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COMMENTARY

CALMODULIN

AN UBIQUITOUS PROTEIN WHICH REGULATES CALCIUM-DEPENDENT CELLULAR FUNCTIONS AND CALCIUM MOVEMENTS

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Calmodulin is a protein of 16,500 daltons which binds Ca^{2+} with high affinity and specificity and is responsible for Ca^{2+}-dependent activation of a variety of enzyme systems involved in a number of fundamental functions in eukaryotic cells. It is ubiquitously distributed in the animal kingdom and now appears to play a role of multifunctional intracellular Ca^{2+} receptor [1, 2].

The influence of the extracellular Ca^{2+} level on heart contraction was reported in 1883 by Ringer [3], and the contractile effect of intracellularly injected Ca^{2+} ions was first demonstrated by Heilbrunn and Wiercinsky in 1947 [4]. After these pioneering studies, many experiments have demonstrated that a transient elevation of intracellular free Ca^{2+} concentration from 10^{-8} or 10^{-7} to 10^{-6} or 10^{-5} M triggers not only muscle contraction but also major phenomena such as secretions, glycogenolysis or cell division. The possibility that the intracellular effects of Ca^{2+} might require the presence of a binding protein was first suggested by Meyer *et al.* [5], who found that Ca^{2+} was necessary for the activity of muscle phosphorylase kinase. Until the two last years, the attention was mostly focused on troponin C, which was discovered in 1969 by Ebashi [6] and which activates the contraction in sarcomeric (skeletal and cardiac) muscles. The more recent discovery of other intracellular calciproteins, that is proteins that bind Ca^{2+} with an affinity consistent with the role of intracellular mediator, provides new insight into molecular events triggered by calcium. These calciproteins include parvalbumin, vitamin D-dependent and vitamin K-dependent Ca^{2+} binding proteins, aequorin, spasmin, protein S-100 and calsequestrin. They were found in some tissues such as intestinal mucosa (vitamin D-dependent protein) or skeletal muscle (parvalbumin) where they play specialized and more or less elucidated roles [7]. Calmodulin is by far the most widely distributed calciprotein. Its structure has been maintained throughout the animal kingdom during evolution [8]. It probably represents the most ancient molecule in the family of Ca^{2+} binding proteins, whereas the other calciproteins appear as specialised molecules which partially lost the potentialities of calmodulin during evolution.

The purpose of this article is to discuss some aspects of the functional role of calmodulin with special attention to excitation–contraction and excitation–secretion coupling. Before examining how calmodulin activity is regulated in cells and what may be its participation in the integration of cellular events, the pathway of the discovery of calmodulin and its physicochemical and biochemical properties will be briefly summarised. It is of interest to point out that calmodulin was discovered during studies of adenosine 3′,5′-monophosphate (cyclic AMP) metabolism and that calmodulin may exert its functions either directly or through the cyclic nucleotide system, including 3′,5′-guanosine monophosphate (cyclic GMP). Furthermore, both calmodulin itself and cyclic nucleotides may regulate Ca^{2+} fluxes. Thus, calmodulin seems to play a pivotal role in concerted cellular regulation by Ca^{2+} and cyclic nucleotides.

Discovery and identification of calmodulin

In 1970, Cheung [9] and Kakiuchi *et al.* [10, 11] reported independently that the Ca^{2+} dependent activation of brain 3′,5′-nucleotide phosphodiesterase requires the presence of a heat stable protein activator, and 2 years later Wolff and Siegel [12] purified a Ca^{2+} binding protein from bovine brain. The activities of 3′,5′-phosphodiesterase from various tissues (see below), of adenylate cyclase from brain [13–15] and of a variety of enzyme systems were then found dependent on Ca^{2+} for optimal activity. The physicochemical and biological properties of the activator protein from all examined tissues were similar if not identical to those of the phosphodiesterase activator from brain. Furthermore, the tissue content in activator protein was greater than necessary for full activation of phosphodiesterase [16, 17]. These findings led to the view that the same ubiquitous protein plays the role of multifunctional regulator in many cells. During the course of identification and purification, this protein was called "Ca^{2+}-dependent regulator" or "modulator", or "troponin C like protein" [18]. It is now commonly known as calmodulin. This term was proposed by Cheung *et al.* [19] because the protein modulates enzyme activities dependent on Ca^{2+} and Ca^{2+} movements.

Finally calmodulin has been purified to homogen-

city from bovine brain [20, 21] and from other tissues, including rat testis and uterus [22, 23] and ram testis [24]. The amino acid compositions and sequences [25] of the proteins from these tissues and from the marine coelenterate *Renilla Reniformis* [26] were found to be very similar if not identical, as well as their physicochemical and biological properties (see below). In the following discussion, the term 'calmodulin' will be used to designate the protein and the term 'calmodulin activity' to designate the biological activity of more or less purified tissue preparations characterized and assayed by their ability to induce Ca^{2+}-dependent enzyme activation (see below).

Physicochemical and biochemical properties

Calmodulin is a protein of 16,500 daltons [25]. Its thermal stability in the presence of Ca^{2+} and its acidic nature have been used in purification procedures, which generally include successively ammonium sulfate fractionation, heat treatment, anion exchange chromatography and gel filtration [20–25].

Calmodulin has 148 amino acid residues and is characterised by the presence of trimethyl-lysine and the absence of cystein and tryptophan [27]. This allows the use of tyrosine fluorescence to study conformational changes induced by Ca^{2+}.

It is well established that calmodulin contains four calcium binding sites [22, 25], with a dissociation constant within the range of 10^{-6} M [28, 29]. Upon Ca^{2+} binding, calmodulin undergoes a conformational change which is necessary for its biological activity [25, 28]. In spite of the significant internal homology between calmodulin and troponin C in and around the Ca^{2+}-binding sites, a recent report suggests that these two proteins exhibit different ion binding properties. The conformational changes revealed by monitoring tyrosine fluorescence appeared to occur on binding of two Ca^{2+} ions per mole calmodulin at high affinity sites I and II [30], whereas troponin C high affinity sites were found located in domains III and IV [31].

The mechanism of activation of enzyme systems by calmodulin is schematically depicted in Fig. 1, where n stands for the number of Ca^{2+} ions bound to one calmodulin molecule. According to the studies mentioned above, $n = 2$. However results from Means and Deadman [2] suggest that fractional occupancy may lead to activation of one or the other of the enzyme systems, since these authors found that 3′,5′-phosphodiesterase could be maximally activated with only one Ca^{2+} bound, whereas microtubule depolarisation required all four sites to be occupied.

The enzyme systems mentioned in Fig. 1 play a role in important cell functions such as regulation of cyclic nucleotide level, contraction of muscle and non muscle cells, phosphorylation of various proteins, intracellular Ca^{2+} level, energetic metabolism, etc. Calmodulin has also been implicated in regulation of other enzyme and cellular processes, such as activation of phospholipase A_2 [43] neurotransmitter release [44] and microtubule disassembly [45], for instance. Some of the calmodulin-dependent enzymes have been purified using affinity chromatography on Sepharose–calmodulin [46]. The evidence suggesting an effect of calmodulin on some of the above mentioned enzyme systems (such as guanylate cyclase, for instance) or cellular processes (for example neurotransmitter release) is still preliminary. It is beyond the scope of this article to discuss all calmodulin-regulated processes. The possible role of calmodulin in a variety of cellular functions has recently been reviewed by different authors [1, 2, 47–49].

It is noteworthy to mention that, at least in some biological materials, calmodulin has been found associated with inhibitor proteins. A thermolabile calmodulin-binding protein has been purified from bovine brain [46, 50, 51]. It has a molecular weight of 85,000 daltons, binds calmodulin in the presence of Ca^{2+}, inhibits activation of phosphodiesterase by calmodulin but does not effect the basal activity of the enzyme. Other proteins inhibit Ca^{2+}-dependent

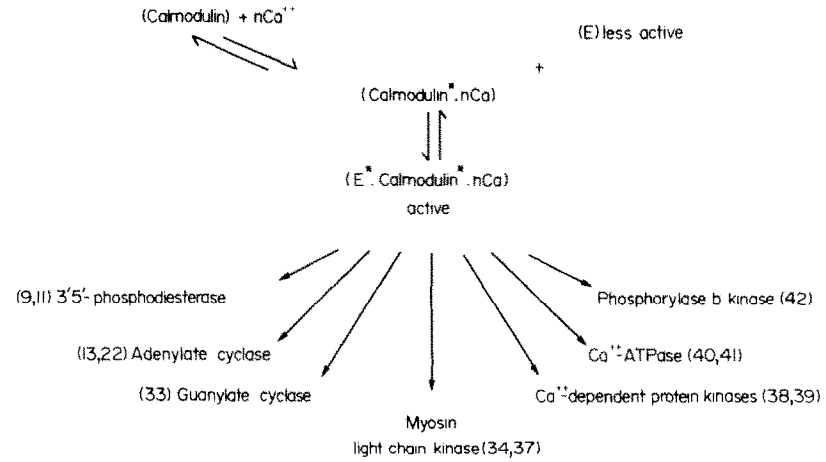

Fig. 1. Schematic representation of the mechanism of activation of some enzyme systems by calmodulin in the presence of Ca^{2+}. E represents the apoenzyme, n the number of Ca^{2+} ions bound per mole calmodulin and the asterisk (*) indicates a new conformation. Numbers in parenthesis indicate selected references.

3′,5′-nucleotide phosphodiesterase from various tissues, but they are thermostable and their molecular weights are different [52–54]. The role of calmodulin inhibitor proteins is unknown.

Calmodulin levels and localisation

Calmodulin activity contained in mammalian tissues have first been estimated using phosphodiesterase activation assay [55, 56]. Activation of turkey gizzard myosin light chain kinase is more specific for calmodulin [57]. More recently, highly specific calmodulin antibodies were produced in several laboratories [58–60] and led to the development of a radioimmunoassay [58]. In all tested tissues and cells, the immuno-activity of calmodulin was found to be greater than the activity measured by enzyme activation. This difference may perhaps be ascribed to the presence in tissue extracts of calmodulin binding proteins competing for Ca^{2+}-dependent enzyme activation, whereas the radioimmuno-assay was performed in the presence of a Ca^{2+} chelator to prevent interference with calmodulin binding proteins.

Studies on calmodulin levels were performed in various cells, using the radioimmuno-assay. As a whole, the results suggest that regulation of calmodulin-activated enzymes is due to changes in Ca^{2+} level rather than to alterations in calmodulin content, since calmodulin levels remained constant under hormonal stimulation, and increased due to a general increase in protein synthesis in virally transformed cells [2].

Immunocytochemical techniques have been used to localise calmodulin in a variety of animal cells. Calmodulin is found throughout the cytoplasm, but appears to be excluded from the nucleus [61]. It is associated with various cellular structures, especially actin containing filaments, microtubules in mitotic cells [2], and various membranes [62] including postsynaptic membranes where it is localised on the inner surface [63]. This association with multiple cellular structures is presumably related to multiple roles of calmodulin in the regulation of actomyosin ATPase activity, in the assembly–disassembly of microtubules, in synaptic transmission, and in other cell functions.

Inhibitors of calmodulin activity

Levin and Weiss [64], studying inhibition of brain cyclic nucleotide phosphodiesterase by phenothiazine neuroleptics, found that trifluoperazine and related phenothiazine derivatives are potent inhibitors of the Ca^{2+}-sensitive phosphodiesterase, at a concentration which has no influence on the basal activity of the enzyme in absence of Ca^{2+}. They subsequently demonstrated that these drugs bind to calmodulin in its Ca^{2+}-activated form (dissociation constant $K_D = 10^{-6}$ M, in the case of trifluoperazine) and inhibit its biological properties [65, 66]. These findings were confirmed by other authors [67] and extended to various antipsychotic agents [68] and to compounds such as *N*-(6-aminohexyl)-5-chloro-1-naphthalene-sulphonamide (W7) [69]. The antipsychotic effect of neuroleptics is unrelated to the inhibition of calmodulin activity [70], but trifluoperazine and other inhibitors of calmodulin activity have been used to inhibit and identify the role of calmodulin-regulated reactions in various cells [49, 69, 71, 72]. It should be mentioned, however, that the tricyclic structure of the phenothiazine compounds may also interact with hydrophobic environment [70]. It is therefore important to ascertain whether the effects of these compounds on the test systems are due to their specific action on calmodulin, as recently stated by Cheung [1].

Calmodulin and cyclic nucleotide metabolism

As mentioned above, calmodulin affects the synthesis as well as the hydrolysis of cyclic nucleotides in acellular systems.

Different molecular forms of phosphodiesterase have been physically separated from practically all mammalian tissues and characterized by their substrate specificity, their kinetic properties and their sensitivity to activators and inhibitors. Some of them can be activated by calmodulin and others cannot. The phosphodiesterase forms have been reviewed by Wells and Hardman [73]. These authors pointed out the difficulties to summarise the results without oversimplification, in view of the extreme diversity of the methods employed and of the heterogeneity of most tissues. One of the major forms found in many tissues (i.e. brain, heart, smooth muscles, etc.) can be activated by calmodulin. It has recently been purified to chromatographic and electrophoretic homogeneity [74]. This form has a greater maximal velocity (V_{max}) and a higher Michaelis constant (K_m) for cyclic AMP than for cyclic GMP (40–100 and 2–4 μM, respectively). For this reason, it is described as the 'high-K_m' phosphodiesterase. The effect of calmodulin is to increase its V_{max} on cyclic AMP and, to a lesser extent, on cyclic GMP. The role of this enzyme, which is soluble, is unknown. According to a theoretical analysis based on the assumption that adenylate cyclase and low-K_m phosphodiesterase would be bound to the plasma membrane whereas high-K_m phosphodiesterase would be cytosolic, the high-K_m enzyme would be ineffective to regulate the cyclic AMP concentration near the membrane, but would be effective to regulate the cyclic nucleotide level further inside the cell. It would then generate a concentration gradient of cyclic AMP from the plasma membrane to the interior of the cell [75]. If this hypothesis is true, the effect of activation of the high-K_m phosphodiesterase by calmodulin might be to oppose to an increase in cyclic AMP concentration inside the cell, without preventing a local increase in cyclic AMP concentration near the membrane, (where adenylate cyclase is stimulated and calcium simultaneously released under the influence of hormones and neuromediators). Association between adenylate cyclase stimulation and elevation in intracellular Ca^{2+} level has been described in a number of circumstances [76, 77], such as beta-adrenergic heart stimulation [78]. It should be mentioned that other calmodulin-sensitive phosphodiesterase forms have been described in some tissues. They differ from the previous one by their substrate specificity and kinetic properties. One of them hydrolyzes identically both cyclic AMP and cyclic GMP and has a low-K_m value for both substrates [79].

Calmodulin was also found to stimulate Ca^{2+}-dependent adenylate cyclase in membrane prep-

arations from brain [13, 32] and other tissues [80]. The mechanism by which calmodulin activates adenylate cyclase and the question whether hormone and neurotransmitter receptors are coupled to a Ca^{2+}-dependent or to a Ca^{2+}-independent adenylate cyclase have been discussed in recent reviews [47, 48]. There are conflicting reports on the Ca^{2+} requirement for dopamine stimulation of striatal adenylate cyclase. It has been suggested by Costa's group that sensitivity of adenylate cyclase to the neurotransmitter is regulated by the level of membrane-bound calmodulin [81] and that cyclic AMP-dependent phosphorylation of a membrane protein produces the release of calmodulin from the membrane to the cytosol [82]. This hypothesis rested on the finding that incubation of membranes with cyclic AMP, cyclic AMP-dependent protein kinase and ATP resulted in translocation of calmodulin activity from the membrane to the supernatant and in a parallel decrease in dopamine sensitivity of the adenylate cyclase. From these results Costa's group also suggested that the increase in cyclic AMP level produced by the neurotransmitter and the subsequent translocation of calmodulin decreases the synthesis of the cyclic nucleotide by adenylate cyclase and increases its hydrolysis by a cytosolic phosphodiesterase. This mechanism may lower the cyclic AMP level, thereby allowing calmodulin to reassociate to the membranes. The assumptions inherent to this hypothesis have been recently discussed elsewhere [47].

According to the model described above, changes in the compartmentalisation of calmodulin may also account for receptor-mediated changes in adenylate cyclase sensitivity to neurotransmitters. Chronic blockade of dopamine receptors by neuroleptics causes both supersensitivity of striatal adenylate cyclase to dopamine and an increase in calmodulin activity in the membrane [82]. Conversely, persistent stimulation by dopaminergic agonists produces an increase in calmodulin associated with cytosolic phosphodiesterase [83].

The effects of calmodulin on cyclic GMP metabolism seems to parallel its effects on cyclic AMP, but are less documented. As mentioned above, calmodulin activates a cytosolic phosphodiesterase which displays a low-K_m value (micromolar) for cyclic GMP. In view of the estimated intracellular concentration of cyclic GMP, which is in the 10^{-7} M range [84], this enzyme may be effective in lowering cyclic GMP as well as cyclic AMP inside the cell. One would expect this mechanism to decrease the intracellular level of cyclic GMP in the presence of sufficient intracellular Ca^{2+} concentrations, for instance those which produce muscle contraction. This phenomenon has not been observed in many experiments in which cyclic nucleotide levels were monitored during contraction [85]. On the contrary, the level of cyclic GMP was reported to increase in a Ca^{2+}-dependent fashion during contraction of some smooth muscles [86]. This may perhaps be due to the Ca^{2+}-dependent stimulation of guanylate cyclase. Calmodulin activation of this enzyme has been reported [33].

In conclusion, the role of calmodulin in regulating cyclic nucleotide metabolism seems important, but remains to a large extent unclear. Calmodulin-dependent stimulation of both synthesis and degradation of cyclic nucleotides may lead to rapid and long term changes in their local (compartmentalised ?) intracellular concentrations. These changes may in turn cause translocation of calmodulin within the cell and variations in its sensitivity to neurotransmitters.

Calmodulin-dependent and cyclic AMP-dependent phosphorylations

It is well established that in eukaryotes cyclic AMP exerts intracellular effects through the activation of cyclic AMP-dependent protein kinases [87]. It has been known for several years that some cyclic AMP regulated functions such as glycogen metabolism [5] or smooth muscle contraction [88] require Ca^{2+}, and it has recently been recognised that in some cases Ca^{2+} exerts its role through a calmodulin dependent protein phosphorylation. Examples of such mechanisms are provided by the activation of phosphorylase kinase [42] in the case of glycogen metabolism and by the activation of myosin light chain kinase [36] in the case of smooth muscle contraction. The latter example will be discussed below to illustrate concerted regulation of a cell function by calmodulin and cyclic AMP.

Results obtained in the chicken gizzard suggest that an increase in free Ca^{2+} concentration in smooth muscle cells activates a kinase which phosphorylates the 20,000 daltons light chain myosin and thereby allows the actin–myosin interaction [89, 90]. Recently, the activator protein of myosin light chain kinase was identified to be calmodulin [34–37] and was reported to be a component of smooth muscle kinase. Thus, calmodulin seems responsible for the triggering action of Ca^{2+} on contraction in chicken gizzard. Results from Hidaka *et al.* suggest that the same mechanism operates in other smooth muscles in which selective inhibitors of calmodulin inhibit contraction and actomysin ATP-ase activity and superprecipitation [69, 91]. Altogether these findings suggest that in vertebrate smooth muscles calmodulin operates as a co-factor of myosin light chain kinase to induce myosin phosphorylation and contraction. The same mechanism seems to be involved in non muscle cells such as platelets, in which Ca^{2+}-calmodulin-dependent myosin light chain phosphorylation causes contraction and mediates secretion [72]. Thus, the role of calmodulin in regulating the actomyosin ATPase in smooth muscle and in non muscle cells appears comparable to that of troponin C in regulating the actomyosin ATPase of sarcomeric (skeletal and cardiac) muscles. Interestingly, cyclic AMP-dependent phosphorylation of chicken gizzard myosin light chain induces an inhibition of this enzyme by a decreased affinity for calmodulin in presence of Ca^{2+} [92]. It was recently reported that cyclic AMP dependent phosphorylation inhibits Ca^{2+}-dependent activation of porcine carotid actomyosin ATPase and also inhibits contraction of chemically skinned smooth muscle fibres of guinea pig taenia coli in conditions where Ca^{2+} concentration is held constant [93]. Thus, inhibition of myosin light chain kinase might account for the relaxing action of cyclic AMP in smooth muscle. This might explain, for instance, the relaxing action of beta-

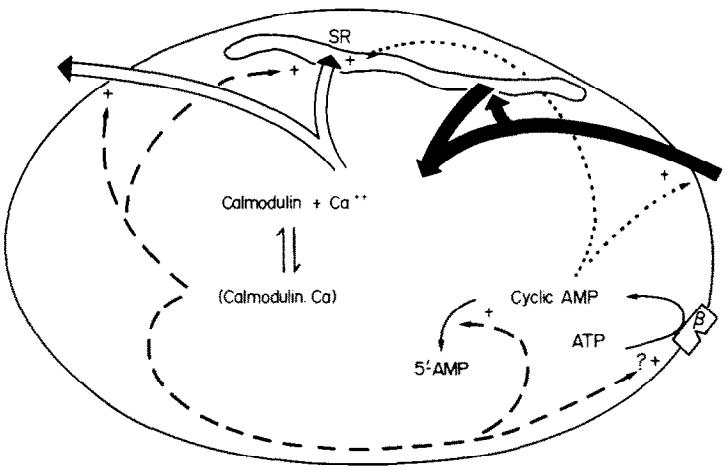

Fig. 2. Schematic diagram of presumed reciprocal interactions between Ca^{2+} and cyclic AMP levels in heart cells. Effects of the complex (calmodulin–Ca) (– – – –) and of cyclic AMP-dependent phosphorylations (...) are referred to as + since they cause activation of the indicated function. The question mark denotes an effect which has not been reported in heart cells but which has been studied in other cells. SR stands for sarcoplasmic reticulum.

adrenergic and other adenylate cyclase stimulating agents, since, in this case, relaxation occurs without any increase in Ca^{2+} efflux [94]. One may speculate that the same mechanism might also account for the functional antagonism between Ca^{2+} and cyclic AMP in some cell functions such as stimulus-coupled secretion processes in platelets [72] and in other cells [95]. At the present time, however, the experimental evidence in favour of this suggestion is still lacking.

There are other cell functions in the regulation of which Ca^{2+}-calmodulin and cyclic AMP cooperate positively rather than negatively as in the cases of functional antagonism mentioned above. An example of this type of interaction between Ca^{2+} and cyclic AMP is provided by the regulation of Ca^{2+} transport in heart sarcoplasmic reticulum. This example is discussed below.

Calmodulin in the regulation of intracellular calcium

In addition to binding a large proportion of the total Ca^{2+} intracellularly released upon stimulation and to mediate the intracellular effects of Ca^{2+}, calmodulin may act to decrease Ca^{2+} level. This action would constitute a self regulating mechanism for returning to a low steady state Ca^{2+} concentration and terminating the effects of Ca^{2+}.

Calmodulin has been reported to stimulate a plasma membrane Ca^{2+} ATPase which is coupled to an active Ca^{2+} pump that extrudes Ca^{2+} from the cytoplasmic compartment [40, 41, 96, 97]. Other mechanisms for restoring the low intracellular Ca^{2+} level have been described, particularly a $Na^+:Ca^{2+}$ exchange. It would be important to know if calmodulin affects this exchange and what is the relative participation of the various mechanisms to Ca^{2+} extrusion in various cell types. In the squid axon, the Ca^{2+} pump seems predominant in the physiological intracellular Ca^{2+} range [98].

In heart muscle cells, calmodulin stimulates Ca^{2+} transport in sarcoplasmic reticulum, as a consequence of the activation of a membrane bound protein kinase that phosphorylates phospholamban [99]. It has been known for several years that phospholamban can also be phosphorylated at a different site by a cyclic AMP-dependent protein kinase, and this phosphorylation also increases Ca^{2+} transport [100]. Thus, in heart cells both calmodulin and cyclic AMP cooperate positively to decrease intracellular free Ca^{2+} concentration. This mechanism may account for the reduction of cardiac systole which characterizes the effects of beta-adrenergic agents, together with a simultaneous increase in force of contraction. Cyclic AMP also seems to be implicated in the latter effect, since cyclic AMP-dependent phosphorylation of a plasma membrane protein [101] may be responsible for the increase in Ca^{2+} conductance that occurs under beta-adrenergic stimulation of heart cells [102]. This would allow more Ca^{2+} to penetrate the cell and to induce a larger release of Ca^{2+} from sarcoplasmic reticulum [103], thereby increasing the force of contraction.

The complex interactions between Ca^{2+} and cyclic AMP are tentatively illustrated in Fig. 2 in the case of beta-adrenergic stimulation of heart cells. Cyclic AMP produced by activation of plasma membrane adenylate cyclase may increase Ca^{2+} influx. Alternatively, beta-adrenoceptors may be coupled to a Ca^{2+} channel in the membrane. Whatever the mechanism, intracellularly released Ca^{2+} binds to troponin C (not shown) and to calmodulin, thereby producing contraction and activation of enzyme systems which contribute to the intracellular response. The complex Ca–calmodulin also enhances both cyclic AMP breakdown in the cytoplasm and Ca^{2+} pumps lowering free intracellular Ca^{2+} level. In addition, local activation of plasma membrane bound calmodulin may amplify the cyclic AMP response at its beginning, but this mechanism has not been reported in the heart and, in the cells where it has been found, its amplitude seems to decrease with time [81, 82] through an autoregulatory process. Independently of the presence or the absence of the latter mechanisms in the heart, calmodulin can modulate cyclic nucleotide levels in this tissue and regulate Ca^{2+}

fluxes and transport either directly or through the cyclic AMP system.

Conclusion

The concept that calmodulin plays the role of multifunctional Ca^{2+} receptor has gained considerable experimental basis during the past two years [103]. It appears that, together with cyclic nucleotides, calmodulin integrates the regulatory processes allowing responses of eukaryotic cells to extracellular signals identified by plasma membrane specific receptors. Binding of hormones or neurotransmitters to these receptors induce transient elevations of intracellular Ca^{2+} and cyclic AMP levels. On the one hand, calmodulin cooperates with cyclic AMP to regulate cell functions, for instance through the activation of protein kinases. In this respect, cyclic AMP-dependent phosphorylation seems to modulate the all or none effects produced by binding of Ca^{2+} to calmodulin. Activation of phosphorylase kinase and sarcoplasmic reticulum Ca^{2+} pump provide examples of this type of mechanism. On the other hand, calmodulin activates enzyme systems which decrease both Ca^{2+} and cyclic AMP levels. This appears as autoregulatory mechanisms which are prominent features of the Ca^{2+} and cyclic AMP intracellular signals. The coupling between the two second messengers was foreseen by Rasmussen [76], but its mechanism is only beginning to be understood.

Our knowledge of the activation of many enzyme systems by calmodulin is very recent, and there are still considerable gaps in our understanding of the role of calmodulin in many tissues. Many of the enzymes systems involved in calmodulin effects have only been partially purified. Even when one understands the interaction of calmodulin with these enzyme systems, it will be necessary to elucidate the role of calmodulin within cells on a quantitative and temporal basis. At the present time the implication of calmodulin in many cell functions is mostly supported by indirect evidence obtained in acellular systems, and the model discussed above in the case of heart cells is only tentative and probably oversimplified. Conceivably, partial or total saturation of the four binding sites of calmodulin depending on Ca^{2+} local concentration might cause activation of one system or the other. In this respect, compartmentalisation of Ca^{2+} concentration within cells might be particularly important. Further insight into these phenomena is necessary to understand the role of calmodulin which already appears as essential in cellular regulation.

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