Calmodulin and Excitation-Contraction Coupling

Susan L. Hamilton, Irina Serysheva, and Gale M. Strasburg

Excitation-contraction coupling in cardiac and skeletal muscle involves the transverse-tubule voltage-dependent Ca\(^{2+}\) channel and the sarcoplasmic reticulum Ca\(^{2+}\) release channel. Both of these ion channels bind and are modulated by calmodulin in both its Ca\(^{2+}\)-bound and Ca\(^{2+}\)-free forms. Calmodulin is, therefore, potentially an important regulator of excitation-contraction coupling. Its precise role, however, has not yet been defined.

Excitation-contraction (E-C) coupling is the process by which depolarization of the muscle fiber membrane, elicited by a nerve action potential, triggers the release of Ca\(^{2+}\) from the sarcoplasmic reticulum (SR) (1). The resulting rise in intracellular Ca\(^{2+}\) concentration activates the troponin complex, thereby initiating the contraction of the muscle. The two primary proteins involved in the initial events of E-C coupling are the dihydropyridine receptor (DHPR) and the ryanodine receptor (RYR), which are both Ca\(^{2+}\) channels (Fig. 1). Skeletal and cardiac muscle have different isoforms of both the DHPR and RYR. The skeletal muscle DHPR, which is an L-type Ca\(^{2+}\) channel, is composed of four subunits: \(\alpha_{1S}\) (190–212 kDa), \(\alpha_\delta\) (125 kDa), \(\beta\) (52–58 kDa), and \(\gamma\) (25 kDa). The cardiac DHPR has three known subunits: \(\epsilon_{1c}\) (240 kDa), \(\alpha_\delta\) (125 kDa), and \(\beta\) (62 kDa). The \(\gamma\)-subunit has not yet been identified as a subunit of the cardiac channel. The \(\alpha_1\)-subunit of the DHPR forms the channel pore and contains the binding sites for channel-specific drugs.

In mammalian skeletal muscle, the DHPR functions as both a voltage sensor and a voltage-dependent Ca\(^{2+}\) channel, but influx of Ca\(^{2+}\) via the DHPR is not required for E-C coupling. The two skeletal muscle proteins are thought to be physically coupled, such that depolarization-induced changes in the conformation of the DHPR are directly sensed by the RYR, triggering its opening. In cardiac muscle the mechanism of E-C coupling appears to involve Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR). The cardiac DHPR serves as a functional voltage-dependent Ca\(^{2+}\) channel allowing entry of extracellular Ca\(^{2+}\), which raises the local intracellular Ca\(^{2+}\) concentration. Binding of Ca\(^{2+}\) to the cytoplasmic domain of the cardiac muscle RYR (RYR2) then induces the channel to open, releasing Ca\(^{2+}\) from the SR. Although the primary signal in skeletal muscle appears to be the mechanical signal, CICR may serve to propagate the Ca\(^{2+}\) signal by activating skeletal muscle RYRs (RYR1s) that are not coupled to DHPRs.

Release of Ca\(^{2+}\) from the SR is controlled by the Ca\(^{2+}\) release channel or RYR. The RYR1 and RYR2 are both homotetramers with a subunit molecular mass of ~565 kDa, and they share 66% sequence identity and ~80% overall homology. Approximately 4/5 of the RYRs are predicted to be cytoplasmic, with only 1/5 of the molecular at the carboxy terminus forming the luminal and membrane-spanning domains.

In addition to the primary mechanisms of regulation of E-C coupling (mechanical gating or CICR), Ca\(^{2+}\) release is likely to be modulated by other proteins bound to the DHPR or to RYR. One of these proteins, calmodulin (CaM), regulates the activity of the DHPR (8, 15), RYR1 (11), and RYR2 (13). On the basis of its interactions with these channels, CaM is likely to play an important role in both cardiac and skeletal muscle E-C coupling.

CaM as an intracellular Ca\(^{2+}\) sensor

CaM is a ubiquitously expressed Ca\(^{2+}\) binding protein composed of an amino terminal and a carboxy terminal lobe connected by an eight-turn \(\alpha\)-helix. The structures of CaM in its Ca\(^{2+}\)-free (apoCaM) (5) and Ca\(^{2+}\)-bound (Ca\(^{2+}\)CaM) forms (obtained from the Brookhaven Protein Databank website: http://cmm.info.nih.gov/modeling/pdb_at_a_glance.html) are shown in Fig. 2. CaM binds most target proteins in a Ca\(^{2+}\)-dependent manner. Both the amino terminal and carboxy terminal lobes have two E-F hand Ca\(^{2+}\) binding sites that undergo Ca\(^{2+}\)-dependent conformational changes that expose hydrophobic binding pockets, allowing binding to amphipathic \(\alpha\)-helical domains within the target proteins (9). Ca\(^{2+}\)CaM undergoes another major change in conformation on binding to its target. The structure of CaM bound to an amphipathic \(\alpha\)-helix from myosin light chain kinase is shown in Fig. 3B (4).

In addition to the Ca\(^{2+}\)-dependent binding of CaM to target proteins, CaM binds to some proteins in the absence of Ca\(^{2+}\) (9). Several types of Ca\(^{2+}\)-independent binding sites for CaM have been reported (9). One type of Ca\(^{2+}\)-independent CaM binding site is an IQ motif (IQXXXRGXXX). Some IQ motifs, however, can also bind Ca\(^{2+}\)CaM. In binding to an IQ motif,
apoCaM also undergoes a major conformational change. The structure of apoCaM bound to an IQ motif from myosin is shown in Fig. 3A (3).

CaM and the voltage-dependent Ca²⁺ channel

CaM is both a positive and negative regulator of the cardiac L-type Ca²⁺ channel. Elevations in intracellular Ca²⁺ concentration produce a conformational change in CaM, tethered to the channel, producing L-channel inactivation (8).

Zühlke et al. (15) have shown that CaM serves as a Ca²⁺ sensor for both positive and negative regulation of the cardiac L-type Ca²⁺ channel (8). A mutant CaM that cannot bind Ca²⁺ at any of the four Ca²⁺ binding sites blocks the effects of Ca²⁺CaM on the L-type Ca²⁺ channel, suggesting that both the Ca²⁺-free and Ca²⁺-bound forms of CaM can bind to this channel. This ability of Ca²⁺CaM to inhibit the channel appears to be mediated via its binding to an IQ motif in the cytoplasmic carboxy tail of the α₁-subunit. If the isoleucine of this motif is mutated to an alanine, the Ca²⁺-dependent inactivation is lost, and this unmask a strong facilitation by CaM. If the isoleucine is converted, however, to a glutamate, both of the effects of CaM (inactivation and facilitation) are lost (15). These findings suggest that either apoCaM and Ca²⁺CaM are binding in the same region of the DHPR (probably with different determinants for binding) or that Ca²⁺CaM binding sites are allosterically regulated by the binding of apoCaM.

CaM and RYR1

Dual regulation by CaM is also seen with RYR1. RYR1 is the major CaM binding protein of SR membranes (11). Tripathy et al. (11) have shown that CaM bound to SR Ca²⁺ release channel (RYR1) at nanomolar Ca²⁺ concentrations activates the channel. In contrast, CaM bound to RYR1 at micromolar Ca²⁺ concentrations inhibits Ca²⁺ release channel activity. Our studies demonstrate the existence of a single CaM binding site per subunit of RYR1 at both high and low Ca²⁺ concentrations (6). The affinity of both apoCaM and Ca²⁺CaM for RYR1 is in the range of 5–50 nM (6, 14, 11). Wagenknecht and colleagues (12) have identified the binding site for CaM in the three-dimensional reconstructions of RYR1, and the approximate location on RYR1 is shown in Fig. 1.
RYR1 itself binds Ca\(^{2+}\) in the absence of CaM, creating a complicated picture of the response of this channel to Ca\(^{2+}\) in the presence of CaM. In the absence of CaM, Ca\(^{2+}\) in the 1–300 µM range activates RYR1, but at concentrations of >500 µM it is inhibitory. In the presence of CaM, a biphasic dependence of RYR1 activity on Ca\(^{2+}\) is still seen, except both activation and inhibition take place at lower Ca\(^{2+}\) concentrations. A mutant CaM that does not bind Ca\(^{2+}\) at any of the four binding sites is an activator of the channel at all Ca\(^{2+}\) concentrations (10). The results of Fruen et al. (2) suggest that, in contrast to its interaction with RYR1, apoCaM binds with much lower affinity to RYR2 than to RYR1, and it does not increase RYR2 activity.

The binding sites for both apoCaM and Ca\(^{2+}\)-CaM on RYR1 appear to be close to amino acids 3630–3637. Bound CaM (either Ca\(^{2+}\)-bound or Ca\(^{2+}\)-free) can protect this region from either proteolytic cleavage after amino acid 3630 and after amino acid 3637 or modification of cysteine 3635 by either oxidants or N-ethylmaleimide (7). Cysteine 3635 can form a disulfide bond with an unidentified cysteine on an adjacent subunit (6). CaM bound to RYR1 can prevent this oxidation-induced intersubunit cross-linking. Conversely, oxidation blocks CaM binding (14). One interpretation of these findings is that CaM binds at a site of intersubunit contact and, in doing so, could protect the channel from the effects of oxidants (Fig. 4), for example, during periods of oxidative stress associated with strenuous exercise.

A role for CaM in E-C coupling?

There are a number of unanswered questions that limit our ability to define the role of CaM in skeletal and cardiac muscle E-C coupling. Previous studies on DHPR have focused on its activity in transverse-tubule vesicles enriched in DHPR or on the purified form reconstituted in lipid bilayers. Likewise, studies on regulation of RYR have been conducted on SR vesicles enriched in RYR or on the purified protein in lipid bilayers. These studies indicate that both RYR and the DHPR can bind CaM in vitro, but can they both bind CaM in vivo (for example, when the DHPR is physically coupled to RYR1)? In skeletal muscle, only every other RYR1 appears to be coupled to a voltage sensor. Are coupled and uncoupled RYRs modulated by CaM, or is CaM only bound to the uncoupled channels, thereby increasing their sensitivity to Ca\(^{2+}\)-triggered
perturbation of enteric GABA receptors presents potential new target sites for drug development.

subserve hormonal and paracrine signaling. Disruption in gastrointestinal function following

for apoCaM binding are different from those for Ca\(^{2+}\)CaM


References


In summary, it seems likely that CaM binding to the DHPR and RYR must, in some way, modulate E-C coupling in both cardiac and skeletal muscle. Its role may be to regulate the Ca\(^{2+}\)-dependent enhancement and inhibition of activity or, possibly, to prevent or enhance coupling between the DHPR and RYR1. Since CaM plays a wide variety of roles in both development and cell function, it would be difficult to create a CaM knockout animal model to determine the functional role of CaM in regulation of either cardiac or skeletal muscle E-C coupling. Instead, the most likely approach will be to mutate its binding sites on RYR1 and/or the DHPR, express these mutated channels in cells that are deficient in one of these proteins, and assess the functional consequences. This approach will require the further identification of the molecular determinants on RYR and the DHPR involved in both apoCaM and Ca\(^{2+}\)-CaM binding. If the amino acids needed for apoCaM binding are different from those for Ca\(^{2+}\)-CaM binding, it may be possible to selectively destroy the binding of one form of CaM without greatly altering the interaction of the other form. This would allow the assessment of the functional contributions of the different forms of CaM.


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