Characterization of Ca^{2+} Release from the Cardiac Sarcoplasmic Reticulum

N. S. DHALLA[1], P. V. SULAKHE[2], J. M. J. LAMERS[3] and P. K. GANGULY[1]

1 *Experimental Cardiology Section, Department of Physiology, Faculty of Medicine, University of Manitoba, Winnipeg, Canada R3E OW3*
2 *Department of Physiology, College of Medicine, University of Saskatchewan, Saskatoon, Canada*
3 *Department of Biochemistry I, Faculty of Medicine, Erasmus University, Rotterdam, The Netherlands*

Abstract. The characteristics of Ca^{2+} release in relation to Ca^{2+} binding were studied in sarcoplasmic reticulum vesicles isolated from canine myocardium. The Ca^{2+} binding appeared to be dependent on ATP as a 4 fold increase in Ca^{2+} binding was observed upon the addition of ATP. In the presence of a suboptimal ATP concentration (20 μmol/l; without ATP regenerating system) a rapid release of Ca^{2+} started within 2 min. The rate of Ca^{2+} release was increased by increasing the concentration of Ca^{2+} in the preincubation medium when studied by diluting preloaded vesicles in medium free of Ca^{2+} and ATP; an apparent saturation was reached at 5 mmol/l Ca^{2+} but Ca^{2+} release again increased between 5 and 10 mmol/l Ca^{2+}. High pH (8.0) enhanced the Ca^{2+} release process. When Ca^{2+} loaded vesicles were treated with various phospholipases and proteases, an enhanced Ca^{2+} release was observed in comparison to the control values. The release of Ca^{2+} was also increased by pharmacological agents like caffeine, ether and halothane. The Ca^{2+} release rate was stimulated by the p-chloromercurybenzoate treatment, which decreased ATP dependent Ca^{2+} binding and Ca^{2+}-stimulated ATPase activities of the sarcoplasmic reticulum vesicles. The effect of temperature when evaluated by Arrhenius plots showed a higher energy of activation of Ca^{2+} release (66.15 kJ/mol) in comparison to that for Ca^{2+} binding (41.03 kJ/mol). These results indicate that, although Ca^{2+} release and Ca^{2+} binding activities of the cardiac sarcoplasmic reticulum appears to be related, Ca^{2+} release is probably a distinct process and is controlled differently. It seems that the Ca^{2+} release site in sarcoplasmic reticulum membranes is lipoprotein in nature.

Key words: Ca^{2+} release — Ca^{2+} binding — Ca^{2+}-stimulated ATPase — Pharmacological agents — Dog heart sarcoplasmic reticulum

Introduction

There is a considerable evidence to suggest that sarcoplasmic reticulum (SR) plays a major role in the regulation of intracellular Ca^{2+} concentration and thus participates in the excitation-contraction coupling process in both cardiac and skeletal muscles (Ebashi and Endo 1968; Martonosi 1972; Endo 1977; Dhalla et al. 1977; Van Winkle and Entman 1979). However, both mammalian cardiac and skeletal muscles appear to differ in many biochemical and pharmacological aspects, particularly, in relation to their SR function. Differences have been reported (Sumida et al. 1978) in cardiac and skeletal SR Ca^{2+} metabolism mainly with respect to phosphorylation-dephosphorylation processes and Ca^{2+} dissociation. Furthermore, differences in the ATP-dependent Ca^{2+} transport system of SR have been also described in these tissues (Van Winkle and Entman 1979). Although biochemical and functional correlates have been elucidated well in skeletal SR using various physical as well as enzymatic methods (Hidalgo and Ikemoto 1977; Davis et al. 1976), virtually no detailed study has been carried out to characterize cardiac SR membrane in relation to Ca^{2+} binding and release activities.

An understanding of Ca^{2+} movement across SR membranes is crucial as it may provide information regarding factors which change the movement of Ca^{2+} in favour of net efflux in cardiac muscle. There are reports to indicate that ATP induces a rapid Ca^{2+} binding to cardiac SR which is followed by a spontaneous Ca^{2+} release (McCollum et al. 1972; Entman et al. 1972). Accordingly, it is believed that Ca^{2+} release is thermodynamically and kinetically a distinct process representing a separate phase of the ATP-dependent binding-release cycle. Kirchberger and Wong (1978) have demonstrated that Ca^{2+} efflux from the isolated cardiac SR is increased by micromolar concentrations of extravesicular Ca^{2+} and have argued that a component of the Ca^{2+} pump participates in Ca^{2+} efflux. The characteristics of Ca^{2+} release due to reversal of the Ca^{2+} pump has been described in the skeletal muscle where the Ca^{2+} release mechanisms appear to be quite independent of the Ca^{2+} pump in their behavior; for example, conditions enhancing Ca^{2+} release do not appreciably affect Ca^{2+} uptake activity (Endo 1977). Some experiments have also been interpreted to suggest that there are Ca^{2+} efflux channels present in SR (Katz et al. 1976) where gating is regulated by drugs like ryanodine or by physiological parameters such as Ca^{2+} concentration, pH (Jones et al. 1979) or a transmembrane potential (Farmen et al. 1979). Although these reports provide some informations on Ca^{2+} release process of both the cardiac and skeletal muscle, the characteristics of Ca^{2+} release from the cardiac SR are still far from being understood. In the present study therefore we have examined the effects of ATP limitation, Ca^{2+} preloading, temperature, pH and SH group inhibition on Ca^{2+} binding and release processes in the SR from canine myocardium. The effects of various agents which alter membrane integrity were also studied in order to gain some insight into the nature of Ca^{2+} release site in cardiac SR.

Material and Methods

Isolation of SR vesicles. Cardiac SR was isolated from mongrel dogs in a manner similar to that previously reported by Harigaya and Schwartz (1969) with slight modification (Dhalla et al. 1970). After removing the connective tissue and fat, ventricles were thoroughly washed in 0.25 mol/l sucrose containing 1 mmol/l EDTA, pH 7.0 and homogenized in 10 volumes of a medium containing 10 mmol/l $NaHCO_3$, 5 mmol/l sodium azide and 10 mmol/l Tris-HCl, pH 6.8 in a Waring blender at 0—5 °C for 45 sec. The homogenate was filtered through four layers of gauze and centrifuged at 10,000 × g for 20 min to remove cell debris, myofibrils, nuclei and mitochondria. The 10,000 × g supernatant fraction was then centrifuged at 40,000 × g for 45 min. The pellet thus obtained was thoroughly washed and resuspended in 8 volumes of 0.6 mol/l KCl and centrifuged at 40,000 × g for 45 min. The sediment was suspended in a solution containing 0.3 mol/l sucrose, 10 mmol/l Tris-maleate, pH 7. This fraction was referred to as sarcotubular vesicles (sarcoplasmic reticulum). The data concerning biochemical marker enzyme activities, sensitivity to inhibitors of mitochondrial transport and electron microscopic studies revealed negligible mitochondrial or myofibrillar contamination (Dhalla et al. 1970).

Ca^{2+} binding assay. SR vesicles (0.4—0.6 mg/ml) were incubated in a standard medium containing (in mmol/l): 100 KCl, 5 $MgCl_2$, 20 Tris-maleate (pH 6.8) and different concentrations of ATP and $^{45}CaCl_2$ as required. The amount of radioactive Ca^{2+} taken up by SR was determined by measuring residual Ca^{2+} in the medium after removal of the reticulum by rapid filtration through millipore filters (0.45 μm). In all experiments the Ca^{2+} concentration was maintained greater than that was shown to result in an optimal Ca^{2+} binding (> 10 μmol/l).

Ca^{2+} release. The vesicles of SR (0.6—0.8 mg) were loaded with $^{45}CaCl_2$ in standard medium described above for Ca^{2+} binding. These vesicles were then subjected to release Ca^{2+} into a Ca^{2+} free medium under different experimental situations. For Ca^{2+} exchange experiments, the Ca^{2+} loaded SR (0.3—0.6) mg/ml) was incubated in the standard medium containing 200 μmol/l $CaCl_2$ (non radioactive). The other experimental conditions are given in legends to the figures.

Effects of pharmacological agents, phospholipases and proteases on Ca^{2+} release process. Release of accumulated $^{45}Ca^{2+}$ from cardiac SR on addition of pharmacological agents like caffeine, ether and halothane and different phospholipases and proteases was determined in almost identical condition as described above. The SR vesicles were incubated in the presence of 100 mmol/l KCl, 5 mmol/l $MgCl_2$, 20 mmol/l Tris-maleate (pH 6.8), 5 mmol/l ATP and 0.1 mmol/l $^{45}CaCl_2$ at 25 °C for 5 min, centrifuged at 40,000 × g for 20 min and suspended in 0.3 mol/l sucrose, 10 mmol/l Tris-maleate (pH 7.0). These vesicles were then incubated in presence of various agents for different time intervals and serial samples were obtained by filtration. All incubations with volatile substances were carried out in sealed flasks.

ATPase measurement. Total (0.1 mmol/l $CaCl_2$, 5 mmol/l $MgCl_2$) and basal (0.5 mmol/l EGTA, 5 mmol/l $MgCl_2$) ATPase activities were determined in an incubation medium similar to that used for Ca^{2+} binding assay, except that the reaction was initiated by the addition of 4 mmol/l ATP (with 3 min preincubation in presence of 30—60 μg of membrane protein). The reaction was terminated after 5 min by 1 ml of 12% (w/v) cold trichloroacetic acid. Inorganic phosphate liberated into the protein-free filtrate was assayed by the procedure of Taussky and Shorr (1953). The Ca^{2+}-stimulated Mg^{2+} dependent ATPase activity reported here is the difference between the total and basal ATPase activities.

All experiments were repeated at least two to five times. The values presented in this paper are representative from single experiment carried out on the same day. Freshly prepared SR membranes were employed in all experiments.

Results

When the concentration of ATP in reaction mixture was 3 mmol/l or 20 μmol/l with an ATP regenerating system, maximal Ca^{2+} binding occurred within 1 min (Fig. 1). This Ca^{2+} binding appeared to be dependent on the presence of ATP as a fourfold increase in Ca^{2+} binding occurred in the presence of ATP. When suboptimal ATP concentration was used (20 μmol/l without regenerating system), a rapid release of Ca^{2+} ion started from the bound Ca^{2+} after a few minutes. Furthermore, this release was stopped and almost the maximal binding was restored by readdition of 20 μmol/l ATP. These results indicate that Ca^{2+} binding sites were saturated so long as a sufficient amount of ATP was present.

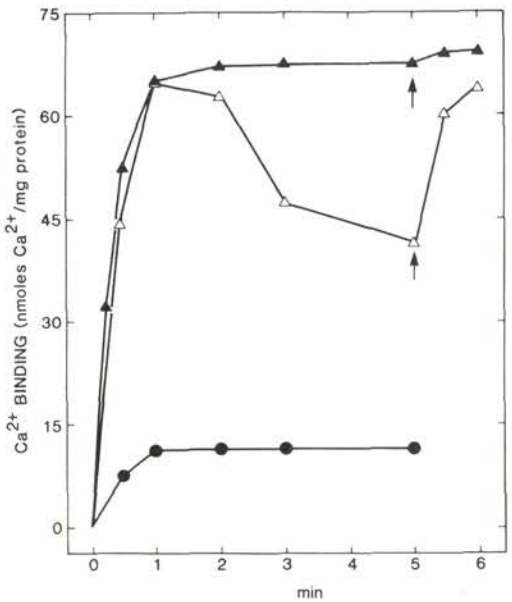

Fig. 1. Dependence of Ca^{2+} binding and release on ATP. The sarcotubular vesicles were incubated in a medium containing (in mmol/l): 100 KCl, 5 $MgCl_2$, 0.1 $^{45}CaCl_2$, 20 Tris-maleate (pH 6.8) and different concentrations of ATP at 30 °C for times indicated. The aliquots were removed, filtered through millipore filters (0.45 μm) and Ca^{2+} binding calculated. (▲)—Reaction medium contained 3 mmol/l ATP. Similar results were obtained by using 20 μmol/l ATP with ATP regenerating system (4 mmol/l phosphoenolpyruvate and 0.1 mg/ml of pyruvate kinase). (Δ)—Reaction medium contained 20 μmol/l ATP. (●)—Reaction medium contained no ATP which is otherwise complete. The arrows indicate the addition of 20 μmol/l ATP.

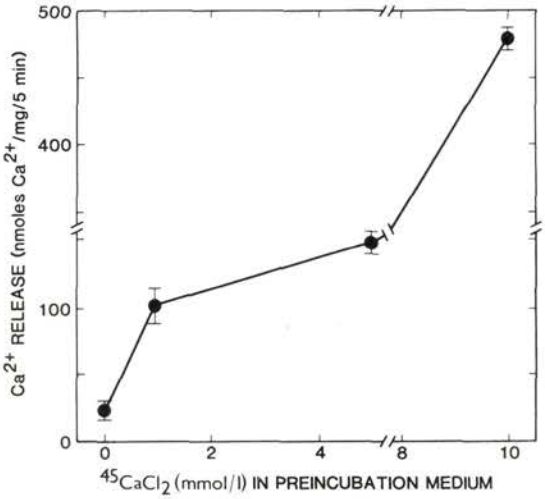

Fig. 2. The relationship between the concentration of Ca^{2+} in the preloading phase and the rate of Ca^{2+} release. The sarcotubular vesicles were incubated at 25 °C with different concentrations of $^{45}CaCl_2$ (as indicated) in the standard medium and were then allowed to release the bound Ca^{2+} into Ca^{2+} free medium (100 mmol/l KCl, 5 mmol/l $MgCl_2$ and 20 mmol/l Tris-maleate; pH 6.8). The samples were filtered at 1 min after the initiation of the release of Ca^{2+} by addition of loaded vesicles into the Ca^{2+} free medium and the activity of the filtrate counted. Values are mean ± S.E.M. of five experiments.

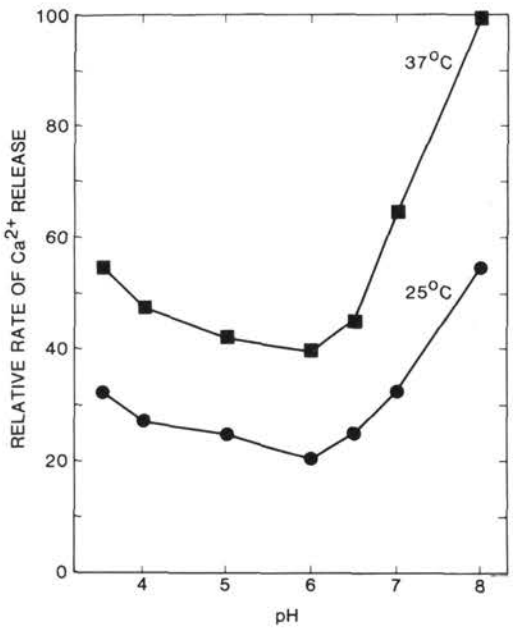

Fig. 3. Effect of pH on the relative rate of Ca^{2+} release from the sarcoplasmic reticulum measured at 25 ° and 37 °C. Ca^{2+} release at pH 8 at 37 °C was equivalent to 100.

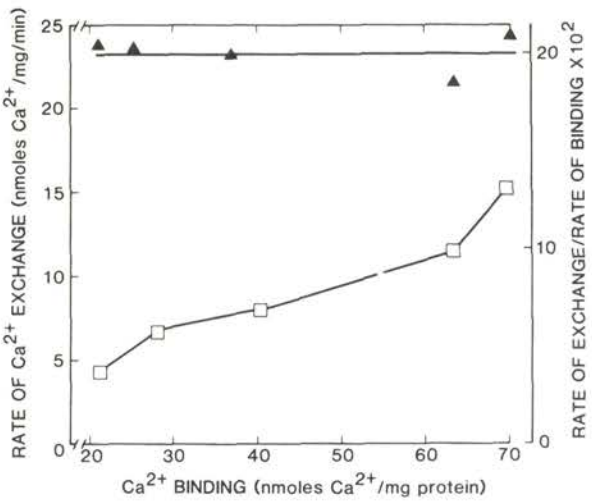

Fig. 4. Effect of the amount of Ca^{2+} bound on the rate of Ca^{2+} exchange. The sarcoplasmic vesicles were loaded with 0.1 mol/l $^{45}CaCl_2$ in the standard medium at 25 °C for 30 sec, 1 min, 2 min, 5 min, and 8 min and were then centrifuged at 40,000 × g for 20 min and suspended in 0.3 mol/l sucrose, 10 mmol/l Tris-maleate, pH 7. These suspended vesicles were the incubated in a medium containing 0.2 mmol/l $CaCl_2$ (non radioactive), 100 mmol/l KCl, 5 mmol/l $MgCl_2$ and 20 mmol/l Tris-maleate; pH 6.8 for one min. The aliquots were filtered through millipore filters and the activity of the filtrate counted. (□) — Rate of exchange. (▲) — Rate of exchange/Rate of binding × 10^2.

Increasing the concentration of Ca^{2+} in the preincubation medium was found to be associated with a large increase in Ca^{2+} release as assayed by dilution of the preloaded SR vesicles in Ca^{2+} free medium (Fig. 2). An apparent saturation was reached at approximately 5 mmol/l indicating that Ca^{2+} release in the present experiment was not due to a simple diffusion. Fig. 2 also shows that between 5 and 10 mmol/l Ca^{2+} in the preincubation medium, Ca^{2+} release activity was again increased. While these data on Ca^{2+} release was obtained by using millimolar concentrations of Ca^{2+}, it is pointed out that the affinity of Ca^{2+} binding sites for Ca^{2+} in the SR membranes has been shown to be in the micromolar range (Martonosi 1972). In a separate study, the Ca^{2+} release rate was determined over a broad pH range at 25 ° and 37 °C and the results are shown in Fig. 3. Both low (3.5) as well as high (8.0) pH stimulated Ca^{2+} release rates.

In another set of experiments, the membranes were preloaded with $^{45}CaCl_2$ at different time intervals so that the amount of Ca^{2+} bound varied. These SR vesicles were then diluted in a medium containing a high (200 μmol/l) concentration of unlabelled Ca^{2+}. Fig. 4 shows that there was a close correlation between the amount of Ca^{2+} bound and Ca^{2+} efflux rate. At higher amount of Ca^{2+} bound, the rate of Ca^{2+} efflux was increased; however, the ratio of rate of Ca^{2+} exchange and the rate of Ca^{2+} binding did not change (Fig. 4).

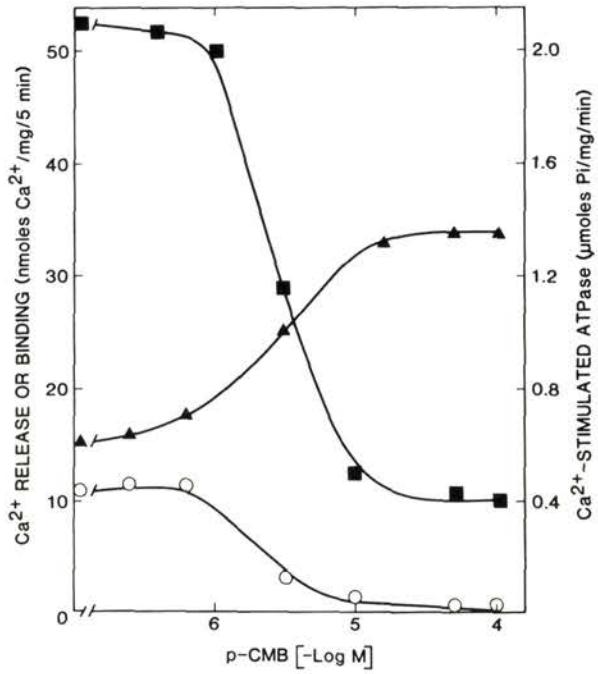

Fig. 5. Effect of p-chloromercurybenzoate (pCMB) treatment of sarcoplasmic reticulum on Ca^{2+}--stimulated ATPase, Ca^{2+} binding and release. Experimental conditions were same as before only the final reaction media contained different concentrations of pCMB. (○)–Ca^{2+}-stimulated ATPase. (■)–Ca^{2+} binding and (▲)–Ca^{2+} release.

The Ca^{2+}-stimulated ATPase was inactivated by micromolar amounts of pCMB (Fig. 5) suggesting that thiol groups (SH) were essential in maintaining enzyme activity. Along with this effect of ATPase, a marked decrease of Ca^{2+} binding was observed, indicating a linkage between Ca^{2+} stimulated ATPase and Ca^{2+} binding. Ca^{2+} release rate, however, was markedly stimulated in the same range of pCMB concentration. This finding, therefore, suggests that thiol reagent dissociates Ca^{2+} binding from the Ca^{2+} release process.

Both Ca^{2+} release and Ca^{2+} binding were determined at three temperatures 15°, 25° and 37 °C; temperature lower than 15 °C was not selected in this study because a deviation of linearity could be expected in a temperature range from 5° to 15° (Entman et al. 1972). Linear relationships were obtained in Arrhenius diagrams in this temperature range for Ca^{2+} binding as well as release process (Fig. 6). A definite difference was observed between activation energy for the Ca^{2+} release (66.15 kJ/mol) and the Ca^{2+} binding (41.03 kJ/mol) processes.

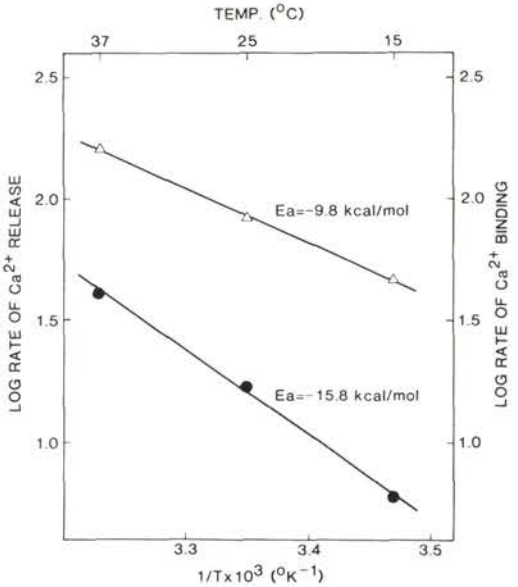

Fig. 6. Arrhenius plot of the rate of Ca^{2+} binding and Ca^{2+} release. Both Ca^{2+} binding and Ca^{2+} release experiments were done at indicated temperatures in a standard medium as described previously. Energy of activation (E_A) was then calculated from the data obtained in these experiments. (△)–Ca^{2+} binding. (●)–Ca^{2+} release.

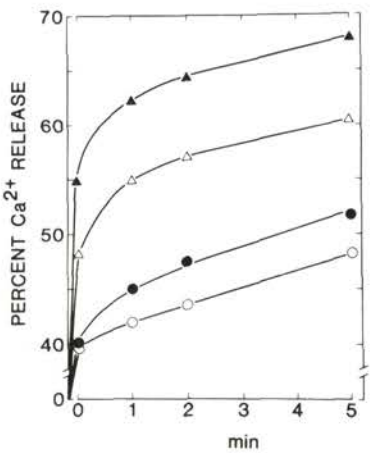

Fig. 7. Effects of phospholipases A, C and D treatment on Ca^{2+} release from sarcotubular vesicles. The SR vesicles were incubated in the presence of 0.1 mmol/l $^{45}CaCl_2$ in the standard medium, centrifuged at 40,000 × g for 20 min and suspended in 0.3 mol/l sucrose, 10 mmol/l Tris-maleate (pH 7.0). The suspended vesicles were then incubated with 0.25 mg/mg protein phospholipase A (▲), 0.25 mg/mg protein phospholipase C (△) or 0.25 mg/mg protein phospholipase D (●) for different time intervals. Control experiments (○) were done similarly except no membrane perturbing agent was used. Values are expressed as percent Ca^{2+} release of loaded Ca^{2+} vesicles obtained at various times.

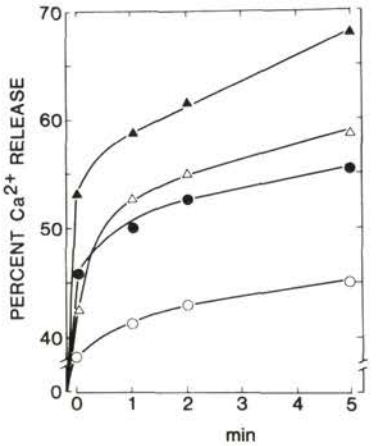

Fig. 8. Effects of pronase, trypsin and pepsin on Ca^{2+} release from sarcotubular vesicles. Experimental conditions were same as those of Fig. 7 except that 0.1 mg/mg protein pronase (▲), 0.1 mg/mg protein trypsin(△) or 0.1 mg/mg protein pepsin (●) were used during final incubation. (○)–control experiment.

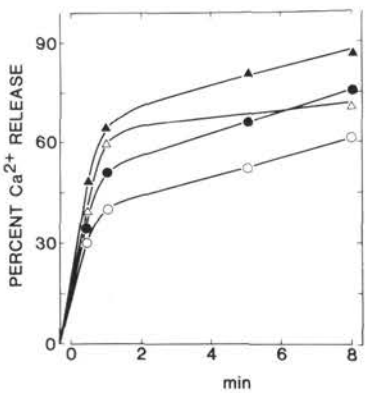

Fig. 9. Effects of various pharmacological agents like halothane, caffeine and ether on Ca^{2+} release from sarcotubular vesicles. Experimental conditions were same as those of Fig. 7 except that 6.5 mmol/l halothane (▲), 5 mmol/l caffeine (●) or 300 mmol/l ether (△) were used during final incubation. (○)–control experiment.

In order to understand the nature of Ca^{2+} release from cardiac SR, the Ca^{2+} loaded vesicles were treated with various agents. Fig. 7 shows that the treatment of phospholipases A, C and D leads to a time dependent increase in Ca^{2+} release. Both phospholipases A and C enhanced Ca^{2+} release to a greater extent in comparison to that was seen with phospholipase D treatment. The proteolytic enzymes like pronase, trypsin and pepsin also increased Ca^{2+} release from the loaded SR vesicles (Fig. 8); the maximal effect was seen with pronase treatment. In another series of experiments, the Ca^{2+} loaded vesicles were incubated with different pharmacological agents like halothane, caffeine and ether. All these agents enhanced Ca^{2+} release from the SR vesicle (Fig. 9).

Discussion

The present work confirms the previous observations that ATP-dependent Ca^{2+} binding may represent saturation of a finite number of individual sites on the SR. Ca^{2+} was bound to these sites as long as a sufficient amount of ATP was present. The data in this study suggest that in the presence of ATP the Ca^{2+} binding sites may undergo phasic changes for Ca^{2+} affinity and thus result in Ca^{2+} binding and release. However, no direct relationship was evident between the affinity of Ca^{2+} for the binding sites and the dependence of Ca^{2+} release on the Ca^{2+} concentration in the preloading phase (Fig. 2). The affinity of Ca^{2+} for release could not be estimated because the Ca^{2+} release process exhibited no saturation kinetics with respect to the amount of Ca^{2+} bound. Although from the calculation carried out by Weber et al. (1966) and Ogawa (1970) it appears that in the absence of a permeant anion such as oxalate, most of the available Ca^{2+} is bound in SR membrane, it is possible that a part of Ca^{2+} bound is taken up in the intravesicular compartment. So Ca^{2+} release function as estimated in this paper could be partly due to a simple diffusion of Ca^{2+}. Fig. 4 in this paper shows that the rate of Ca^{2+} release is dependent on the amount of Ca^{2+} bound in which experiment the contribution of diffusion to release was lowered by studying Ca^{2+} efflux in a medium containing high unlabelled Ca^{2+}. It, therefore, seems that Ca^{2+} release from the cardiac SR could be determined by the rate of Ca^{2+} binding but their relationship may be a complex one and thus a great deal of caution should be exercised while interpreting data obtained from in vitro experiments.

Kirchberger and Wong (1978) have suggested that a component of the Ca^{2+} pump participates in the release of Ca^{2+} from isolated cardiac SR. According to their report both Ca^{2+} release and Ca^{2+} uptake were stimulated by micromolar concentrations of outside Ca^{2+}, increased by protein-kinase-catalyzed phosphorylation and decreased by high intravesicular Ca^{2+} concentrations. However, it is interesting to note from our experiments that inhibition of the SH groups in SR membranes completely destroyed the relationship between Ca^{2+} binding and

release. Both Ca^{2+}-stimulated ATPase as well as Ca^{2+} binding were decreased when the rate of Ca^{2+} release was enhanced by the effect of p-chloromercury-benzoate treatment. Furthermore, the Arrhenius plots revealed an apparent energy (E_A) of 41.03 kJ/mol for the binding and 66.15 kJ/mol for the release. Similar differences under different experimental conditions were also observed by Entman et al. (1972). Such a difference of E_A of both processes suggests that release may represent a separate change of conformation of SR membrane with a high energy of activation. Therefore, it is likely that these two phases i. e., binding and release may be distinctly different from each other.

It is now believed that Ca^{2+} may be released from SR by a slow inward current of Ca^{2+} that flows across the sarcolemma during the plateau phase of the cardiac action potential. In contrast to skeletal muscle, in cardiac muscle the Ca^{2+}-induced Ca^{2+} release mechanism may play an important physiological role as suggested by Ebashi (1976). Recently Miyamoto and Racker (1982) have reported that the physiological Ca^{2+} release from the skeletal muscle SR is induced by depolarization of the SR membrane and the mechanisms of Ca^{2+}-induced Ca^{2+} release and of depolarization-induced Ca^{2+} release are mutually interrelated. On the other hand, it may be noted that caffeine is considered to induce twitching and contracture by the Ca^{2+}-induced Ca^{2+} release mechanism (Endo 1975). Furthermore, halothane-induced malignand hyperpyrexia (Britt and Kalow 1970) has been also thought to be due to Ca^{2+}-induced Ca^{2+} release mechanism. In the present study we have shown that caffeine, halothane and ether were capable of enhancing Ca^{2+} release from the cardiac SR and this effect seems to be of a direct nature due to the interaction of these pharmacological agents with SR membranes. While on the basis of the existing information it is difficult to exclude the Ca^{2+}-induced Ca^{2+} release from SR as a site of action of these agents, Pretorius et al. (1969) have shown a reduction of Ca^{2+} accumulation and an increase in Ca^{2+} efflux by caffeine in SR of both cardiac and skeletal muscles. Furthermore, Fuchs et al. (1968) have suggested that many drugs including volatile anesthetics may depress cardiac contractility by depleting SR Ca^{2+} via inhibition of the Ca^{2+} pumping mechanism. Thus it appears that both Ca^{2+} release and Ca^{2+} binding sites in the SR membranes may be involved in the actions of some drugs on the cardiac and skeletal muscle contraction. In this study we have demonstrated that the Ca^{2+} release process of cardiac SR was enhanced by treatment with different phospholipases and proteases. It seems that the Ca^{2+} release is dependent on the protein and the phospholipid components which are integrated into the organized structure of SR membranes. The selective removal of a membrane component, thus, alters membrane integrity and subsequently changes the Ca^{2+} permeability. Therefore, it is not difficult to conceive that Ca^{2+} release is dependent on the specific interactions of the membrane components and the release site is lipoprotein in nature.

Acknowledgements. This work was supported by a grant from the Manitoba Heart Foundation. Dr. Lamers was partly supported by the Dutch Heart Foundation.

References

Britt B. A., Kalow W. (1970): Malignant hyperthermia. A statistical review. Can. Anaesthesiol. Soc. J. **17,** 293—315

Davis D. G., Inesi G., Gulik-Krzywicki T. (1976): Lipid molecular motion and enzyme activity in sarcoplasmic reticulum membrane. Biochemistry **15,** 1271—1276

Dhalla N. S., Sulakhe P. V., Khandelwal R. L., Hamilton, I. R. (1970): Excitation-contraction coupling in heart. II. Studies on the role of adenyl cyclase in the calcium transport by dog heart sarcoplasmic reticulum. Life Sci. **9,** 625—632

Dhalla N. S., Ziegelhoffer A., Harrow J. A. C. (1977): Regulatory role of membrane systems in heart function. Can. J. Physiol. Pharmacol. **55,** 1211—1234

Ebashi S., Endo M. (1968): Calcium and muscle contraction. Progr. Biophys. Mol. Biol. **18,** 123—183

Ebashi S. (1976): Excitation-contraction coupling. Annu. Rev. Physiol. **38,** 293—313

Endo M. (1975): Mechanism of action of caffeine on the sarcoplasmic reticulum of skeletal muscle. Proc. Jpn. Acad. **51,** 479—484

Endo M. (1977): Calcium release from the sarcoplasmic reticulum. Physiol. Rev. **57,** 71—108

Entman M. L., Bornet E. P., Schwartz A. (1972): Phasic components of calcium binding and release by canine cardiac relaxing system (sarcoplasmic reticulum fragments). J. Mol. Cell. Cardiol. **4,** 155—169

Farmen R. H., Jones L. R., Besch H. R. Jr. (1979): Transmembrane potential and Ca^{2+} release from cardiac sarcoplasmic reticulum vesicles: Modulation of selective cation ionophores. Circulation (Suppl. II), II—223

Fuchs F., Gertz E. W., Briggs F. N. (1968): Effect of quinidine on calcium accumulation by isolated sarcoplasmic reticulum of skeletal and cardiac muscle. J. Gen. Physiol. **52,** 955—968

Harigaya S., Schwartz A. (1969): Rate of calcium binding and uptake in normal animal and failing human cardiac muscle. Circ. Res. **25,** 781—794

Hidalgo C., Ikemoto N. (1977): Disposition of proteins and aminophospholipids in the sarcoplasmic reticulum membrane. J. Biol. Chem. **252,** 8446—8454

Jones L. R., Besch H. R. Jr., Sutko J. L., Willerson J. T. (1979): Ryanodine-induced stimulation of net Ca^{2+} uptake by cardiac sarcoplasmic reticulum vesicles. J. Pharmacol. Exp. Ther. **209,** 48—55

Katz A. M., Dunnett J., Repke D. I., Hasselbach W. (1976): Control of calcium permeability in the sarcoplasmic reticulum. FEBS Letters **67,** 207—208

Kirchberger M. A., Wong D. (1978): Calcium efflux from isolated cardiac sarcoplasmic reticulum. J. Biol. Chem. **253,** 6941—6945

Martonosi A. (1972): Biochemical and clinical aspects of sarcoplasmic reticulum function. In: Curr. Topics in Membranes and Transport (Eds. F. Bonner and A. Kleinzeller), Vol. 3, pp. 83—197, Academic Press, New York

McCollum W. B., Besch H. R. Jr., Entman M. L., Schwartz A. (1972): Apparent initial binding rate of calcium by canine cardiac-relaxing system. Amer. J. Physiol. **223,** 608—614

Miyamoto H., Racker E. (1982): Mechanism of calcium release from skeletal sarcoplasmic reticulum. J. Membrane Biol. **66,** 193—201

Ogawa Y. (1970): Some properties of frog fragmented sarcoplasmic reticulum with particular reference to its response to caffeine. J. Biochem. **67,** 667—683

Pretorius P. J., Pohl W. G., Smithen C. S., Inesi G. (1969): Structural and functional characterization of dog heart microsomes. Circ. Res. **25,** 487—499

Sumida M., Wang T., Mandel F., Froehlich J. P., Schwartz A. (1978): Transient kinetics of Ca^{2+} transport of sarcoplasmic reticulum. A comparison of cardiac and skeletal muscle. J. Biol. Chem. **253,** 8772—8777

Taussky H., Shorr E. (1953): A microcolorimetric method for the determination of inorganic phosphorus. J. Biol. Chem. **202,** 675—685

Van Winkle W. B., Entman M. L. (1979): Comparative aspects of cardiac and skeletal muscle sarcoplasmic reticulum. Life Sci. **25,** 1189—1200

Weber A., Herz R., Reiss I. (1966): Study of the kinetics of calcium transport by isolated fragmented sarcoplasmic reticulum. Biochem. Z. **345**, 329—369

Received January 18, 1983 / Accepted March 1, 1983