Calcium Transport Systems in Cardiac Sarcolemma and Their Regulation by the Second Messengers Cyclic AMP and Calcium-Calmodulin*

J. M. J. LAMERS

Department of Biochemistry I, Erasmus University, P. O. Box 1738, 3000 DR Rotterdam, The Netherlands

Abstract. The purpose of this survey is to describe the importance of cyclic AMP and Ca^{2+}-calmodulin as mediators of the effects of β-adrenergic agonists on cardiac sarcolemma. First, the basic characteristics of the three sarcolemmal Ca^{2+}-transporting systems, the slow Ca^{2+} channel, the Ca^{2+}-pumping ATPase and the Na^+/Ca^{2+} antiporter, are described. These different pathways for in- and outflux of Ca^{2+} play a crucial role in the excitation-contraction coupling and relaxation of heart muscle. Catecholamines in the myocardium cause an increase in the rate and extent of tension development during systole, and in the rate of relaxation during diastole. These functional changes may largely be brought about by cyclic AMP-induced phosphorylation of membrane proteins that increases both the probability of opening the slow Ca^{2+} channels and the rate of Ca^{2+} pumping ATPase. It is generally believed that the effects on Ca^{2+} transport systems are due to direct actions of β-adrenergic agonists leading to an increased cytosolic Ca^{2+} level during systole. Indirectly, an increase in systolic Ca^{2+} can amplify the primary effect of catecholamine on the Ca^{2+} pumping ATPase and probably also on the Na^+/Ca^{2+} antiporter through Ca^{2+}-calmodulin-dependent phosphorylation of membrane proteins. The intimate involvement of calmodulin in the operation of several sarcolemmal Ca^{2+}-transporting systems is discussed in the light of the unknown mechanism of action of the so-called Ca^{2+} channel blockers, a class of drugs that have a very important potential to provide information on the fundamental reaction steps in excitation-contraction coupling. Some of these drugs are potent inhibitors of Ca^{2+}-calmodulin-regulated enzymes.

Key words: Heart muscle — Sarcolemma — Ca^{2+}-pumping ATPase — Slow Ca^{2+} channel — Na^+/Ca^{2+} antiporter — Phosphorylation — Cyclic AMP — Calmodulin — Catecholamines — Ca^{2+} channel blockers

* Presented at the 2nd International Symposium on Membrane Receptors and Ionic Channels, May 21—26, 1984, Smolenice, Czechoslovakia

Introduction

Mammalian cardiac muscle contains at least three systems for the transport of Ca^{2+} across the sarcolemma, a voltage-dependent slow Ca^{2+}channel (Reuter 1983), a Ca^{2+}-stimulated Mg^{2+}-ATPase (Hui et al. 1976; Sulakhe and St. Louis 1976; Wollenberger and Will 1978; Lamers et al. 1980; Caroni and Carafoli 1981a) and an electrogenic Na^+/Ca^{2+} antiporter (Pitts 1979; Reeves and Sutko 1980; Miyamoto and Racker 1980; Caroni et al. 1980; Lamers and Stinis 1981). The Ca^{2+} concentration in the extracellular fluid is some 10,000 times higher than that of the cytosol under resting conditions of the heart muscle. Depolarization of the sarcolemma opens slow channels through which Ca^{2+}, that penetrates by this manner into the cytosol,will activate troponin C either directly or indirectly by producing a Ca^{2+}-induced Ca^{2+} release (Fabiato and Fabiato 1978) from the intracellular stores in the sarcoplasmic reticulum. During the plateau phase of the action potential extracellular Ca^{2+} can also enter the cytosol via the Na^+/Ca^{2+} antiporter. It is allowed to operate in the Na^+ out/Ca^{2+} in direction due to reduction of both the membrane potential and the Na^+ gradient. This Ca^{2+} influx will bear a current although its direction will be opposite to that produced by Ca^{2+} flowing through the slow channel. Therefore, to what extent Ca^{2+} entering by Na^+/Ca^{2+} antiporter contributes to the total Ca^{2+} influx during the plateau phase of cardiac action potential can hardly be estimated by electrophysiological techniques. After contraction a great part of Ca^{2+} bound to the myofibrillar proteins will leave the myocyte again by energy-requiring processes: in exchange for extracellular Na^+ by the Na^+/Ca^{2+} antiporter and ejected by the Ca^{2+}-calmodulin-activated Ca^{2+} pumping ATPase. During relaxation of the muscle another part of troponin C-bound Ca^{2+}, as it was released before from internal storage sites,will again be taken up by the action of the sarcoplasmic reticulum Ca^{2+} pumping ATPase. The extent of myocardial contraction and the velocity of muscle relaxation depends on the intracellular Ca^{2+} concentration in the cytosol during the systole and on the rate of the removal of Ca^{2+} from the myofibrils, respectively.

It is well known that both contraction force and relaxation rate of the heart muscle are hormonally regulated. In particular; β-adrenergic agonists not only increase contraction force but also enhance the rate of relaxation. A considerable number of papers have indicated that cyclic AMP as intracellular messenger of catecholamines affects physiological target systems (enzymes and transporters) by stimulating a protein kinase (for reviews, see Sulakhe and St. Louis 1976; Wollenberger and Will 1978; Tada and Katz 1982; Reuter 1983; England 1983). The changes are largely brought about by cyclic AMP-induced phosphorylation of sarcolemmal, cytosolic, contractile and sarcoplasmic reticular proteins. Now it has become apparent that cyclic AMP-induced phosphorylation reactions do regulate force and shortening properties of cardiac muscle by manipulating intracellular

concentration of Ca^{2+} ions. This paper gives an outline of the present consensus on how cyclic AMP does so by modulating sarcolemmal Ca^{2+} transporting systems. Another concept that evolved quite recently and with which will be dealt here is that Ca^{2+}-calmodulin dependent phosphorylation processes may have a role in amplifying the catecholamine response (Haiech and Demaille 1983). On the other hand, these reactions may have an essential role in protecting the myocyte against Ca^{2+} overload originating from β-adrenergic overstimulation (Louis and Maffit 1982). In the myocardium, pharmacological agents known under the groupnames: β-blockers and Ca^{2+} channel blockers share a common property, namely both drugs reduce Ca^{2+} entry during the plateau phase of cardiac action potential (Nayler et al. 1984). However, it has been shown that the mechanisms of their inhibition are quite different. One group of agents apparently interacts with the cyclic AMP-mediated events, and the other may either interact directly with the slow Ca^{2+}-channel or with Ca^{2+}-calmodulin-dependent regulation of the Ca^{2+} channel. The second group of drugs may also affect the Ca^{2+}-pumping ATPase or the Na^+/Ca^{2+} antiporter. Before describing these mechanisms, though, it is useful to start with a description of the phosphorylation sites in cardiac sarcolemma and the ultimate effect of phosphorylation on the functioning of Ca^{2+} transporters.

Phosphorylation of Sarcolemma Proteins in vivo and in vitro

Several membrane proteins have previously been described in sarcolemma that were substrates for an intrinsic cyclic AMP-dependent protein kinase with apparent M_r values of 53,000; 26,000; 15,000 and 9000 (Lamers and Stinis 1980; Lamers and Weeda 1984). The protein of M_r 9000 appears to be a substrate protein for an intrinsic Ca^{2+}-calmodulin-dependent protein kinase as well, the latter being in addition able to phosphorylate a protein of M_r 55,000 (Lamers et al. 1981). This characteristic phosphoprotein pattern of sarcolemma has been described in several other reports (Jones et al. 1981; Rinaldi et al. 1982; Flockerzi et al. 1983). It should be noted that the sarcolemmal membrane proteins before being processed for electrophoresis, are treated with SDS at 95 °C, which has been shown (Lamers et al. 1981; Lamers and Weeda 1984; Rinaldi et al. 1982) to be a prerequisite for the detection of the M_r 9000 protein. The protein would otherwise be present in the M_r 23—24,000 region of the gel. This was shown to be due to the fact that the M_r 9000 protein is a monomer derived from the 23—24,000 phospholamban-like protein (Fig. 1) which apparently is composed of several electrophoretically indistinguishable monomers M_r (9—11,000 each), containing a different site for phosphorylation by cyclic AMP and Ca^{2+}-calmodulin-dependent protein kinase (Wegener and Jones 1984). The latter results were obtained with the M_r 9000 protein present in cardiac sarcoplasmic reticulum where it originally

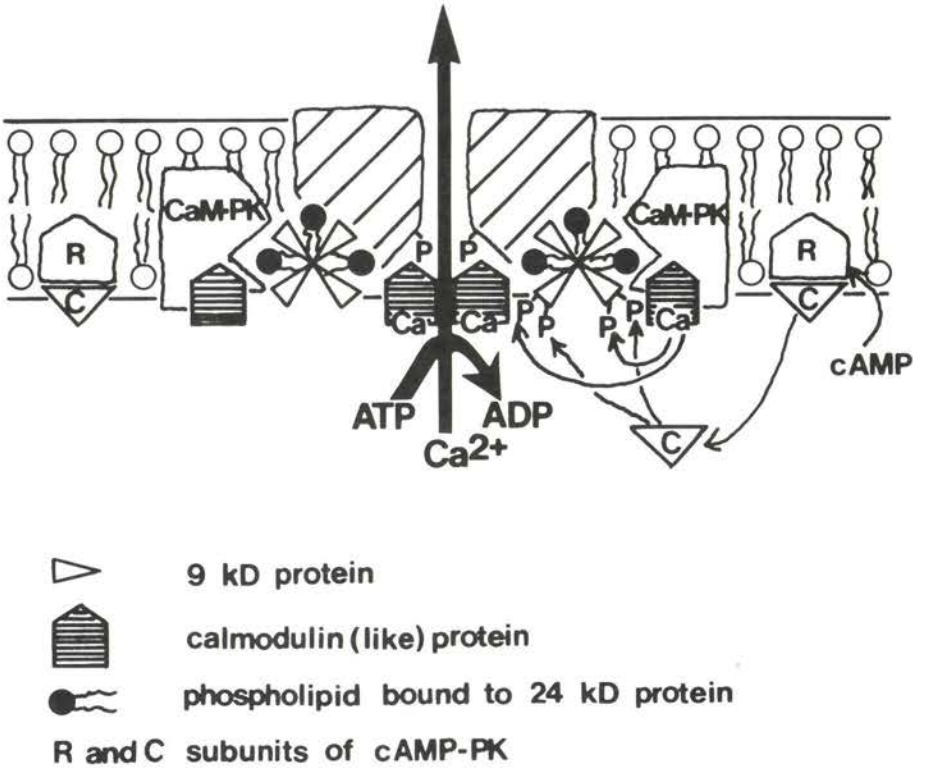

Fig. 1. Schematical presentation of the regulation of Ca^{2+} pumping ATPase localized in cardiac sarcolemma. CaM-PK is an abbreviation for Ca^{2+}-calmodulin-dependent protein kinase. Introduction of phosphate groups into serine side residues of 9 kD subunit proteins has been shown to induce an increase in the rate of Ca^{2+}-pumping ATPase.

was discovered in the high molecular weight form (M_r 22,000) (see Tada and Katz 1982). Those two different sites on the sarcolemmal M_r 9000 protein, phosphorylated by cyclic AMP, and Ca^{2+}-calmodulin-dependent protein kinase, were previously demonstrated by following the time-course of ^{32}P incorporation in isolated sarcolemmal vesicles (Lamers et al. 1981). It is interesting to note that, very recently, phospholamban-like protein in sarcolemma has also been found to be a substrate protein for an intrinsic Ca^{2+}-activated phospholipid-dependent protein kinase (Iwasa and Hosey 1984). Phosphorylation of the phospholamban-like protein and the M_r 15,000 protein in intact heart can be made apparent by perfusion of $^{32}P_i$-preperfused hearts with added catecholamine (Huggins and England 1983; Walsh et al. 1979). In the latter experiments it was shown that the increase in ^{32}P incorporation into the M_r 9000 protein preceded the inotropic response by 5—10 s. No data were presented concerning the time at which the

heart showed an increased rate of relaxation. The data support the hypothesis that phosphorylation of the M_r 9000 and 15,000 protein in cardiac sarcolemma mediate changes in Ca^{2+} transporting properties induced by catecholamines.

The possibility that the phospholamban-like protein in sarcolemma represents a sarcoplasmic reticulum contaminant has been proposed by Manalan and Jones (1982) and Katz et al. (1983). However, this possibility has been ruled out by other recent reports (Lamers and Stinis 1983; Flockerzi et al. 1983; Huggins and England 1983; and Capony et al. 1983), which demonstrated the abundant presence of M_r 9000 protein molecules in purified sarcolemmal vesicles.

Regulation of the Sarcolemmal Ca^{2+} Pumping ATPase by Phosphorylation

Studies with heart sarcolemmal vesicles have demonstrated that Ca^{2+}-pumping ATPase is modulated either by cyclic AMP- or Ca^{2+}-calmodulin-dependent phosphorylation (Sulakhe and St. Louis 1976; Hui et al. 1976; Wollenberger and Will 1978; Ziegelhöffer et al. 1979; Lamers et al. 1981; Caroni and Carafoli 1981b; Vetter et al. 1982). The phosphorylation, leading to activation of the Ca^{2+} pump, is catalyzed by cyclic AMP-dependent protein kinase, either intrinsically present or exogenously added (Fig. 1). The same is true for the activation through Ca^{2+}-dependent phosphorylation. In order to add the Ca^{2+}-dependent protein kinase exogenously, Ca^{2+}-calmodulin-dependent phosphorylase kinase was used (Sulakhe and St. Louis 1976; Caroni et al. 1983). In most cases, the activation of the Ca^{2+} pump originates from a change in the V_{max} and $K_{0.5}$ for Ca^{2+}. In contrast, in our studies on dog heart sarcolemma (Lamers et al. 1981) cyclic AMP-dependent phosphorylation increased the affinity for Ca^{2+} ions, and no effects could be shown if membranes were phosphorylated with Ca^{2+}-calmodulin-dependent protein kinase. The specificity of the cyclic AMP-induced activation of the Ca^{2+} pumping ATPase could be demonstrated by its sensitivity to less than 10 μg/ml of heat-stable protein kinase inhibitor. In this respect it is important to note that in another study a 10-fold higher concentration of the protein kinase inhibitor was needed to partially block the cyclic AMP-induced increase in V_{max} rate of the Ca^{2+}-pumping ATPase (Caroni and Carafoli 1983). At present it is not clear which phosphoprotein is the direct activator of the Ca^{2+}-pumping ATPase. In the light of the dual activation by cyclic AMP and Ca^{2+}-calmodulin it seems likely that the M_r 9000 protein is involved (Fig. 1). The protein has been shown to be present in more than 5:1 stoichiometry with the monomeric ^{32}P intermediate of the Ca^{2+} pumping ATPase (Lamers and Stinis 1983).

A comparison of Ca^{2+} activation patterns between the sarcolemmal Ca^{2+} pumping ATPase and Ca^{2+} calmodulin-dependent protein kinase was made previously (Lamers et al. 1981). It can be concluded from these results that rather

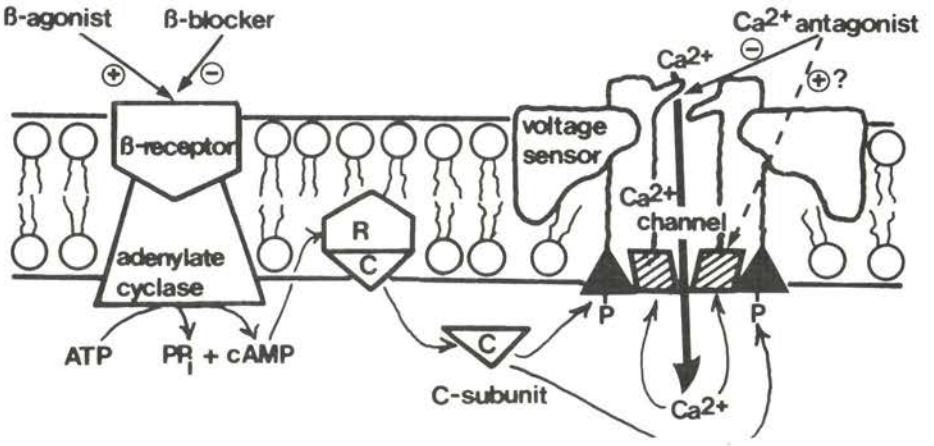

Fig. 2. Regulation of the cardiac slow Ca^{2+} channel by β-receptor stimulation. Interaction sites of β-blockers and Ca^{2+} antagonists are also indicated. C-subunit stands for the active component of cyclic AMP-dependent protein kinase. The phosphorylatable subunit (full triangles) and Ca^{2+}-dependent closing gate protein (stripped squares) are presented at the cytosolic part of the transmembrane Ca^{2+} channel protein.

high concentrations of Ca^{2+} are needed to activate half-maximally Ca^{2+}-calmodulin-dependent protein kinase. Calmodulin-dependent protein kinase thus will have a minimal activity at 0.3 μmol/lCa^{2+}, a level at which the cyclic AMP-dependent protein kinase-induced activation is maximal.

Regulation of the Sarcolemmal Slow Ca^{2+} Channel by Phosphorylation

Reuter (1983) has shown that treatment of isolated heart cells with catecholamines increases the probability of opening of the slow Ca^{2+} channels. Therefore it was postulated that one of the targets of cyclic AMP-dependent protein kinase should be a component of the slow Ca^{2+} channel, the phosphorylation of which would facilitate voltage-dependent opening of the Ca^{2+} channel (Fig. 2). This postulate was supported by experiments in which a purified catalytic subunit of cyclic AMP-dependent protein kinase was injected into isolated heart cells, resulting in an increased amount of Ca^{2+} entering the cells during depolarization (Osterrieder et al. 1982). Rinaldi et al. (1982) studied Ca^{2+} channel activity in isolated sarcolemmal vesicles and observed an increase in depolarization-induced Ca^{2+} uptake if the vesicles were phosphorylated by cyclic AMP-dependent protein kinase. However, this *in vitro* measurable Ca^{2+} channel requires an outwards directed Na^+ gradient for activity, and it is relatively insensitive to Ca^{2+} entry blockers (Philipson 1983). These properties are inconsistent with known properties

of the cardiac muscle Ca^{2+} channel. Also, other evidence has raised serious concerns regarding the interpretation of the „passive" flux of Ca^{2+} ions through a channel (Katz et al. 1983). At any rate, Flockerzi et al. (1983) have shown recently that a very small portion of the depolarization-induced Ca^{2+} uptake might indeed represent a flux through open voltage-operated channels, but no effect of cyclic AMP-dependent phosphorylation could be detected.

Regulation of the Na^+/Ca^{2+} Antiporter by Phosphorylation

In heart, one may expect that Ca^{2+} enters the cell by the Na^+/Ca^{2+} exchanger during the depolarization phase and leaves it during repolarization of the plasma membrane (Mullins 1979). Thus, cyclic AMP-dependent regulation of the Na^+/Ca^{2+} antiporter alone by phosphorylation would be the least complicated mechanism for explaining the β-adrenergic stimulation of both Ca^{2+} entry during systole and Ca^{2+} efflux during diastole. However, no effect of cyclic AMP-dependent protein kinase on the Na^+/Ca^{2+} antiporter in cardiac vesicles could be demonstrated (Rinaldi et al. 1982). The Na^+/Ca^{2+} antiporter is however subject to Ca^{2+}-calmodulin-dependent phosphorylation, which increase both its affinity for Ca^{2+} and its V_{max} rate (Caroni and Carafoli 1983). The $K_{0.5}$ value did change from 10 to 2 μmol/l for Ca^{2+} ions (Fig. 3). Such a phosphorylation occurs in response to rather high Ca^{2+} concentrations ($K_{0.5} = 2$ μmol/l), however, the addition of calmodulin appeared to increase the Ca^{2+} affinity of the protein kinase ($K_{0.5} = 0.8$ μmol/l). It is proposed that the activation mechanism would provide a positive feed-back mechanism to potentiate the cyclic AMP-dependent increase of penetration of Ca^{2+} into the cell as well as the increase of rate of Ca^{2+} extrusion from the myocyte. However, the proposed stimulation of Ca^{2+} entry will be marginal in the light of the saturating concentrations of Ca^{2+} and Na^+ that permanently exist at the outside of the sarcolemma. The Ca^{2+}-calmodulin-dependent inactivating enzyme (a protein phosphatase with $K_{0.5}$ of 3 μmol/l for Ca^{2+}) would represent a negative feed-back mechanism designed to protect the cardiac cell from Ca^{2+} overload (Caroni and Carofoli 1983).

Calmodulin-Like Proteins as Ca^{2+}-Dependent Regulators of Sarcolemmal Ca^{2+} Transporting Systems

Recently, it has been discovered by us and others (Caroni and Carafoli 1981a; Lamers et al. 1981; Lamers and Stinis 1983; Tuana et al. 1981; Mas-Oliva et al. 1983) that Ca^{2+}-pumping ATPase contains a tightly bound calmodulin-like protein that directly regulates Ca^{2+} sensitivity of the pump (see also Fig. 1). By hypotonic treatment immediately followed by hypertonic wash in the presence of EGTA (Caroni and Carafoni 1981a) or by calmidazolium treatment (Lamers and Stinis

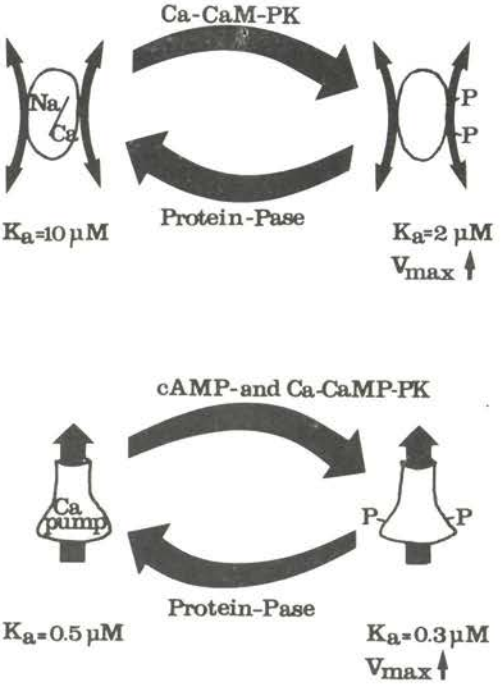

Fig. 3. Comparison between regulation of the Ca^{2+}-pumping ATPase and the Na^+/Ca^{2+} antiporter by phosphorylation-dephosphorylation mechanisms. K_a refers to the affinity of the Ca^{2+} transporter for Ca^{2+} ions.

1983) of sarcolemmal vesicles the calmodulin-like subunit can be removed from the Ca^{2+} pump. The extracted M_r 15—17,000 protein has been shown to activate brain phosphodiesterase (Lamers and Stinis 1983) and erythrocytic Ca^{2+} pumping ATPase (Caroni and Carafoli 1981a). However, amino acid sequencing would be required to prove its identity with calmodulin. The stimulation of brain phosphodiesterase by sarcolemmal calmodulin-like protein has been shown to be Ca^{2+} dependent, thus it can be assumed that we have to deal with a Ca^{2+} binding protein. Louis and Jarvis (1982), using an affinity labeling technique, were able to show that the M_r 8—11,000 phosphoprotein (phospholamban) in sarcoplasmic reticulum cross-linked with calmodulin in a Ca^{2+}-dependent manner.

These findings may suggest that the phospholamban-like protein in sarcolemma is also an acceptor of calmodulin in Ca^{2+}-pumping ATPase. Calmidazolium, the most potent anti-calmodulin drug so far described (Van Belle 1981; Lamers and Stinis 1983) is a strong inhibitor of the sarcolemmal Ca^{2+}-pumping ATPase. The drug decreases the affinity of the pumping ATPase for Ca^{2+} ions. A comparative measurement was done with a dihydropyridine Ca^{2+} entry blocker (Fleckenstein 1977) felodipine. Whereas calmidazolium and felodipine both blocked

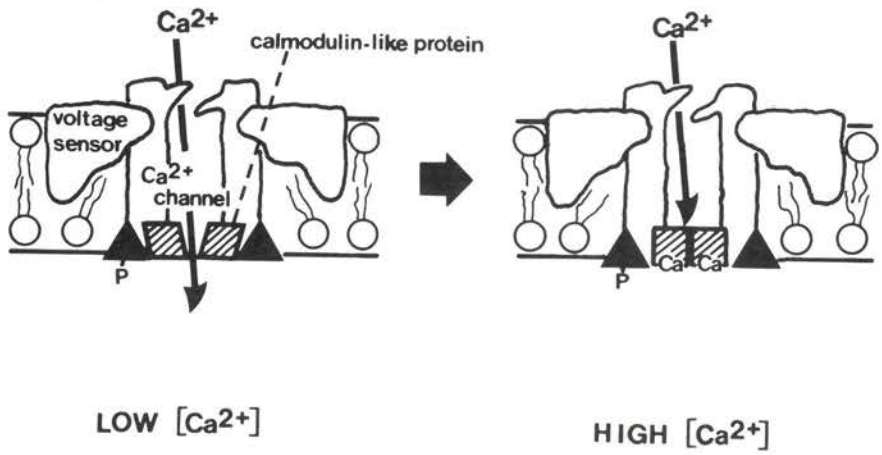

Fig. 4. A schematical picture of the possible role of a Ca^{2+} binding protein (calmodulin-like protein) as the inner side closing gate of the slow Ca^{2+} channel (compare also Johnson 1984).

the Ca^{2+}-calmodulin-dependent brain phosphodiesterase, felodipine, in contrast to calmidazolium, interacted non-competitively with Ca^{2+} on the Ca^{2+}-pumping ATPase (results not shown). In conclusion, it is very likely that calmidazolium and felodipine are interacting in a different manner with the calmodulin-like protein of the Ca^{2+}-pumping ATPase. It should be noted that another dihydropyridine Ca^{2+} entry blocker, nifedipine, did not inhibit the Ca^{2+}-pumping ATPase. The drug was even slightly stimulating the enzyme (results not shown).

Recent studies by the group of Johnson and Wittenauer (1983); Johnson et al. (1983) and Johnson (1984), on binding of calmidazolium, felodipine and prenylamine to fluorescent calmodulin are very interesting in the light of the effects on the Ca^{2+}-pumping ATPase observed in our laboratory. By fluorescence measurements it was demonstrated by Johnson et al. (1983) that interaction occurred between high affinity binding sites on calmodulin of Ca^{2+}, calmidazolium or felodipine, respectively. On the basis of these results and the efficiency of felodipine to block the Ca^{2+} channel the authors proposed that a calmodulin-like protein might also be present in the slow Ca^{2+} channel protein complex localized at the inside of the sarcolemma (Fig. 4). This Ca^{2+} binding protein would regulate the Ca^{2+} dependent closing of the slow Ca^{2+} channel as it is proposed to occur at the inner gate. This gate would be open at low Ca^{2+} and closed at higher levels. Ca^{2+} entry blockers would interact with this calmodulin-like protein to increase its affinity for Ca^{2+}, thereby closing the slow Ca^{2+} channel more rapidly (compare Fig. 2). Similarly, these drugs could interact with the calmodulin-like protein of the Ca^{2+}-pumping ATPase, thereby increasing its affinity for Ca^{2+} ions. Both mechanisms would explain Ca^{2+} antagonistic action of the drug. Indeed, a stimulating

effect has recently been reported on the sarcolemmal ATP-dependent Ca^{2+} uptake at low concentrations of nifedipine (David-Dufilho et al. 1984).

Acknowledgement. The author wishes to thank Miss M. I. Wieriks for her assistance in the preparation of the manuscript, and Mr. J. Cysouw for carrying out the experiments on drug effects on the calmodulin-dependent enzymes.

References

Capony J. P., Rinaldi M. L., Guilleux F., Demaille J. G. (1983): Isolation of cardiac membrane proteolipids by high pressure liquid chromatography: a comparison of reticular and sarcolemmal proteolipids, phospholamban and calciductin. Biochim. Biophys. Acta **728**, 83—91

Caroni P., Carafoli E. (1981a): The Ca^{2+} pumping ATPase of heart sarcolemma.Characterization, calmodulin dependence and partial purification. J. Biol. Chem. **256**, 3263—3270

Caroni P., Carafoli E. (1981b): Regulation of Ca^{2+} pumping ATPase of heart sarcolemma by phosphorylation-dephosphorylation process. J. Biol. Chem. **256**, 9371—9373

Caroni P., Reinlib L., Carafoli E. (1980): Charge movements during the Na^+—Ca^{2+} exchange in heart sarcolemmal vesicles. Proc. Nat. Acad. Sci. USA **77**, 6354—6358

Caroni P., Carafoli E. (1983): The regulation of the Na^+—Ca^{2+} exchanger of heart sarcolemma. Eur. J. Biochem. **132**, 451—460

Caroni P., Zurini M., Clarke A., Carafoli E. (1983): Further characterization and reconstitution of the purified Ca^{2+} pumping ATPase of heart sarcolemma. J. Biol. Chem. **258**, 7305—7310

David-Dufilho M., Devynck M. A., Kazda S., Meyer P. (1984): Stimulation by nifedipine of calcium transport by cardiac sarcolemmal vesicles from spontaneously hypertensive rats. Eur. J. Pharmacol. **97**, 121—127

England P. (1983): Cardiac function and phosphorylation of contractile proteins. Phil. Trans. R. Soc. Lond. B. **302**, 83—90

Fabiato A., Fabiato F. (1978): Calcium-induced calcium release from sarcoplasmic reticulum of adult human, dog, cat, rabbit, rat and frog hearts and fetal and new-born rat ventricles. Proc. N. Y. Acad. Sci. **307**, 491—522

Fleckenstein A. (1977): Specific pharmacology of calcium in myocardium, cardiac pacemakers and vascular smooth muscle. Annu. Rev. Pharmacol. Toxicol. **17**, 149—166

Flockerzi V., Mewes R., Ruth P., Hofmann F. (1983): Phosphorylation of purified bovine cardiac sarcolemma and potassium-stimulated calcium uptake. Eur. J. Biochem. **135**, 131—142

Haiech J., Demaille J. G. (1983): Phosphorylation and the control of calcium fluxes. Phil. Trans. R. Soc. Lond. B. **302**, 91—98

Hui C. W., Drummond M., Drummond G. I. (1976): Calcium-accumulation and cyclic AMP-stimulated phosphorylation in plasma membrane-enriched preparations of myocardium. Arch. Biochem. Biophys. **173**, 415—427

Huggins J. P., England P. J. (1983): Sarcolemmal phospholamban is phosphorylated in isolated rat hearts perfused with isoprenaline. FEBS Lett. **163**, 297—302

Iwasa Y., Hosey M. M. (1984): Phosphorylation of cardiac sarcolemma proteins by the calcium-activated phospholipid-dependent protein kinase. J. Biol. Chem. **259**, 534—540

Johnson J. D. (1984): A calmodulin-like Ca^{2+} receptor in the Ca^{2+} channel. Biophys. J. **45**, 134—136

Johnson J. D., Wittenauer L. A. (1983): A fluorescent calmodulin that reports the binding of hydrophobic inhibitory ligands. Biochem. J. **211**, 473—479

Johnson J. D., Wittenauer L. A., Nathan R. (1983): Calcium, Ca^{2+} channels and calmodulin in muscle contraction. J. Neural Transmission **18**, (suppl.), 97—111

Jones L. R., Maddock S. W., Hathaway D. R. (1981): Membrane localization of the type II cyclic AMP-dependent protein kinase activity. Biochim. Biophys. Acta. **641**, 242—253

Katz A. M., Colvin R. A., Ashavaid T. (1983): Phospholamban and calciductin. J. Mol. Cell. Cardiol. **15**, 795—797

Lamers J. M. J., Stinis J. T. (1980): Phosphorylation of low molecular weight proteins in purified preparations of rat heart sarcolemma and sarcoplasmic reticulum. Biochim. Biophys. Acta **624**, 443—459

Lamers J. M. J., Stinis J. T. (1981): An electrogenic Na^+/Ca^{2+} antiporter in addition to the Ca^{2+} pump in cardiac sarcolemma. Biochim. Biophys. Acta **640**, 521—534

Lamers J. M. J., Stinis J. T. (1983): Inhibition of Ca^{2+}-dependent protein kinase and Ca^{2+}/ /Mg^{2+}-ATPase in cardiac sarcolemma by the anti-calmodulin drug calmidazolium. Cell Calcium **4**, 281—294

Lamers J. M. J., Weeda E. (1984): Methods for studying phosphorylation in cardiac membranes. In: Methods in Studying Cardiac Membranes (Ed. N. S. Dhalla) Chapter 13, CRC Press Inc. Boca Raton

Lamers J. M. J., Stinis J. T., De Jonge H. R. (1981): On the role of cyclic AMP and Ca^{2+}-calmodulin-dependent phosphorylation in the control of $(Ca^{2+} + Mg^{2+})$-ATPase of cardiac sarcolemma. FEBS Lett. **127**, 139—143

Louis C. F., Maffit M. (1982): Characterization of calmodulin-mediated phosphorylation of cardiac muscle sarcoplasmic reticulum. Arch. Biochem. Biophys. **218**, 109—118

Louis C. F., Jarvis B. (1982): Affinity labeling of calmodulin-binding components in canine cardiac sarcoplasmic reticulum. J. Biol. Chem. **257**, 15187—15191

Manalan A. S., Jones L. R. (1982): Characterization of the intrinsic cAMP-dependent protein kinase activity and endogenous substates in highly purified cardiac sarcolemmal vesicles. J. Biol. Chem. **257**, 10052—10062

Mas-Oliva J., De Meis L., Inesi G. (1983): Calmodulin stimulates both adenosine 5'-triphosphate hydrolysis and synthesis by a cardiac calcium ion dependent adenosinetriphosphatase. Biochemistry **22**, 5822—5825

Miyamoto H., Racker E. (1980): Solubilization and partial purification of the Na^+/Ca^{2+} antiporter from the plasma membrane of bovine heart. J. Biol. Chem. **255**, 2656—2658

Mullins L. J. (1979): The generation of electric currents in cardiac fibers by Na^+/Ca^{2+} exchange. Am. J. Physiol. **5**, C 103-C 110

Nayler W. G., Dillon J. S., Daly M. J. (1984): Cellular sites of action of calcium antagonists and β-adrenergic blockers. In: Calcium Antagonists and Cardiovascular Disease (Ed. Opie L. H.) Vol. 9, pp. 181—192, Raven Press, N. Y.

Osterrieder W., Brum G., Hescheler J., Trautwein W., Flockerzi V., Hofmann E. (1982): Injection of subunits of cyclic AMP-dependent protein kinase into cardiac myocytes modulates Ca^{2+} current. Nature (London) **298**, 576—587

Philipson K. D. (1983): "Calciductin" and voltage-sensitive calcium uptake. J. Mol. Cell. Cardiol. **15**, 867—869

Pitts B.J.R.(1979): Stoichiometry of the sodium-calcium exchange in cardiac sarcolemmal vesicles. J. Biol. Chem. **254**, 6232—6235

Reeves J. P., Sutko J. L. (1980): Sodium-calcium exchange activity generates a current in cardiac membrane vesicles. Science **208**, 1461—1464

Reuter H. (1983): Calcium channel modulation by neuro-transmitters, enzymes and drugs. Nature **301**, 569—574

Rinaldi M. L., Capony J. P., Demaille J. G. (1982): The cyclic AMP-dependent modulation of cardiac slow calcium channels. J. Mol. Cell. Cardiol. **14**, 279—289

Sulakhe P. V., St. Louis P. J. (1976): Membrane phosphorylation and calcium transport in cardiac and skeletal muscle membranes. Gen. Pharmac. **7**, 313—319

Tada M., Katz A. M. (1982): Phosphorylation of the sarcoplasmic reticulum and sarcolemma. Annu. Rev. Physiol. **44**, 401—423

Tuana B. S., Džurba A., Panagia V., Dhalla N. S. (1981): Stimulation of heart sarcolemmal calcium pump by calmodulin. Biochem. Biophys. Res. Commun. **100**, 1245—1250

Van Belle H. (1981): R 24571: A potent inhibitor of calmodulin-activated enzymes. Cell Calcium **2**, 483—494

Vetter R., Haase H., Will H. (1982): Potentiation effect of calmodulin and catalytic subunit of cyclic AMP-dependent protein kinase on ATP-dependent Ca^{2+} transport by cardiac sarcolemma. FEBS Lett. **148**, 326—330

Walsh D. A., Clippinger M. S., Sivaramakrishnan S., McCullough T. E. (1979): Cyclic adenosine monophosphate dependent and independent phosphorylation of sarcolemma membrane proteins in perfused rat heart. Biochemistry **18**, 871—877

Wegener A. D., Jones L. R. (1984): Phosphorylation-induced mobility shift in phospholamban in sodium dodecyl sulphate polyacrylamide gels. J. Biol. Chem. **259**, 1834—1841

Wollenberger A., Will H. (1978): Protein kinase-catalyzed membrane phosphorylation and its possible relationship to the role of calcium in the β-adrenergic regulation of cardiac contraction. Life Sci. **22**, 1159—1178

Ziegelhöffer A., Anand-Srivastava M. B., Khandelwal R. L., Dhalla N. S. (1979): Activation of heart sarcolemmal Ca^{2+}/Mg^{2+}-ATPase by cyclic AMP-dependent protein kinase. Biochem. Biophys. Res. Commun. **89**, 1073—1081

Received May 25, 1984/Accepted August 7, 1984