figures in Ríos et al. (1993), illustrates some features of the effects of perchlorate that characterize it as a couplon agonist. Fig. 3 A shows the EC coupling "transfer function," which describes the couplon functionally, relating its input (the displacement of intramembranous charge of the voltage sensor) to its output (the flux or rate of Ca^{2+} release). Normally, this transfer function is deeply concave upwards, a relationship which suggests cooperativity (i.e., that the movement of multiple sensor particles is necessary to effect channel opening). As shown, the effect of perchlorate is profound; it linearizes the transfer function, causing a greater response at low charge displacement. A second defining aspect of perchlorate as couplon agonist, not shown, is that it causes a left shift of the relationship between charge displacement and membrane voltage V_m; i.e., it affects the behavior of both couplon proteins. Fig. 3 B depicts a model that uses the MWC formalism to describe allosteric interactions between voltage sensors and release channels. This model accounts well for the change in transfer function (as shown by the simulations in Fig. 3 C). It also predicts the change in V_m dependence of charge displacement.

The effect of perchlorate, in principle an RyR agonist, on functional manifestations of the associated DHPR is not unique. Indeed, activation of intracellular Ca^{2+} release and intramembranous charge movement were shifted to lower voltage in fibers of transgenic mice expressing heterozygously the MH RyR1 Y522S mutation (Andronache et al., 2009). An even greater negative shift of Ca^{2+} release activation was found in porcine myotubes homozygous for the MH R515C mutation (Dietze et al., 2000). As we do here, these authors justified the shifts in voltage dependence of charge movement (and other functions of the DHPR) using the allosteric association with the RyR, and thereby supporting the evidence for "longitudinal" spread of functional alterations, bidirectionally, along a mechanically linked couplon. The view of dantrolene as a couplon antagonist should be testable; if it is correct, dantrolene effects on the RyR should have repercussions on other couplon molecules and functions, including the V_m dependence of the voltage sensor and the EC coupling transfer function.

Congenital muscle disorders with cores. MacLennan and Zvaritch (2011) list seven skeletal myopathies that present with "cores," areas inside myofibers formed by compacted and contracted myofibrils, devoid of staining for oxidative enzymes and essentially devoid of mitochondria. The most common is central core disease (CCD), which is reportedly more frequent than MH (Durham et al., 2007). CCD has a more overt and variable functional phenotype than MH, characterized by early onset muscle weakness and delayed motor development. However, many patients share features of MH and CCD, which is not surprising as most cases of CCD are linked to mutations in RyR1. Although the pathogenic mechanisms and mutual relationships of MH and CCD have not been fully elucidated, an influential systematization,

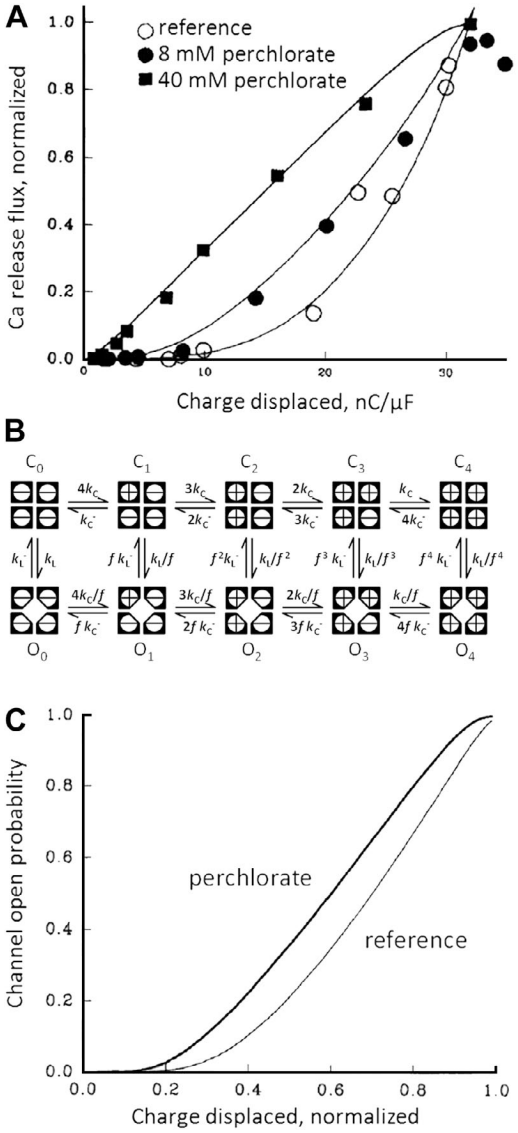

Figure 3. Perchlorate as a couplon agonist. (A) The EC coupling transfer function; relationship between amount of voltage sensor charge displaced and peak Ca^{2+} release flux (measured in voltage-clamped cell of the frog semitendinosus muscle). Perchlorate linearizes the relationship in a dose-dependent manner. (B) An allosteric model, which uses the MWC formalism to describe the activation of release channel opening (represented by the transition from states C_j to O_j) by the operation of four voltage sensors (circles); upon membrane depolarization these move to the activating position (represented as +) in a sequence progressing from left to right. The model reproduces many features of Ca^{2+} release activation, including the effects of perchlorate. This anion is assumed to increase the single parameter *f*, which embodies the activating effect of each moving voltage sensor. Changes in the transfer function (represented in C) and charge displacement then ensue. Panels are modified from Ríos et al. (1993).

put forward by G. Avila, R.T. Dirksen, and colleagues (Avila and Dirksen, 2001; Avila et al., 2003), attributes both MH and MH + CCD phenotypes to mutations distributed along the whole RyR1 sequence, but concentrated in "hotspots" 1 and 2 (located within the large cytosolic-facing moiety of the protein; e.g., MacLennan and Zvaritch, 2011). In this scheme, the two disorders share a primary functional defect, an excessive propensity of the channel to open, and differ only in degrees; slight alteration of p_{open} will cause pure MH (also known as MHS, a susceptibility without an overt disease phenotype), whereas greater p_{open} will bring about increase in $[Ca^{2+}]_{cyto}$ and decrease in $[Ca^{2+}]_{SR}$ and in total releasable Ca at rest, a phenotype described as "leaky" channels (e.g., Treves et al., 2008). As for the pure CCD presentations,[3] which lack any evidence of MHS, they are mostly attributed to mutations in C-terminal hotspot region 3, specifically in the putative transmembrane segments of the channel-forming region (Avila and Dirksen, 2001; Avila et al., 2003; Kraeva et al., 2013b).

Somewhat surprisingly, the pure CCD phenotype appears to have a pathophysiology radically different from that of the MH + CCD disease. The former phenotype, which has been called "uncoupled," is attributed to poorly functioning channels, with reduced Ca^{2+} conductance or p_{open}, in the presence of conserved $[Ca^{2+}]_{cyto}$ and total SR calcium load. In contrast, the MH and MH + CCD diseases share a pathophysiology of excessive RyR p_{open}. This dichotomy is highlighted in a recent presentation by Murayama et al. (2015. 59th Annual Meeting of the Biophysical Society. Abstr. #1363) of 15 mutants in the central region of RyR1 expressed in HEK cells, in which every MH + CCD mutant caused a greater ER leak than any of the pure MH mutants.

Mechanistic considerations provide three additional insights. The first is based on a long-known basic law, first demonstrated by Friel and Tsien (1992) and recast more recently as the cell boundary theorem (Ríos, 2010), which establishes that any long-term change in concentration within a cell (or an intracellular organelle) must be accompanied by and caused by changes in the transport properties of the boundary (respectively the cell or the organelle membrane). The implication for present purposes is that a leaky couplon cannot determine per se a steady increase in $[Ca^{2+}]_{cyto}$. Changes directly caused by the leak can only be transient. The cell boundary theorem establishes that steady changes must depend on a modification in the properties of the plasmalemma. Indeed, changes at the plasmalemmal level have been found in MH models and other cells with leaky SR (Lopez et al., 1986; Duke et al., 2010; Eltit et al., 2010a,b, 2011). When the primary defect is in the SR membrane, as is the case with mutations that increase RyR p_{open}, the alteration of plasmalemmal properties (typically involving increase in store-operated calcium entry) will be a downstream consequence of SR depletion, acting as an important link to increased $[Ca^{2+}]_{cyto}$ in the pathogenic pathway.

The qualification of CCD cells as "uncoupled" is popular but seems inadequate. Indeed, in nearly every mutation studied, the affected RyRs appear to have diminished capability to pass current but normal sensitivity to activation by voltage (or pharmacologic stimuli). These properties are clearly apparent for instance in I4895T mice, which express one of the most frequent and typical human CCD mutations (Loy et al., 2011). Although the channels are non- or underperforming, their coupling seems to work well. The term uncoupled applies well only to the pore region mutant R4842W, which specifically fails to respond to voltage while maintaining a nearly normal response to caffeine (Avila et al., 2003).

Comparing the skeletal and cardiac couplons provides a third insight. Although there is a close correspondence between MH and CPVT1, both of which are understood to emerge from RyR gain of function, there is no clear correspondence between CCD (which results from loss of function) and a cardiac disease. One reason is a relative dearth of examples of RyR2 mutations homologous to loss of function RyR1 mutations. In their comprehensive survey, Durham et al. (2007) listed 13 similar mutations found in homologous spots of RyR1 and RyR2, of which only one, RyR1 T4637I/A, causes CCD. The homologous mutation of RyR2, S4565R, causes CPVT, which is surprising because CPVT is seen as a consequence of primary gain rather than loss of function. Clearly, there ought to be more such mutations in RyR2. It is possible that in the truly "hot" region for loss of function (the putative pore lining region or selectivity filter, residues 4891–4901 and 4819–4830, respectively, in human RyR1 and RyR2) the RyR2 mutants are not viable, explaining to some extent the observed deficit. However, and in view of the numerous examples now identified in RyR1, other corresponding RyR2 loss of function mutations ought to exist and manifest in less severe disease.

What sort of phenotype could be expected in viable loss of function mutations of RyR2? Zhao et al. (2015) engineered a mouse to express RyR2-A4860G, a mutation which causes a catecholaminergic ventricular fibrillation in affected patients (Priori et al., 2002) and produces channels with lower p_{open} (Jiang et al., 2007). The disease phenotype of the heterozygous A4860G mouse, listed as "unconventional CPVT1" in Table 1, was unique in showing distortions of the action potential known as early afterdepolarizations (EADs) instead of the DADs usually associated with CPVT1 (Zhao et al., 2015). Whatever the interpretation of these interesting observations might be, it is clear that ventricular tachycardia and fibrillation do not necessarily require an

[3]Every patient with a CCD diagnosis is considered potentially MHS, deserving special precautions during anesthesia.

underlying gain of RyR function. Instead, the example supports the expectation that Ca^{2+} signaling changes caused by RyR2 loss of function mutations will cause varied alterations in cardiac rhythm, which go beyond the characteristic parameters of CPVT. See Priori and Chen (2011) for additional consideration of RyR2 mutations that cause loss of function.

CPVT caused by mutations in triadin and calsequestrin. Continuing along the couplon, we find forms of CPVT associated with mutations in triadin (CPVT5) and in calsequestrin 2 (CPVT2). 3 mutations of triadin (Roux-Buisson et al., 2012) and 15 mutations in calsequestrin 2 have been linked to CPVT (Faggioni and Knollmann, 2012); CPVT2 accounts for 3–5% of all CPVT cases. No junctin defects were apparent in the families exhibiting the CPVT linked to triadin mutations (Roux-Buisson et al., 2012). Therefore, junctin cannot substitute functionally for triadin, even though both proteins cooperate to tether calsequestrin to the junction and keep cardiac cisternae thin and compact (Tijskens et al., 2003; Franzini-Armstrong et al., 2005). Note that all triadin mutations and most of those in calsequestrin 2 are associated with genetically recessive disease.

This group of diseases constitutes another condition, in addition to MH, where the "couplon" explanation for genetic heterogeneity works; in this case, mutations at various levels of the cardiac couplon cause similar CPVT phenotypes. But, how good is the analogy? Do triadin and calsequestrin mutations alter the RyR2 gating properties in the same, allosteric, way that $Ca_V1.1$ mutations alter the control of RyR1 to cause MH?

The answer has implications for an ongoing controversy about control of Ca^{2+} release by intra-SR $[Ca^{2+}]$. A striking manifestation of this control is store overload-induced Ca^{2+} release (SOICR), the spontaneous Ca^{2+} release seen in normal cardiac SR upon Ca overload (Jiang et al., 2005). Is this control direct, by luminal SR Ca^{2+} acting on the RyR, or does it require calsequestrin? Experiments in bilayers and cardiomyocytes show that calsequestrin exerts allosteric control, essentially an inhibitory role of gating of the RyR (e.g., Györke et al., 2009; Qin et al., 2009). Given the demonstrated interactions of calsequestrin with junctin and triadin, this control must be physically mediated by one or both of these proteins. In experiments with bilayer-reconstituted channels, different calsequestrin mutations modify the interaction in different ways, resulting, for example for the case of R33Q, in a partial loss of the inhibition of the release channel attributed to the presence of wild-type calsequestrin or a total loss of this inhibition for the L167H mutant (Qin et al., 2008). These observations explain the mutation-linked arrhythmia as a consequence of release from inhibition while the mutated calsequestrin remains attached to the couplon. A somewhat different view is suggested by other features of the CPVT2 phenotype, also found in the triadin-linked form of CPVT: one is the near complete loss of calsequestrin in most cases and the other is their autosomal-recessive inheritance. Both indicate that mutation of attached calsequestrin is not sufficient to establish the phenotype, suggesting a simpler mechanism, whereby channel gain of function results when the protein is lost from the couplon (Chen et al., 2013). Both views, however, invoke a "gating" change in the RyR, induced by calsequestrin as it is modified or disappears from the couplon.

An alternative "buffering" theory (proposed by MacLennan and Chen [2009]) explains CPVT1, CPVT2, and the recently described CPVT5 as instances of facilitation of SOICR, without recourse to allosteric control. In this view, SOICR reflects direct activation of the RyR by luminal Ca^{2+}, acting at a binding site that may have been identified (Chen et al., 2014). In the case of CPVT1, the causative RyR2 mutation would increase the sensitivity to luminal Ca^{2+} (i.e., SOICR threshold would be lowered). In the case of CPVT2, the threshold value of $[Ca^{2+}]_{SR}$ for triggering SOICR would not change (as RyRs are not affected) but would be reached more easily upon any perturbation, given the lowered levels of calsequestrin 2 observed in this form of the disease. This explanation should also apply to the CPVT linked to triadin mutations, as these mutations result in near or complete absence of calsequestrin at SR cisternae.

Both the "gating" and the "buffering" explanations of the gain of function syndrome found upon mutations of calsequestrin 2 (or triadin) are plausible. The gating mechanism is consistent with observations in bilayers, with the autosomal-recessive mode of inheritance (as the putative gating control may still be exerted when the concentration of calsequestrin is reduced), and is indirectly supported by observations consistent with control of RyR1 gating by calsequestrin 1 (Sztretye et al., 2011). In contrast, the "buffering" model is appealing in its simplicity and explains best the linkage of the phenotype, with variable severity, to mutations that cause varied degrees of loss of calsequestrin (Faggioni and Knollmann, 2012). There is a distinct possibility that both are correct.

Diseases of skeletal muscle linked to mutations in triadin and calsequestrin. Although the cardiac diseases caused by mutations of triadin and calsequestrin 2 are rare, diseases caused by corresponding alterations of the skeletal couplon are even more so. We believe that this robustness against disease is related to the lesser stability of calcium control in heart muscle, manifested for instance in the greater propensity for spontaneous discharge of SR Ca^{2+} of cardiomyocytes relative to skeletal myofibers. We argue that these are consequences of the radical difference in buffering power of their Ca^{2+} stores.

Consider first the phenotype of calsequestrin 1– and triadin-null mice. Calsequestrin 1–null mice (Paolini et al., 2007) have moderate muscle weakness and characteristic

changes in cytosolic Ca^{2+} transients, caused by loss of Ca storage capacity (Canato et al., 2010; Olojo et al., 2011).[4] Triadin-null mice (Shen et al., 2007; Oddoux et al., 2009), which lack all skeletal and cardiac products of the single triadin gene, combine a cardiac phenotype of CPVT and a skeletal phenotype of weakness, together with a sharply reduced expression of calsequestrin 1.

In patients with CPVT-linked triadin mutations, triadin is effectively absent (Roux-Buisson et al., 2012). Not surprisingly therefore, the functional phenotype is similar to that in triadin-null mice, including skeletal muscle weakness. As we stated earlier, the CPVT phenotype is attributed in most cases to a gain of function at the couplon level, whereas skeletal muscle weakness is usually caused by a reduced cytosolic Ca^{2+} signal, loss of function. How could the same mutation cause divergent phenotypes in the two types of muscle? Clues can be found in the only human disease known to be linked to a mutation in the gene encoding calsequestrin 1.

This disease, described as a "protein aggregate myopathy" by Rossi et al. (2014), cosegregates with the calsequestrin 1 mutation D244G in an autosomal-dominant manner. Its phenotype is a clear loss of function (muscle weakness, together with reduced sensitivity to caffeine) accompanied by structural alterations, electron-dense bodies containing calsequestrin plus other couplon proteins. The formation of calsequestrin aggregates is attributed to misfolding and/or abnormal polymerization caused by loss of the electric charge of aspartate at a site involved both in high-affinity Ca^{2+} binding and in (low-affinity) Ca^{2+}-dependent interaction between calsequestrin protomers (Rossi et al., 2014).

We now have the elements to justify the discrepancy between the phenotype of loss of function in triadin- and calsequestrin 1–linked diseases of skeletal muscle (and in animal models where the proteins are absent) and the gain of function in cardiac diseases caused by mutations in triadin and calsequestrin 2. As stated earlier, the allosteric effect of calsequestrin demonstrated in bilayers (e.g., Chen et al., 2013) and shown to operate physiologically (Sztretye et al., 2011) is an inhibition of RyR channel opening. The loss of function phenotype linked to loss or misplacement of D244G calsequestrin 1 cannot therefore be attributed to a diminished allosteric effect, which should result in greater channel activity. We believe that it is instead caused by loss of releasable Ca, consequent to the misplacement of calsequestrin (and perhaps other couplon proteins) plus the degradation of its buffering properties caused by defective polymerization. Tellingly, and unlike most forms of CPVT2, the D244G-linked disease is transmitted in a dominant manner. The putative allosteric inhibition should therefore persist in these patients, exerted by the WT calsequestrin still present in them; no gain of function should be expected. In contrast, total loss of the protein as seen in calsequestrin 1–null mice causes an MH-like gain of function phenotype (Protasi et al., 2009). This effect, of obviously recessive propagation, is consistent with relief from the basal-repressive action that the protein is believed to exert. Thus, we believe that the loss of function disease phenotype associated with mutations of calsequestrin 1 or triadin is fully explained by the loss of buffer.

Beyond this fairly elaborate explanation, there is a fundamental difference between the skeletal and cardiac EC coupling machineries, which is probably at the root of the divergent phenotypes that result from homologous mutations in the two couplons. This is the notable difference in volume, calsequestrin content, and therefore capacity of the calcium stores (Fig. 1). In cardiac cells, decay in $[Ca^{2+}]_{SR}$ during a local or global Ca^{2+} transient reduces the resting concentration by 50 to nearly 100%, depending on the estimation methods. In contrast, it took averaging 6,000 events to quantify the minute "skraps" of SR Ca^{2+} depletion that accompany Ca^{2+} sparks of skeletal muscle (Launikonis et al., 2006); the depletion that results from Ca^{2+} release during an action potential is also small (Pape et al., 1993; Launikonis et al., 2006). The more robust support of Ca^{2+} release by the calcium store in skeletal muscle implies that this muscle will have more resilience in the face of calsequestrin mutations and will be less prone to swings in SR Ca^{2+} concentration, spontaneous discharges of Ca^{2+} content, and the ensuing alterations of RyR gating. This resilience should narrow the range of mutations that will result in overt disease to those that affect the RyR directly, or via its DHPR link. Consistent with these expectations, the calsequestrin 1 variant M87T, found in 16 of 205 MH probands by Kraeva et al. (2013a), did not cosegregate with a disease phenotype in spite of being scored "probably damaging" by commercial software.

We conclude that the allosteric effects by triadin, junctin, and calsequestrin are either less relevant in skeletal muscle or not radically altered within the more limited range of changes in $[Ca^{2+}]_{SR}$ that occur there during physiological Ca cycling. Ca^{2+} release should also be more tolerant to changes in Ca^{2+} buffering power in skeletal muscle because of the much greater SR volume and calsequestrin endowment, which provide a superior functional reserve. These considerations help explain the different functional phenotypes that result from homologous mutations in the two tissues, as well as the lower incidence of uncontrolled Ca^{2+} release events and the lower prevalence and comparatively milder phenotypes of couplonopathies in skeletal muscle.

Large-scale alterations disrupt couplon functions in a transmission-specific manner. Several diseases are not primarily associated with mutations but still involve changes in the structure and function of the couplon and its component

[4]These manifestations of loss of function are somewhat offset by others that cause effective functional gain, including loss of the inhibitory effect on Ca^{2+} release that manifests upon partial SR depletion (Sztretye et al., 2011) and an MH-like reaction to high temperature (Protasi et al., 2009).

proteins. We include here two types of alterations: (1) changes in the properties and mutual relationships of the membranes where couplons reside, often associated with a modified supramolecular arrangement and physical size of the couplons, and (2) posttranslational modifications, which are multiple and alter the functions of couplon molecules in many ways. Posttranslational modifications will not be considered other than to note that they may coexist with large alterations in structure, or even cause them, as documented in rat cardiomyocytes for the arrangement of RyRs in checkerboard clusters, which is promoted by RyR phosphorylation (Asghari et al., 2014). Following the comparative approach adopted in this article, we predict that similar disruptions in structure should have different consequences in cardiac and skeletal muscle.

In cardiac muscle, large alterations in couplon structure occur in cardiac failure (HF). This involves a major remodeling of transverse tubules, with comparatively minor changes in the SR. These changes (extensively reviewed in a recent issue of *Cardiovascular Research*, starting with Kohl and Lehnart [2013]) may include loss of T tubule network density, extent, and connections to plasmalemma, increase in mean distance between L-type channels and RyRs (caused by loss of T tubules or increase in physical distance between membranes), loss of density of L-type channels, and reduction of couplon size and numbers of RyRs per couplon. The chief functional alteration found in HF patients and animal models consists in a loss of coupling between L-type current and Ca release, interpreted since Gómez et al. (1997) as the degradation of CICR caused by the structural changes. In late stages of HF, this is compounded with loss of stored calcium, which both reduces Ca release flux and hastens its termination by reducing the inter-RyR feedback that sustains release (Gillespie and Fill, 2013; Laver et al., 2013).

There is no condition comparable with HF in skeletal muscle, but major alterations in transverse tubule membrane structure have been induced by extracellular solutions of high osmolarity (demonstrated in detail by Apostol et al. [2009]). Similar structural changes have been observed in amphibian muscle upon fatiguing stimulation, where they are attributed to the intratubular accumulation of osmolytes, largely lactate (Lännergren et al., 2000, 2002). As in cardiac muscle, these changes, including dilation and clipping of transverse tubules, should cause misalignment of triadic junctions and an alteration of coupling between DHPRs and RyRs. In contrast with cardiac muscle, where the HF-associated structural changes are accompanied by EC coupling loss of function, the consequences of osmotically induced distortion in skeletal muscle should be put in the gain of function category, as they consist largely of local calcium release events[5] and a diffuse increase in $[Ca^{2+}]_{cyto}$ (Apostol et al., 2009).

[5]ECRE or elementary events of Ca release is the term preferred by Apostol et al. (2009), as these include events similar to Ca sparks and others that are very different (Kirsch et al., 2001).

It is not surprising that the effects of structural disruption in cardiac and skeletal muscle differ, given the differences in transmission mechanisms. However, the emergence of local and global Ca release in skeletal muscle is not easy to explain. Kirsch et al. (2001) and Wang et al. (2005) attributed their appearance to the release of the basal inhibition of RyR1 that according to Shirokova et al. (1999) is exerted by DHPRs (see also Zhou et al. [2006]). Apostol et al. (2009), however, demonstrated that a full response to the osmotic treatment requires DHPRs in a functionally available state. A conclusion consistent with all observations is that the alterations of EC coupling upon fatiguing exercise and osmotic disruption of the skeletal couplon do not reflect a simple breakage of the mechanical DHPR–RyR1 connection.

A control mechanism unique to skeletal muscle. Finally, we apply our mechanistic and comparative approach to the functional deficits caused by variants in JP-45, a protein outside the main longitudinal allosteric chain of couplon molecules. The location of JP-45 as mechanical bypass between DHPR and calsequestrin (Fig. 2) is suggestive of a servomechanism for control of the voltage sensor by $[Ca^{2+}]_{SR}$. That the link has functional consequences on the DHPR is demonstrated by the observation that two variants of JP-45 (which do not cause an actual couplonopathy in humans) induce a similar right shift of the V_m dependence of sensor charge movement and activation of Ca release flux (Yasuda et al., 2013). That calsequestrin 1 is instrumental to this control is proven by the observation of a massive increase in Ca^{2+} influx via L-type channels in mice with knockout of both JP-45 and calsequestrin 1 (Mosca et al., 2013) but not in single calsequestrin 1 nulls.

If present in cardiac muscle, JP-45 could be an effective control device, as it would tether DHPRs to calsequestrin molecules exposed to much greater swings in $[Ca^{2+}]_{SR}$. However, JP-45 interacts exclusively with calsequestrin 1 (Zorzato et al. 2015. 59th Annual Meeting of the Biophysical Society. Abstr. #1355), which implies that the regulation mediated by JP-45 is specific to skeletal muscle. This form of modulation of the DHPR of skeletal muscle thus joins the set of major differences between the two couplons. An extra control seems appropriate for $Ca_V1.1$ given that it performs two functions, voltage sensor and Ca^{2+} influx pathway, which are separate to the point of requiring different conformational transitions in $Ca_V1.1$ (e.g., Feldmeyer et al., 1990).

Conclusions

We define a novel category of diseases of striated muscle, the couplonopathies, as those that have in common a substantial disruption of the functional unit of Ca^{2+} release for EC coupling, the couplon. Consideration of similarities and differences between the couplons

of skeletal and cardiac muscle affords insights into the pathogenesis of several couplonopathies, including MH and CPVT. Specifically, we argue that the allosteric connection among couplon proteins $Ca_V1.1$ and RyR1 is required for the MH phenotype usually linked to mutations in the RyR channel to also associate with mutations in $Ca_V1.1$. As an extension of this idea, we propose that the same allosteric interaction underpins the beneficial effects of dantrolene. The absence of a corresponding mechanical connection in cardiac muscle explains the absence of CPVT diseases caused by mutations in $Ca_V1.2$. Based on mechanistic considerations applicable to both couplons, we identify the plasmalemma as a site of alterations in transport properties, typically consisting of an increase in store-operated calcium entry, secondary to couplon mutations that promote Ca^{2+} release. These secondary changes constitute significant factors in the pathogenesis of MH. Mutations in triadin and calsequestrin have tissue-specific consequences: in the heart they cause couplonopathies associated with either loss of the allosteric control putatively exerted by these proteins on the Ca^{2+} release channel or loss of Ca^{2+} buffer capacity in the SR. In skeletal muscle, the phenotypes are milder or nonexistent because of the narrower range of physiological $[Ca^{2+}]_{SR}$ visited during function, as well as the much greater functional reserve of Ca storage that is present in this tissue. Finally, the effects of variants or ablation of JP-45 demonstrate a control of the DHPR that is unique to skeletal muscle and may be prescribed by the separate channel and sensor functions of the skeletal muscle DHPR.

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