Review Article

Calmodulin and its roles in skeletal muscle function

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The purpose of this review is to describe the importance of calmodulin as a mediator of the effects of calcium ions in living systems, particularly in the process of skeletal muscle contraction.

Calmodulin is a low molecular weight, acidic, calcium binding protein which mediates the Ca^{2+} regulation of a wide range of physiological processes throughout eukaryotic organisms. At low free Ca^{2+} concentrations, such as exist in resting muscle sarcoplasm, calmodulin exists in the Ca^{2+}-free form in which state it does not generally interact with a target protein. Following an appropriate stimulus, the free Ca^{2+} concentration rises whereupon Ca^{2+} binds to calmodulin which undergoes a conformational change enabling it to interact with a target protein(s). The overall result of this protein–protein interaction is a physiological effect, e.g., Ca^{2+} binding to calmodulin in smooth muscle allows it to interact with and activate myosin light chain kinase which catalyzes the phosphorylation of myosin. This reaction results in contraction of the smooth muscle. Recent studies have implicated calmodulin in the Ca^{2+} control of three enzymes in skeletal muscle: phosphorylase kinase, myosin light chain kinase and a protein kinase of the sarcoplasmic reticulum. Various classes of drugs, including certain local anaesthetics, have been shown to affect calmodulin-dependent processes. It is likely that the effects of such drugs result from their interaction with calmodulin.

Key words

MUSCLE, SKELETAL: calcium, calmodulin, glycogen metabolism, myosin phosphorylation, sarcoplasmic reticulum.

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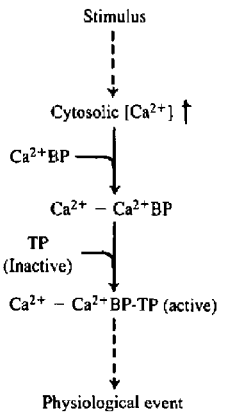
Calcium ions are recognized to be important intracellular messengers involved in a host of physiological responses to nervous and hormonal stimuli, the effects of Ca^{2+} being mediated by specific Ca^{2+}-binding proteins.[1] The second messenger concept in relation to calcium ions is summarized in Figure 1.

An appropriate stimulus leads to an elevation of cytosolic Ca^{2+} concentration, this Ca^{2+} coming from intracellular stores or the extracellular space. This cytosolic Ca^{2+} then interacts with one or more calcium-binding proteins, depending on the tissue under consideration. These calciproteins form a family of structurally homologous proteins, examples of which are calmodulin (the subject of this article), troponin C (which is a component of the primary Ca^{2+} regulatory mechanism in striated muscles), and parvalbumins (the soluble relaxing factors of fast-twitch skeletal muscle). The resultant Ca^{2+}-calcium binding protein complex is then capable of interaction with one or more target proteins, again depending on the tissue under consideration, to form a ternary complex of Ca^{2+}-calcium binding protein-target protein. The effect is generally to convert the target protein (which is often an enzyme) from an inactive to an active state. The end-result of the target protein activation is a physiological event which may be the immediate consequence of target protein activation or may result from a series of intermediate reactions (usually protein phosphorylations) triggered by the target protein activation.

An obvious example to quote is contraction of skeletal muscle fibers in response to nervous stimulation which leads to an elevation of sarcoplasmic Ca^{2+} concentration. These Ca^{2+} ions interact with troponin C inducing a conformational change in the calcium binding protein which is transmitted to



$Ca^{2+}BP$ = calcium binding protein
TP = target protein.

FIGURE 1 Second messenger concept.

neighbouring subunits of the troponin complex causing a movement of tropomyosin into the actin groove. This permits actin-myosin interaction and contraction of the muscle at the expense of ATP.[2]

Of the numerous calcium binding proteins studied to date, calmodulin is the most widespread. It has been purified to homogeneity from a wide range of species and tissues from higher vertebrates (including man) through the invertebrates, higher plants, fungi, slime molds and unicellular organisms.[3] Calmodulin has been identified in every tissue and species of eukaryote examined; it has not, however, been found in prokaryotes. In keeping with its widespread distribution, calmodulin exhibits a great diversity of function as shown in the Table. Calmodulin was originally identified as the Ca^{2+}-dependent activator of bovine heart and brain cyclic nucleotide phosphodiesterases by Cheung in the U.S.[4] and Kakiuchi in Japan.[5] Soon afterwards it was shown to activate brain adenylate cyclase, also in a Ca^{2+}-dependent manner.[6] Calmodulin has since been implicated in the regulation of a host of other key enzymes and physiological processes (Table).

Because of its widespread functional importance, knowledge of calmodulin and its roles in physiological processes is fundamental to eventual understanding of how living systems operate and in what ways they are affected in disease conditions or following administration of drugs or anaesthetics. For this reason, it is hoped that this review will be useful and interesting to anaesthetists and other clinicians.

Structural aspects

Calmodulin is a monomeric, globular protein of molecular weight ~17,000 daltons. It is a highly acidic protein (pI = 4.0–4.3) due to a high content (~35 per cent) of aspartate and glutamate residues. Calmodulins lack tryptophan and, usually, cysteine. A high ratio of phenylalanine:tyrosine (commonly 8:2) gives rise to a characteristic UV absorption spectrum which is atypical of common globular proteins. Most calmodulins contain a single residue of the unusual amino acid, ε-*N*-trimethyllysine, which presumably arises from a post-translational methylation of a lysine residue. The amino-terminus of calmodulin is blocked by an *N*-acetyl group.

The amino acid sequences of nine calmodulins ranging from human brain to *Tetrahymena pyriformis* (a ciliated protozoan) have been completed or almost completed. The primary structure of calmodulin is remarkably conserved throughout evolution, suggesting the importance of the entire molecule in the diverse functions of this calcium binding protein.

Calmodulin is capable of binding 4 Ca^{2+} ions

TABLE Diversity of calmodulin function

Calmodulin-dependent enzymes	*Physiological role*
Cyclic nucleotide phosphodiesterase	Cyclic nucleotide metabolism
Adenylate cyclase	Cyclic nucleotide metabolism
(Ca^{2+} – Mg^{2+}) ATPases	Ca^{2+} transport
Myosin light chain kinase	Smooth muscle contraction and non-muscle motility
Phosphorylase kinase	Glycogen metabolism
Other calmodulin-dependent kinases	e.g. neurotransmitter release
Phospholipase A_2	Platelet aggregation
Dynein ATPase	Ciliary and flagellar motility
Other calmodulin-regulated processes	
Insulin secretion from pancreatic β cells	
Pancreatic enzyme secretion	
Intestinal secretion	
Platelet release reaction	
Platelet adhesion end plug formation	

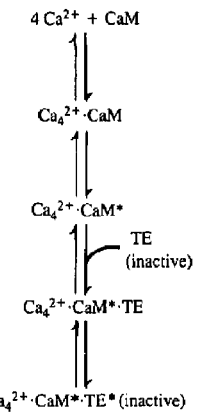
per molecule with affinities in the range of ~0.2–3 μM. Thus in resting cells calmodulin will release its Ca^{2+}, while in excited cells it will be saturated with Ca^{2+}. This provides the basis for calmodulin's roles in biological regulation. The 4 Ca^{2+} binding sites in calmodulin and the individual Ca^{2+} coordinating ligands have been predicted on the basis of the *x*-ray crystallographic structure of the homologous calcium binding protein, carp parvalbumin, and the known amino acid sequence of calmodulin. The calmodulin molecule can be divided into four approximately equal parts, each of which contains one Ca^{2+} binding site.[1] Each of these four domains consists of an α-helical segment, followed by a Ca^{2+} binding loop, followed by another α-helical segment. Not surprisingly, the four domains exhibit a considerable degree of sequence homology.

Mechanism of action

An essential phase in the mechanism of activation of enzymes by calmodulin is a conformational change induced in calmodulin by binding of Ca^{2+}. This results in exposure of a site(s) which can interact with the target enzyme. A considerable amount of evidence has been provided in support of this Ca^{2+}-induced conformational change.[3] Upon binding Ca^{2+}, calmodulin becomes a more compact, globular structure and a hydrophobic site becomes exposed on the surface of the molecule. This hydrophobic site is believed to be involved in the interaction of calmodulin with its target proteins. Figure 2 illustrates the mechanism whereby calmodulin activates most of its target enzymes.

Drug binding

The possibility that certain classes of drugs may exert their pharmacological effects via calmodulin originated with the observation that antipsychotic agents (specifically phenothiazines) inhibit the activities of calmodulin-dependent rat brain adenylate cyclase and cyclic nucleotide phosphodiesterase.[7] The inhibitory effect of antipsychotics on phosphodiesterase was shown to result from binding of the drugs to calmodulin. Antipsychotics are now known to inhibit a number of other calmodulin-dependent enzymes and processes including myosin light chain kinase, phosphorylase kinase and platelet phospholipase A_2. These inhibitory effects can all be explained on the basis of interaction between the drug and the Ca^{2+}-calmodulin complex so as to



*Denotes an active conformation.
CaM = calmodulin.
TE = target enzyme.

FIGURE 2 Mechanism of activation of target enzymes by calmodulin.

block the interaction of calmodulin with its target protein; calmodulin binds 2 moles of trifluoperazine/mole with high affinity (K_d = 1.5 μM) only in the presence of Ca^{2+}. Levin and Weiss[8] made the interesting observation that the phosphodiesterase inhibitory effects of a series of phenothiazines correlated with their clinical effectiveness as antipsychotic drugs. Furthermore, calmodulin-dependent pnosphodiesterase was inhibited by other chemical classes of antipsychotics: butyrophenones, thioxanthenes and diphenylbutylpiperidines. No significant inhibition by antidepressants (amitriptyline and desipramine) or anxiolytics (medazepam and chlordiazepoxide) was observed. Little or no inhibition was seen with known phosphodiesterase inhibitors (theophylline and papaverine) or other centrally acting drugs (amphetamine, (+)-lysergic acid diethylamide, pentobarbital and morphine).

Phenothiazines have been widely used in recent years to implicate calmodulin in various Ca^{2+}-dependent cellular processes. For example, phenothiazines inhibit both myosin phosphorylation and actin-activated myosin Mg^{2+} ATPase activity in smooth muscle actomyosin, and tension development in smooth muscle fibers. These observations

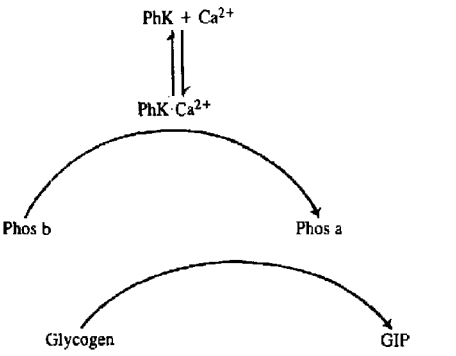


FIGURE 3 Role of calmodulin-dependent phosphorylase kinase in regulation of glycogen metabolism in skeletal muscle.

provided supportive evidence that Ca^{2+}, calmodulin-dependent phosphorylation of myosin plays a central role in the regulation of smooth muscle contraction (see below). Similarly, ATP-dependent transport of Ca^{2+} out of red blood cells by the (Ca^{2+} + Mg^{2+})ATPase was inhibited by phenothiazines and butyrophenones consistent with the proposed role of calmodulin in activation of this Ca^{2+} transport system. Trifluoperazine has been shown to inhibit both glucose-induced insulin release and arginine-induced glucagon release from isolated, perfused rat pancreas. These processes were also inhibited by *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7), a calmodulin antagonist which is chemically unrelated to phenothiazines.

It should be noted, however, that extreme caution must be exercised when interpreting the effects of calmodulin antagonists on biological systems. Phenothiazines do exhibit Ca^{2+}-dependent interaction with calciproteins other than calmodulin, e.g., troponin C and brain S-100b. Furthermore, phenothiazines and butyrophenones have been shown to stabilize membranes in some systems. It is, therefore, not possible to conclude that calmodulin is involved in the regulation of a specific physiological process solely on the basis of the observed effects of antipsychotics on the system. Such an approach can lend only supportive evidence.

Recently, other classes of drugs have been shown to affect calmodulin-dependent processes: R24571 (1-[bis(p-chlorophenyl)methyl]-3-[2,4-dichloro-β-(2,4-dichlorobenzyloxy)phenethyl]imidazolinium chloride), local anaesthetics (dibucaine, QX572, tetracaine and phenacaine) and other drugs with local anaesthetic-like properties (mepacrine, propranolol and proadifen (SKF525A)). In some cases, direct interaction between the drug and calmodulin has been demonstrated, e.g., felodipine (an antihypertensive agent). It is conceivable that potent inhalation agents such as halothane, which is known to cause release of Ca^{2+} from the sarcoplasmic reticulum, may exert their effects by direct interaction with calmodulin.

Calmodulin in skeletal muscle

Calmodulin has, to date, been implicated in three processes in skeletal muscle: (1) regulation of the enzyme phosphorylase kinase, a key enzyme in glycogen metabolism; (2) regulation of the enzyme myosin light chain kinase, which may play a role in modulating actin-myosin interactions in skeletal muscle; and (3) activation of a skeletal muscle sarcoplasmic reticulum (SR) phosphorylating system which may be involved in controlling the release of Ca^{2+} from the SR. The remainder of this article will be concerned with each of these mechanisms in turn.

1 *Phosphorylase kinase*

Phosphorylase kinase functions to mobilize glycogen via the conversion of an inactive form of glycogen phosphorylase b to the active form, phosphorylase a. Phosphorylase kinase is a tetramer of four different subunits, i.e., it has the structure $(\alpha\beta\gamma\delta)_4$. The molecular weights of the individual subunits are: α 145,000, β 128,000, γ 45,000, δ 16,500.[9,10] The molecule, therefore, has an overall molecular weight of 1.3×10^6 daltons. It was known for several years that phosphorylase kinase activity is regulated by Ca^{2+}, but it was only in 1978 that Cohen *et al.*[10] identified the δ subunit as calmodulin; this subunit presumably confers Ca^{2+}-sensitivity to the enzyme. Phosphorylase kinase differs from other calmodulin-dependent enzymes in that the δ subunit (calmodulin) is tightly bound within the phosphorylase kinase complex in the absence of Ca^{2+}. The role of calmodulin-dependent phosphorylase kinase in the regulation of glycogen metabolism in skeletal muscle is summarized in Figure 3.

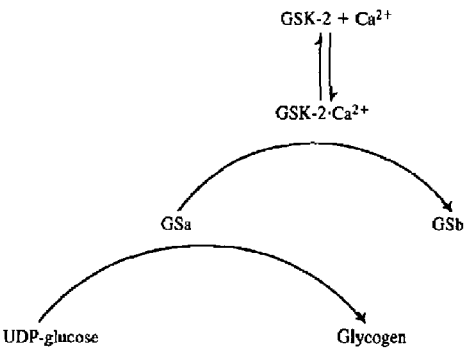


FIGURE 4 Role of calmodulin in inhibiting glycogen synthesis.

Binding of Ca^{2+} to the δ subunit of phosphorylase kinase (PhK) activates the enzyme which then catalyzes the phosphorylation of inactive phosphorylase b (phos b) to active phosphorylase a. The activated phosphorylase initiates the breakdown of glycogen to glucose providing the energy required for muscle contraction.

At the same time, the polymerization of glucose to glycogen is inhibited (Figure 4). Ca^{2+} ions bind to calmodulin which activates the enzyme glycogen synthase kinase-2(GSK-2). This enzyme has recently been identified as phosphorylase kinase itself[11] and it catalyzes the phosphorylation of glycogen synthase (GS), i.e., the conversion of GSa of GSb. In this case the phosphorylation inactivates the enzyme which would otherwise initiate the polymerization of glucose and thereby make it unavailable as a source of energy for muscle contraction. In summary, calcium ions, via calmodulin-dependent activation of phosphorylase kinase, activate glycogen breakdown and inhibit glycogen synthesis and so provide the energy to support muscle contraction.

2 *Myosin light chain kinase*

Skeletal muscle myosin is a hexamer composed of two heavy chains (M_r = 200,000 each) and two pairs of light chains, the so-called alkali light chains, LC_1 and LC_3 (M_r = 22,500 and 16,500, respectively) and the DTNB or phosphorylatable light chain, LC_2. A given myosin molecule of skeletal muscle consists of the two heavy chains, two phosphorylatable light chains, and either two LC_1 alkali light chains or two LC_3 alkali light chains. The myosin molecule contains a globular head region and a long rod-like tail. The tail region is composed exclusively of heavy chains while the globular head contains portions of the heavy chains and both pairs of light chains. The actin binding site and ATPase activity are both associated with the globular head region. The association of the tail portions of many myosin molecules forms the body of the thick filament. One region of the molecule protrudes from the bulk of the thick filament to form a cross-bridge which can interact with the thin actin filaments. This cross-bridge formation between the thick and thin filaments forms the basis of the contractile mechanism, which is believed to occur according to the well-known sliding filament-cross-bridge cycling model.[12,13]

It has been known for many years that the contractile state of skeletal muscle is regulated by the level of sarcoplasmic Ca^{2+} mediated by the troponin system.[2] The possibility that a secondary calcium regulatory system may exist in skeletal muscle arose with the discovery by Perry and co-workers[14] that skeletal muscle contains a Ca^{2+}-dependent myosin light chain kinase (MLCK) which catalyzes the phosphorylation of a specific serine residue on the DTNB light chain of myosin. It is now well-established that MLCK is a calmodulin-dependent enzyme.[15]

While myosin phosphorylation is not a prerequisite for contraction of striated muscles, it is widely believed to be essential for actin-myosin interaction in smooth muscle and various nonmuscle motile systems.[16] The central role of myosin phosphorylation in the regulation of smooth muscle contraction is summarized in Figure 5.

In the resting muscle, the level of sarcoplasmic Ca^{2+} is low ($<10^{-7}$ *M*) and myosin exists in the nonphosphorylated state, in which form it does not interact with actin. The trigger for contraction is an increase in sarcoplasmic Ca^{2+} concentration to ~5 μ*M*. This Ca^{2+} binds to calmodulin which can then interact with the inactive MLCK apoenzyme to form an active ternary complex composed of Ca^{2+}-calmodulin-MLCK. This active enzyme catalyzes the phosphorylation of the 20,000-dalton light chain of smooth muscle myosin. The phosphorylated myosin so formed is then capable of interaction with actin, the result being contraction of the muscle. Relaxation occurs essentially by a reversal of these

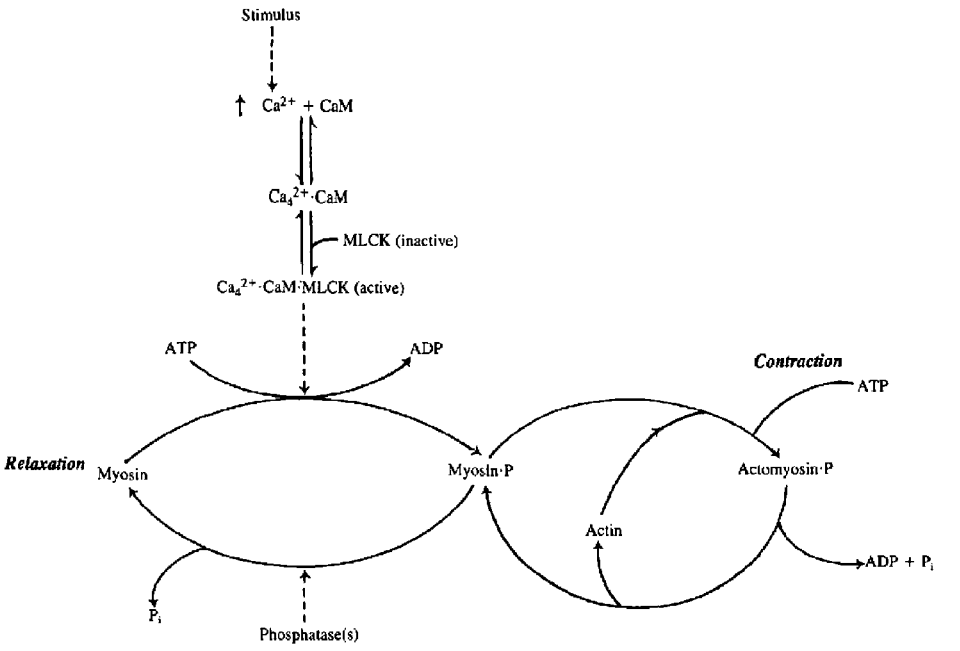


FIGURE 5 Role of myosin phosphorylation in regulation of smooth muscle contraction.

reactions following a return of the sarcoplasmic Ca^{2+} concentration to resting values. MLCK returns to the inactive state so that no further phosphorylation of myosin occurs. The myosin that is already phosphorylated is dephosphorylated by one or more phosphatases and the muscle relaxes.

As mentioned above, while this is believed to be the mechanism of regulation of smooth muscle contraction and nonmuscle motility, it does not represent the primary regulatory system in striated muscles. That role is achieved by the troponin system. It is, however, widely believed that myosin phosphorylation plays a secondary, modulatory role in striated muscles, in some way affecting the kinetics of actin-myosin interaction. Recent work from several laboratories has implicated myosin phosphorylation in two physiological phenomena related to skeletal muscle contraction: (1) post-tetanic potentiation of peak twitch tension; and (2) a decrease in energy utilization during prolonged isometric contractions. These will now be considered in turn.

Post-tetanic potentiation of the peak twitch tension refers to the observation that the maximum tension developed in a muscle increases (or is potentiated) following rapid frequency stimulations. Manning and Stull[17] measured the extent of myosin LC_2 phosphorylation at rest, during an isometric tetanic contraction, and following relaxation. The phosphate content of LC_2 in the resting muscle was 0.1 mole phosphate/mole LC_2. This value did not change significantly during a one-second tetanic contraction. However, within 10–20 seconds following muscle relaxation, the phosphate content had increased to 0.65–0.75 mole phosphate/mole LC_2. There was also a transient increase in peak twitch tension following the tetanic stimulation: a direct correlation was observed between post-tetanic potentiation and myosin LC_2 phosphate content. Subsequently, Klug *et al.*[18] demonstrated that low frequency stimulation, which approximates *in vivo* conditions much more than does a tetanic contraction of rat gastrocnemius, similarly resulted in potentiation of isometric twitch tension.

This potentiation was temporally correlated to the level of myosin LC_2 phosphorylation.

Crow and Kushmerick[19] recently provided evidence that myosin phosphorylation in mouse extensor digitorum longus muscles causes a decrease in the energy cost for isometric force maintenance. The level of myosin LC_2 phosphorylation increased during a prolonged tetanus from ~0.1 mole phosphate/mole LC_2 in the unstimulated muscle to a maximum of 0.55 mole phosphate/mole LC_2. The energy cost for tension maintenance decreased during the tetanus to ~50 per cent of its initial value. A nonlinear correlation was observed between the reduction in energy cost for isometric tetani seen in prolonged stimulations and the extent of myosin LC_2 phosphorylation.

The data of Cooke *et al.*[20] support these findings. They investigated the effect of myosin phosphorylation (using ATP) or thiophosphorylation (using the ATP analog, ATPγS (adenosine 5′-0(3-thiotriphosphate)) on actomyosin ATPase activity in glycerinated rabbit psoas fibers, myofibrils and lightly cross-linked myofibrils. Fifty to eighty per cent thiophosphorylation of myosin was achieved in fibers incubated with MLCK, calmodulin and ATPγS which resulted in an approximately 50 per cent decrease in actomyosin ATPase. The appropriate controls were not thiophosphorylated and exhibited normal ATPase activity. The isometric tension was unaffected by thiophosphorylation. Phosphorylation or thiophosphorylation of myosin in lightly cross-linked myofibrils (which were incapable of shortening) similarly caused an approximately 50 per cent reduction in ATPase activity. The ATPase activity of non-cross-linked myofibrils, on the other hand, was unaffected by myosin phosphorylation. They concluded that myosin phosphorylation in skeletal muscle fibers is a mechanism which modulates the rate of ATP hydrolysis and that the expression of this modulation requires an intact filament array. The possible relationship between the role of myosin phosphorylation in decreasing energy utilization and in post-tetanic potentiation is unknown.

3 *SR protein-phosphorylating system*

Two recent publications[21,22] have implicated calmodulin in a protein-phosphorylating system of skeletal muscle SR. The major findings reported were: (1) calmodulin is associated with SR membranes of fast skeletal muscle; and (2) SR vesicles contain calmodulin-dependent protein kinase activity directed against endogenous substrates (SR proteins). Chiesi and Carafoli[21] demonstrated calmodulin-dependent phosphorylation of SR proteins of M_r = 57,000, 35,000, 20,000, and 13–15,000. The 57,000-dalton protein represented the major phosphorylated substrate, the remainder being relatively minor. Campbell and MacLennan[22] similarly observed a major phosphorylated SR protein of M_r = 60,000 and a minor substrate of M_r = 20,000. Based on the known functions of the SR, one could postulate a role for such a calmodulin-dependent phosphorylating system in regulation of Ca^{2+} uptake, regulation of Ca^{2+} storage, or regulation of Ca^{2+} release from the SR. Calmodulin-dependent phosphorylation of SR proteins was observed to have no effect on Ca^{2+} transport or ATPase activity of SR vesicles. It is, therefore, unlikely that calmodulin is involved in the regulation of Ca^{2+} uptake activity. There is no reason to believe this calmodulin-dependent phosphorylating system is involved in the regulation of Ca^{2+} storage in the SR, a function which has been assigned to calsequestrin. The calmodulin-dependent phosphorylating system may, therefore, be involved in the long-term regulation of Ca^{2+} release from the SR during excitation. Campbell and MacLennan[22] have proposed that calmodulin-dependent phosphorylation of the 60,000-dalton SR protein controls a Ca^{2+} release channel in the SR. When this protein is dephosphorylated, the Ca^{2+} release channel is open. As Ca^{2+} flows out it binds to calmodulin stimulating phosphorylation of the 60,000-dalton protein which closes a gate in the channel. Other Ca^{2+} release channels in the SR membrane are believed to be controlled by proton gradients, the two regulatory mechanisms operating synergistically to control Ca^{2+} release from the SR.

A 22,000-dalton protein, phospholamban, has been identified in cardiac SR membranes and shown to be tightly associated with the Ca^{2+}-transport ATPase. Cardiac phospholamban is phosphorylated by both cyclic AMP-dependent protein kinase and a calcium, calmodulin-dependent protein kinase.[23–25] These phosphorylations have been implicated in the control of the Ca^{2+}-transport ATPase in the heart. Phospholamban has not, however, been demonstrated in skeletal muscle SR.

Concluding remarks

Calmodulin plays a central role in the regulation by Ca^{2+} of diverse biological processes, including muscle contraction. Three calmodulin-dependent enzymes have been recognized in skeletal muscle: phosphorylase kinase, myosin light chain kinase and an SR protein kinase. It is conceivable, and indeed likely, that other calmodulin-dependent enzymes remain to be identified in skeletal muscle. In this context, Grand and Perry[26] have observed two calmodulin-binding proteins (M_r = 150,000 and 61,000) of unknown function in rabbit skeletal muscle. Future efforts will be centred on complete elucidation of the role of myosin phosphorylation and the involvement of calmodulin in Ca^{2+}-induced Ca^{2+} release from the SR, in addition to the identification of new calmodulin-dependent enzymes in skeletal muscle. It is hoped that the acquisition of such knowledge will be accompanied by elucidation of the effects (at the molecular level), and mechanisms of action, of anaesthetics and other agents which may be exerted at the level of calmodulin.

Acknowledgements

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Résumé

Cette revue vise à décrire l'importance de la calmoduline comme médiateur des effets des ions calciques dans les systèmes biologiques, surtout dans le processus de la contraction musculaire squelettique.

La calmoduline est une protéine acide de faible poids moléculaire, liant le calcium, qui agit comme médiateur dans la régulation du calcium pour une variété de processus physiologiques d'organismes eukariotiques. A une basse concentration de calcium libre, celle existant dans le sarcoplasme du muscle au repos, la calmoduline est sous forme libre, non liée au calcium, forme dans laquelle elle ne peut généralement pas réagir avec une protéine cible.

Après un stimulus approprié, la concentration du calcium libre s'élève jusqu'à ce que celui-ci se lie à la calmoduline qui subit alors un changement de configuration la rendant apte à réagir avec une ou des protéine(s) cible(s). Le résultat final de cette interaction protéine-protéine est un effet physiologique: par exemple, le calcium libre se liant à la calmoduline du muscle lisse lui permet de réagir avec la myosine kinase à chaîne légère qui catalyse la phosphorisation de la myosine. Cette réaction amène la contraction du muscle lisse.

Des études récentes ont impliqué la calmoduline dans le contrôle calcique de trois enzymes du muscle squelettique: la phosphorylase-kinase, la myosine-kinase à chaîne légère et la protéine-kinase du réticulum sarcoplasmique. On a démontré que des médicaments dont certains anesthésiques locaux, affectent les processus dépendant de la calmoduline probablement par une inter-réaction avec celle-ci.