Biochimica et Biophysica Acta 900 (1987) 10–16
Elsevier

BBA 73564

Defective Ca^{2+}-pumping ATPase of heart sarcolemma from cardiomyopathic hamster

Tuan H. Kuo, Wayne Tsang and Joseph Wiener

Department of Pathology, Wayne State University School of Medicine, Detroit, MI (U.S.A.)

(Received 17 November 1986)

Key words: ATPase, Ca^{2+}-; Cardiomyopathy; Sarcolemma; Calcium ion transport; (Rat heart)

The Syrian cardiomyopathic hamster has a hereditary disease characterized by a progressive myocyte necrosis and intracellular calcium overload. Several systems in the heart sarcolemma that regulate the rate of Ca^{2+} entry or efflux were examined. There is a selective decrease of Ca^{2+}-pumping ATPase activity in the heart sarcolemma of 40-day-old myopathic hamsters, while the Na^+–Ca^{2+} exchange system and the ouabain-sensitive ($Na^+ + K^+$)-ATPase activity remain intact. This age-dependent decrease in Ca^{2+}-ATPase activity closely parallels the time course of lesion development. Both the affinity for Ca^{2+} (K_m) and the maximal velocity (V_{max}) of the Ca^{2+}-dependent ATP hydrolysis are altered. In addition, there is also an increased number of calcium channel receptor binding sites. Thus the data suggest that the imbalance in Ca^{2+} fluxes across the cardiac plasma membrane may be involved in the pathogenesis of this cardiomyopathy.

Introduction

The hereditary cardiomyopathy of the Syrian hamster is characterized by a very active period of muscle cell death that begins after 30 days with maximal lesions occurring at 60 days of age [1–3]. This is then followed by cardiac hypertrophy, congestive failure and death after 7 months of age [3]. Based on the findings that calcium channel blockers such as verapamil or D600 are effective in preventing the development of necrosis and that there is abnormally high calcium content in the cardiac mitochondria from myopathic hamsters [2,4–6], it has been suggested that this cardiomyopathy is primarily a membrane disease and that intracellular calcium overload may play a role in its pathogenesis [7]. However, very little information is available regarding the calcium homeostasis at the cardiac plasma membrane level in these hamsters. In this report, evidence is presented that a defective sarcolemmal Ca^{2+}-pumping ATPase may be involved in the development of cardiac necrosis in myopathic hamsters.

Correspondence: T.H. Kuo, Department of Pathology, Wayne State University School of Medicine, 540 E. Canfield Avenue, Detroit, MI 48201, U.S.A.

Materials and Methods

Genetically myopathic hamsters Bio 53:58 (female) and age-matched, random bred controls were used.

Isolation of sarcolemma-enriched preparation. Hamster cardiac sarcolemma was prepared according to Kidwai et al. [8] except that protease inhibitors (0.1 mM PMSF, 1 μg/l leupeptin) were included in both the isolation medium and throughout the preparative procedure. Briefly, hearts from four hamsters (1–2 g) were homogenized twice with a polytron PT-20 set at low speed for 5 s in 10% (w/w) sucrose; the homogenate was

filtered through four layers of cheese cloth and then nylon mesh 150. The filtrate was centrifuged at $100\,000 \times g$ for 30 min. The pellet was resuspended in 10% sucrose and applied to a sucrose gradient (30–56% w/w) and centrifuged at $100\,000 \times g$ for 2 h (SW rotor 25). The top band in the gradient that contained sarcolemmal vesicles (density 1.13–1.14) was collected, washed and centrifuged. The final pellet was suspended in 160 mm KCl and 20 mM Tris-HCl (pH 7.4) and stored at $-80\,°C$ until use. The characterization of this preparation indicates that it is approximately 20-fold enriched in ouabain-sensitive ($Na^+ + K^+$)-ATPase as compared to the homogenate. The preparation is free of mitochondrial and nuclear membrane contamination, but not completely free of sarcoplasmic reticulum contamination.

Enzyme assays. The Ca^{2+}ATPase activity of hamster cardiac sarcolemma was measured according to Caroni and Carafoli [9] with modification. Sarcolemmal vesicles (2 μg protein) were preincubated at 37 °C in 1 ml of medium containing 160 mM Tris-HCl (pH 7.4), 1 μg oligomycin, 0.5 mM $MgCl_2$, 10 μM digitoxigenin, 1 μM ionophore A23187, 0.4 mM EGTA, with either no $CaCl_2$ added or with 5 μM free Ca^{2+} as determined by Ca^{2+}-selective electrode. After 5 min, the reaction was started by the addition of 0.5 mM ATP. The P_i released was assayed by colorimetric determination [10]. Ca^{2+}-dependent ATPase activity was calculated by subtracting values obtained in the presence of chelator alone from those obtained with chelator plus Ca^{2+}. This activity is termed total activity. It comprises both the Ca^{2+}-ATPase of sarcolemma and the Ca^{2+}-ATPase from sarcoplasmic reticulum contamination.

The two activities can be distinguished by their different sensitivity toward vanadate inhibition [9]. The K_i (vanadate) for sarcolemmal enzyme is approximately 1.5 μM (our data), whereas the K_i (vanadate) for the sarcoplasmic reticulum enzyme is greater than 50 μM [11]. Thus the assays were carried out in the presence and absence of 3 μM vanadate. Only the vanadate (3 μM) inhibitable activity was referred as the Ca^{2+}-pumping ATPase activity of the plasma membrane.

Ouabain-inhibitable ($Na^+ + K^+$)-ATPase activity was measured according to Besch et al. [12] after pretreatment of the membrane vesicles (0.4 mg protein/ml) with SDS (0.3 mg/ml). 5′-Nucleotidase was measured as described by Mansier et al. [13]. Protein was measured according to Bradford [14] using bovine serum albumin as standard.

Estimation of membrane sideness. In ($Na^+ + K^+$)-ATPase assays in which membrane sideness and leakiness were determined, the procedure of Caroni and Carafoli [15] was used. Ouabain-sensitive ($Na^+ + K^+$)-ATPase activity observed in the presence of SDS pretreatment was defined as total activity, while the activity observed in the absence of SDS pretreatment was considered as due to 'leaky vesicles'. The digitoxigenin-sensitive fraction of the activity observed in the absence of SDS pretreatment but in the presence of 5 μM monensin, was due to 'leaky' and inside out vesicles.

Measurement of Ca^{2+} transport in the sarcolemmal vesicles. ATP-dependent Ca^{2+} uptake was measured isotopically as described by Caroni and Carafoli [9]. The Ca^{2+} accumulation due to the operation of the Na^+–Ca^{2+} exchange system was measured by the method of Pitts [16]. All experiments had blanks that contained a 160 mM NaCl, 20 mM Mops (pH 7.4) incubation medium in order to eliminate the Na^+ gradient from inside to outside of the vesicles. The low values of $^{45}CaCl_2$ uptake (passive Ca^{2+} binding) observed in NaCl medium were subtracted from the values determined in KCl medium to obtain Ca^{2+} uptake due to Na^+–Ca^{2+} exchange.

Membrane lipid analysis. Membranes (1 mg protein) were mixed with 3.75 ml of chloroform/methanol (1 : 2, v/v). Phospholipids were extracted using the method described by Lapetina and Michell [17]. After drying under a stream of N_2, phospholipid extracts were redissolved in chloroform/methanol (2 : 1, v/v) and spotted on precoated Silica gel 60 thin-layer chromatography plates (Merck). Chromatographs were developed in one dimension using chloroform/methanol/acetic acid/water (100 : 30 : 35 : 3, v/v) according to Thomas and Williamson [18]. This solvent system allows the complete separation of sphingomyelin, phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, phosphatidic acid, and cardiolipin.

The individual spots were detected in I_2 vapor and then removed for phosphate determination.

Results

General characteristics of the heart sarcolemma

Various procedures [12,13,16] for the isolation of heart sarcolemma were tested. The method of Kidwai et al. [8] was chosen because of its suitability for small animals, where only a limited supply of hearts were available from which to obtain sarcolemmal preparations of reasonable yield and purity. A comparison of the sarcolemmal ($Na^+ + K^+$)-ATPase activity of the rat heart preparation obtained by Kidwai's procedure or by the more recent method of Mansier et al. [13] was carried out. Both methods yielded preparations that had a ouabain-sensitive ($Na^+ + K^+$)-ATPase in the range of 120–140 μmol/mg per h. This suggests that the preparations from the two methods have similar purity.

Table I shows the general characteristics of the heart sarcolemma from control and myopathic hamsters at 2 months of age, the time when maximal heart lesions occur. The results indicate that there is a significant decrease (48%) of the protein yield in the sarcolemma preparation from the myopathic hearts as compared to controls. This presumably is a result of the severe myocytolysis occurring in myopathic hearts. However, the assay of sarcolemmal enzyme markers indicates that there is no change in the specific activity of either 5′-nucleotidase or ouabain-sensitive ($Na^+ + K^+$)-ATPase. The purity factor of the preparation from the control group was also similar to that from the myopathic animals.

Estimation of the membrane sideness (see Methods) suggested that both preparations from control and myopathic hamsters were frequently represented by sarcolemmal vesicles that were approx. 23% leaky, 77% sealed. Of the sealed vesicles, approx. 17% were inside-out (IO) and 83% were right-side-out (RO). Thus it appears that the sarcolemmal preparations from control and myopathic hearts showed similar membrane orientation as well as ouabain-sensitive ($Na^+ + K^+$)-ATPase activity.

Na^+–Ca^{2+} exchange and Ca^{2+}-ATPase activities

Two systems that transport Ca^{2+} across sarcolemma were examined in the present study. A specific Ca^{2+}-ATPase that has high affinity for Ca^{2+} but a low pumping velocity [9], and a Na^+/Ca^{2+} exchanger that has a low affinity but high capacity for Ca^{2+} [15] were suggested to operate in the Ca^{2+} efflux. Table II shows the sarcolemmal activities of these two systems from hearts of control and myopathic hamsters at 2 months of age. The results indicated no discernible change in the Na^+–Ca^{2+} exchange activity of the preparation from myopathic hamsters as compared to controls. However, the assay of Ca^{2+}-ATPase activities (see Methods) showed that both the total activity as well as the 3 μM vanadate-sen-

TABLE I

GENERAL CHARACTERISTICS OF HEART SARCOLEMMA FROM CONTROL AND MYOPATHIC HAMSTERS AT 2 MONTHS OF AGE

Values are means ± S.D. of four experiments. ($Na^+ + K^+$)-ATPase was measured as activity sensitive to 1 mM ouabain after treatment of sarcolemma vesicles with SDS (see Method). Purity factor was calculated as the ratio of ouabain-sensitive ($Na^+ + K^+$)-ATPase activity in sarcolemmal membrane and heart homogenate.

	Control	Myopathic
Protein yield (μg/g heart)	377 ± 75	196 ± 40 *
5′-Nucleotidase (μmol/mg/h)	270 ± 51	267 ± 55
($Na^+ + K^+$)-ATPase (μmol/mg/h)	89 ± 5	85 ± 4
Purity factor	25 ± 2	26 ± 2

* Significantly different from control value ($P < 0.005$).

TABLE II

Na^+–Ca^{2+} EXCHANGE AND Ca^{2+}-ATPase ACTIVITIES IN HEART SARCOLEMMA FROM CONTROL AND MYOPATHIC HAMSTERS AT 2 MONTHS OF AGE

Values are means ± S.D. of four experiments (see Methods).

	Control	Myopathic
Na^+–Ca^{2+} exchange (nmol/mg/30 s)	30.0 ± 3.2	33.0 ± 5.0
Total Ca^{2+}-ATPase (μmol/P_i/mg/h)	65.4 ± 12.4	22.7 ± 4.8 *
3 μM vanadate-sensitive Ca^{2+}-ATPase (μmol/P_i/mg/h)	48.0 ± 4.2	13.6 ± 2 *

* Significant different from control value ($P < 0.001$).

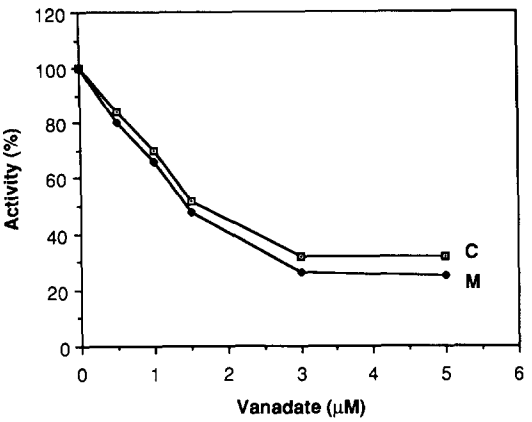

Fig. 1. The inhibition of the Ca^{2+}-ATPase activity by vanadate. Total Ca^{2+}-ATPase activity (expressed as the initial rate of Ca^{2+}-dependent ATP hydrolysis) was estimated as described in Methods. C, control; M, myopathic. 100% activity corresponds to 70 μmol P_i/mg per h. Free Ca^{2+} concentration: 5.0 μM.

sitive activity from the myopathic preparation were depressed by 3–4-fold as compared to controls. Fig. 1 shows the inhibition of Ca^{2+}-ATPase activity by varying concentration of vanadate. The Ca^{2+}-dependent ATPase activities from both the control and myopathic preparationis were inhibited by vanadate in a similar fashion, with maximal inhibition (70–80%) occurring at 3 μM. The concentration of vanadate required for half-maximal inhibition (K_i) is approx. 1.5 μM. These results confirm the earlier report [9] indicating that sarcolemmal Ca^{2+}-ATPase can be distinguished from the sarcoplasmic reticulum Ca^{2+}-ATPase by its high sensitivity toward vanadate inhibition. While the sarcolemmal enzyme can be inhibited by 3 μM vanadate, the reported K_i (vanadate) for the sarcoplasmic reticulum enzyme is about 50 μM [11]. Thus it is valid to use 3 μM vanadate-sensitive activity as an estimation of sarcolemmal Ca^{2+}-ATPase activity.

Since the sarcolemmal Ca^{2+}-dependent ATPase activity is coupled to ATP-dependent Ca^{2+} uptake [9], comparison of the sarcolemmal ATP-dependent Ca^{2+} uptake (see Methods) between the preparations of control and myopathic hamsters were also carried out. The results (not shown) again indicated a significant decrease in the Ca^{2+} transport activity in the preparation from the 2 months old myopathic hamsters.

Kinetic properties of the Ca^{2+}-ATPase of heart sarcolemma

The heart sarcolemma preparations from control and myopathic hamsters were studied for the dependence of the initial rate of ATP hydrolysis on the external free Ca^{2+} or ATP concentration. Fig. 2 shows the effect of varying Ca^{2+} concentrations on the Ca^{2+}-ATPase (3 μM vanadate-sensitive) activity. It is evident that the two preparations from control and myopathic groups differ greatly in their Ca^{2+} requirement (K_m) as well as maximal velocity (V_{max}) of ATP hydrolysis. The Lineweaver-Burk plots (not shown) indicate a V_{max} of 50 μmol/mg per h for the control group and a V_{max} of 14 μmol/mg per h for the myopathic group. Furthermore, there is an increase in the Ca^{2+} requirement to reach half-maximal velocity, i.e. the K_m (Ca) for the myopathic group is increased to 0.64 μM as compared to the controls ($K_m = 0.14$ μM). Fig. 3 shows the effect of varying the ATP concentration on the Ca^{2+}-ATPase activity when the external free Ca^{2+} concentration is kept at 5 μM. Half-maximal activation is seen at approximately 0.16 mM ATP for both control and myopathic groups. However, the V_{max} (ATP) is again reduced in the myopathic group (13 μmol/mg per h) as compared to controls (51 μmol/mg per h).

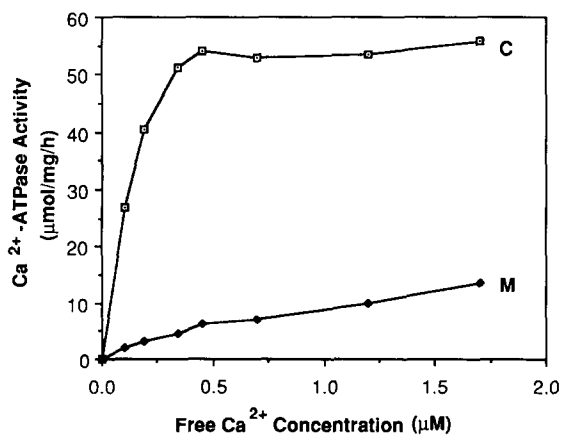

Fig. 2. The effect of free Ca^{2+} concentration on the sarcolemmal Ca^{2+}-ATPase activity. Sarcolemmal Ca^{2+}-ATPase activity (3 μM vanadate-sensitive) was estimated as described in Methods. Free Ca^{2+} concentration (obtained by using EGTA-Ca^{2+} buffer system) was determined by a calcium-selective electrode. C, control; M, myopathic.

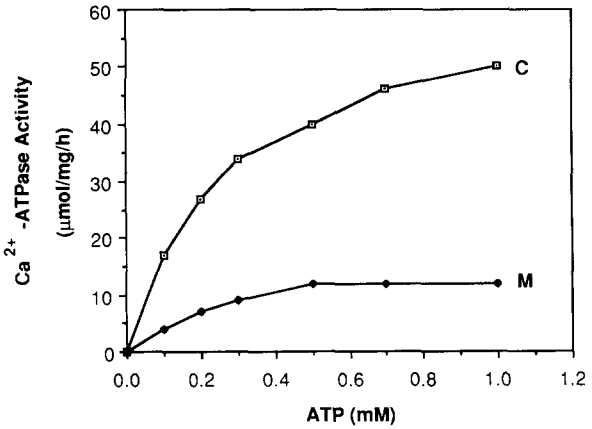

Fig. 3. The effect of ATP concentration on the sarcolemmal Ca^{2+}-ATPase activity. Sarcolemmal Ca^{2+}-ATPase activity (3 μM vanadate-sensitive) was estimated as described in Methods. C, control; M, myopathic.

Sarcolemmal Ca^{2+}-ATPase activity and lesion development. The relationship between Ca^{2+}-ATPase activity and lesion development in the myopathic hamster was studied by using heart sarcolemmal preparations from myopathic and control hamsters of various ages. The results (Fig. 4) indicate that while the Ca^{2+}-ATPase (3 μM vanadate-sensitive) activity of the control group remains constant with age, there is a decrease of enzyme activity in the myopathic preparation after 1 month that reaches a plateau after 2 months of age. This age-dependent decrease in Ca^{2+}-ATPase activity closely parallels the time-course of lesion development [5]. Since the assay of 5′-nucleotidase indicates no differences between the myopathic and control groups throughout the same time span of cardiomyopathy (1–5 months), this eliminates the possibility of membrane preparation artifact as a cause of depressed Ca^{2+}-ATPase activity in the myopathic samples. Furthermore the assay of other ion transporters such as $(Na^+ + K^+)$-ATPase and Na^+–Ca^{2+} exchange systems also indicate no alteration in sarcolemma preparations from 2 and 3 months old myopathic hamsters when the greatest number of lesions occurs. Taken together, these results suggest a specific correlation of the sarcolemmal Ca^{2+}-ATPase activity with lesion development.

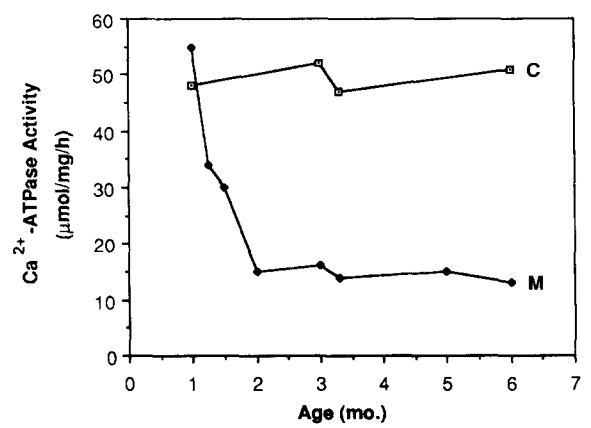

Fig. 4. Sarcolemmal Ca^{2+}-ATPase activity vs. age of hamsters. Heart sarcolemmal preparations were obtained from control (C) and myopathic (M) hamsters at various ages and assayed for 3 μM vanadate-sensitive Ca^{2+}-ATPase activity.

Discussion

The Syrian cardiomyopathic hamster is an useful animal model that mimics human forms of cardiac hypertrophy and failure [3]. Calcium overload of myocytes has been implicated in the pathogenesis of the cardiomyopathy in these animals [7]. The calcium concentration in cardiac myocytes of these hamsters is elevated [6]. Earlier work from this laboratory has identified and purified a calcium-activated protease that together with a mast cell protease may mediate the necrosis of myocytes [5,19]. More recent work is directed at the study of the possible cause of calcium overload in these hamsters. Presumably the calcium overload is the result of an imbalance between the rate of Ca^{2+} entry and rate of efflux across cardiac sarcolemmal membranes. There are two calcium transporters that regulate the rate of Ca^{2+} exit from the cell, i.e. the high-affinity, low-capacity Ca^{2+}-ATPase and the low-affinity, high-capacity Na^+–Ca^{2+} exchange system. In the present study, we report a selective decrease of the Ca^{2+}-pumping ATPase activity in the heart sarcolemma of myopathic hamsters without the alteration of the Na^+–Ca^{2+} exchange system.

Heart sarcolemma preparations were obtained from control and myopathic hamsters by a linear sucrose gradient method [8]. Because both preparations have similar membrane orientation, this fact allows meaningful comparison of Ca^{2+}-pumping ATPase activity in the sarcolemmal vesicles of the two groups. The activity of the Ca^{2+}-pumping

ATPase of the cardiac sarcolemma can be expressed either as the ATP-dependent Ca^{2+} uptake or as the Ca^{2+}-dependent ATP hydrolysis. The two activities are expressions of the same enzyme located in the sarcolemmal membrane [9]. In the present study, we have demonstrated that both activities are depressed in preparations from myopathic hamsters. However, the assay of Ca^{2+}-dependent ATP hydrolysis is more accurate than that of ATP-dependent Ca^{2+} uptake. This is because in the former case, assays have been carried out with and without the presence of 3 μM vanadate to enable the distinction of sarcolemmal vs. sarcoplasmic reticulum Ca^{2+}-ATPase, whereas in the latter case no such distinction has been made due to the limitation of membrane material that is available. Thus only data from the ATP hydrolysis were used for quantitative comparisons between the myopathic and control hamsters.

The sarcolemmal Ca^{2+}-ATPase activity in the present study can be distinguished from the enzyme of sarcoplasmic reticulum origin by its increased sensitivity toward vanadate inhibition (Fig. 1). Furthermore, it is also inhibitable (data not shown) by 30 μM trifluoroperazine, a calmodulin antagonist. These characteristics have been employed in the present study for positive identification of the Ca^{2+}-pumping ATPase of heart sarcolemma [9]. This point is emphasized because most heart sarcolemma preparations including that used in the present study are not 100% pure, and they are invariably contaminated by sarcoplasmic reticulum.

The alteration of the sarcolemmal Ca^{2+}-ATPase activity without affecting the ouabain-sensitive $(Na^+ + K^+)$-ATPase activity in myopathic hamsters during the early phase of lesion development (40–60 days, Table II and Fig. 4) is in contrast to the study of Makino et al. [20]. They have reported a significant depression of $(Na^+ + K^+)$-ATPase without alteration in Ca^{2+}-stimulated ATPase in 40 day old myopathic hamsters. However, a close comparison of the assay conditions indicates that the $(Na^+ + K^+)$-ATPase activity as defined by Makino et al. [20] is not the same as the ouabain-sensitive $(Na^+ + K^+)$-ATPase activity as described by Besch et al. [12], where an unmasking agent such as SDS must be used in order to reveal the latent enzyme activity.

The selective decrease of sarcolemmal Ca^{2+}-ATPase activity in the myopathic hamster reported here is the first report where the calcium-pumping ATPase activity of the myopathic hamster is well identified and characterized. The alterations in both the calcium affinity (K_m) and maximal velocity (V_{max}) suggest possible structural changes or altered regulation of this enzyme in myopathic hamster. It has been shown that the Ca^{2+}-ATPase of plasma membrane can be stimulated by phospholipids such as phosphatidylinositol 4,5-bisphosphate [21]. A comparison of the phospholipid composition of the cardiac plasma membrane from myopathic and control hamsters (Table III) indicates significant changes in the proportion of phosphatidylinositol as well as phosphatidylcholine and phosphatidylserine. Thus it is conceivable that the depressed Ca^{2+}-ATPase activity can also be the result, at least in part, of altered regulation by the membrane phospholipid. In addition to phospholipids, other factors such as calmodulin, fatty acids and phosphorylation-dephosphorylation cycle may also be important in the altered regulation of Ca^{2+}-ATPase.

The parallel relationship between the decrease in enzyme activity and lesion development (Fig. 4) suggests that calcium overload is closely associated with the myocyte necrosis. This suggestion is

TABLE III

PHOSPHOLIPID COMPOSITION OF CARDIAC PLASMA MEMBRANE FROM MYOPATHIC AND CONTROL HAMSTERS (3–5 MONTHS OLD)

Data are expressed as means ± S.D. of four experiments.

	Control	Myopathic
Total phospholipid (nmol/mg protein)	1112 ±146	1119 ±157
Phospholipid composition [a]		
Sphingomyelin	7.5± 2.3	12.7± 3.8
Phosphatidylcholine	45.2± 0.6	40.1± 0.4 *
Phosphatidylinositol	9.3± 0.8	7.1± 0.5 *
Phosphatidylserine	4.8± 0.3	7.0± 0.4 *
Phosphatidylethanolamine	27.2± 4.0	27.9± 4.2
Phosphatidic acid	3.3± 2.3	2.8± 2.3
Cardiolipin	2.2± 2.0	1.5± 1.6

[a] Values of phospholipid composition are expressed as nmol% of total phospholipid phosphorus.

* Significantly different from controls ($P < 0.01$).

supported additionally by the increased calcium channel receptors (B_{max}) in the heart of myopathic hamsters as first reported by Wagner et al. [22] and now confirmed by us (Myopathic: $B_{max} = 523 \pm 115$ fmol/mg; Control: 380 ± 38 fmol/mg ($n = 4$)). The concurrent increase in the calcium channel receptor binding to [^{3}H]PN 200–110 and decrease in Ca^{2+}-pumping ATPase activity suggest an imbalance of calcium fluxes across the cardiac plasma membrane. Whether calcium overloading is the primary cause of cardiomyopathy in these hamsters still requires additional experimentation.

Acknowledgment

Supported in part by United States Public Health Service Research Grants HL 18824 and HL 23603 from the Heart, Lung and Blood Institute, National Institutes of Health.

References

1 Bajusz, E. (1969) Am. Heart J. 77, 686–696
2 Jasmin, G. and Eu, H.Y. (1979) Ann. N.Y. Acad. Sci. 317, 46–58
3 Strobeck, J.E., Factor, S.M., Bhan, A., Sole, M., Liew, C.C., Fein, F. and Sonnenblick, E.H. (1979) Ann. N.Y. Acad. Sci. 317, 59–88
4 Bajusz, E. and Lossnitzer, K. (1968) Trans. N.Y. Acad. Sci. 30, 939
5 Kuo, T.H., Ho, K.L. and Wiener, J. (1984) Biochem. Med. 32, 207–215
6 Wrogemann, K., Haward, A.K. and Blanchaer, M.D. (1979) Ann. N.Y. Acad. Sci. 317, 30–45
7 Fleckenstein, A. (1983) in Calcium Antagonism in Heart and Smooth Muscle, pp. 153–164, John Wiley, New York
8 Kidwai, A.M., Radcliffe, M.A., Duchon, G. and Daniel, E.E. (1971) Biochem. Biophys. Res. Commun. 45, 901–910
9 Caroni, P. and Carafoli, E. (1981) J. Biol. Chem. 256, 3263–3270
10 Zak, B., Epstein, E. and Baginski, E.S. (1977) Ann. Clin. Lab. Sci. 7, 169–172
11 Wang, T., Tsai, L.-I. Solaro, R.J., Grassi de Gende, A.O. and Schwartz, A. (1979) Biochem. Biophys. Res. Commun. 91, 356–361
12 Besch, H.R., Jr., Jones, L.R., Fleming, J.W. and Watanabe, A.M. (1977) J. Biol. Chem. 252, 7905–7908
13 Mansier, P., Charlemagne, D., Rossi, B., Preteseille, M., Swynghedauw, B. and Lelievre, L. (1983) J. Biol. Chem. 158, 6628–6635
14 Bradford, M.M. (1976) Anal. Chem. 72, 248–254
15 Caroni, P. and Carafoli, E. (1983) Eur. J. Biochem. 132, 451–460
16 Pitts, B.J.R. (1979) J. Biol. Chem. 254, 6232–6235
17 Lapetina, E.G. and Michell, R.H. (1972) Biochem. J. 126, 1141–1147
18 Thomas, A.P. and Williamson, J.R. (1983) J. Biol. Chem. 258, 1411–1414
19 Spalla, M., Kuo, T.H. and Wiener, J. (1987) Muscle Nerve 10, 54–59
20 Makino, N., Jasmin, G., Beamish, R.E. and Dhalla, N.S. (1985) Biochem. Biophys. Res. Commun. 133, 491–497
21 Choquette, D., Hakim, G., Filoteo, A.G., Plishker, G.A., Bostwick, J.R. and Penniston, J.T. (1984) Biochem. Biophys. Res. Commun. 125, 908–915
22 Wagner, J.A., Reynolds, I.J., Weisman, H.F., Dudeck, P., Weisfeldt, M.L. and Snyder, S.H. (1986) Science 232, 515–518