Biochimica et Biophysica Acta, 1064 (1991) 49–54

ADONIS 000527369100169C

BBAMEM 75155

Influence of the 53 kDa glycoprotein on the cooperativity of the Ca^{2+}-ATPase of the sarcoplasmic reticulum

Howard Kutchai, Kimberly Boyd, Qing Xu and Christopher P. Weis *

Department of Physiology and Biophysics Program, University of Virginia, Charlottesville, VA (U.S.A.)

(Received 11 July 1990)
(Revised manuscript received 16 October 1990)

Key words: Sarcoplasmic reticulum; ATPase, Ca^{2+}-; Glycoprotein, 53 kDa; Cooperativity

Previous results from this laboratory suggest that the 53 kDa glycoprotein (GP-53) of rabbit skeletal muscle sarcoplasmic reticulum membrane (SR) may influence coupling between Ca^{2+} transport and ATP hydrolysis by the Ca^{2+}-ATPase. Here we report evidence that GP-53 may influence the cooperative behavior of the Ca^{2+}-ATPase. The ATPase activity of the Ca^{2+}-ATPase displays negative cooperative dependence (Hill coefficient $n < 1$) on [MgATP] and has positive cooperative dependence ($n > 1$) on $[Ca^{2+}]_{free}$. We have determined the degree of cooperativity for native SR vesicles, SR preincubated with antiserum against GP-53 or preimmune serum, and SR partially extracted with KCl-cholate. Our results show that SR preincubated with preimmune serum or SR treated with cholate in 50 mM KCl (yielding membranes rich in GP-53) demonstrate a cooperative dependence of Ca^{2+}-ATPase activity on both [ATP] and $[Ca^{2+}]$ similar to that of untreated SR. SR preincubated with anti-GP-53 antiserum (which causes an uncoupling of Ca^{2+} transport from ATP hydrolysis) or SR extracted with cholate in 1 M KCl (yielding membranes depleted of GP-53) displays decreased positive cooperative dependence on $[Ca^{2+}]$ and decreased negative cooperative dependence on [ATP]. The results are consistent with the interpretation that GP-53 may influence the cooperative behavior of the Ca^{2+}-ATPase.

Introduction

The 53 kDa glycoprotein (GP-53), a protein present in the sarcoplasmic reticulum (SR) of all vertebrate muscle that has been examined [1,2], was first identified by Michalak et al. [3] and further characterized by Campbell and MacLennan [4,5]. Chiesi and Carafoli [6] reported that when trifluoperazine binds to GP-53, the affinity of the sarcoplasmic reticulum Ca^{2+}-ATPase for Ca^{2+} is diminished. Chiesi and Carafoli proposed that GP-53 may function to modulate the activity of the Ca^{2+}-ATPase. Leonards and Kutchai [7] found that extraction of sarcoplasmic reticulum (SR) from rabbit skeletal muscle with cholate in the presence of KCl (0.3 M or higher) produced a preparation with normal Ca^{2+}-ATPase activity, but greatly diminished ability to actively transport Ca^{2+}. SDS-polyacrylamide gels of the cholate extracted SR showed that calsequestrin and GP-53 had been largely removed, but that other major protein components of the SR membrane remained. Kutchai and Campbell [8] found that incubating SR with an antiserum against GP-53 markedly inhibited active Ca^{2+} uptake, but had no effect on Ca^{2+}-stimulated ATPase activity. These findings are consistent with the interpretation that GP-53 can influence the function of the Ca^{2+}-ATPase. The cDNA that encodes GP-53 was cloned and sequenced by Leberer et al. [9].

Research by a number of investigators has described the cooperative behavior that characterizes the Ca^{2+}-ATPase of the SR. The dependence of Ca^{2+}-ATPase activity of SR on [ATP] displays negative cooperativity

* Present address: U.S. Environmental Protection Agency, 999 18th Street, Denver Center, Suite 500, Denver, CO 80202-2405, U.S.A.

Abbreviations: SR, sarcoplasmic reticulum; GP-53, 53 kilodalton glycoprotein; GP-160, 160 kilodalton glycoprotein; ATP, adenosine 5′-triphosphate; n, Hill coefficient; SDS, sodium dodecyl sulfate; Mops, 3-(*N*-morpholino)propanesulfonic acid; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; EGTA, ethyeleneglycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; NADH, reduced nicotinamide adenine dinucleotide; K_m, Michaelis constant; Tris, tris(hydroxymethyl)aminomethane; $C_{12}E_8$, octaethyleneglycol dodecyl ether.

Correspondence: H. Kutchai, Department of Physiology, University of Virginia Health Sciences Center, Jordan Hall, Box 449, 1300 Jefferson Park Avenue, Charlottesville, VA 22908, U.S.A.

[10–12]. The dependence of Ca^{2+}-ATPase activity on $[Ca^{2+}]$ is positively cooperative [10,13,14]. Treating the SR with detergents has frequently been found to diminish the cooperativity of the Ca^{2+}-ATPase [11,15,16].

Dr. Patricia M. Sokolove (personal communication) extracted SR with deoxycholate in the presence of high KCl or low KCl. Both preparations had good Ca^{2+}-ATPase activity, but the high KCl preparation displayed less negative cooperativity of its dependence on [ATP]. SDS-polyacrylamide gels showed that the high KCl preparation had lost much of its GP-53. Kutchai et al. [17], in a preliminary study of the transient state kinetics of cholate-extracted SR, had also found evidence of diminished cooperativity in preparations from which GP-53 had been extracted.

The purpose of this study was to investigate the influence of GP-53 on the cooperative behavior of the Ca^{2+}-ATPase of the SR membrane. The dependence of Ca^{2+}-ATPase activity on [ATP] and $[Ca^{2+}]$ was studied in SR from which GP-53 had been extracted and in SR treated with an antiserum against GP-53. The results obtained are consistent with the interpretation that GP-53 may influence the cooperativity of the Ca^{2+}-ATPase.

The kinetic basis for the cooperative behavior of the Ca^{2+}-ATPase remains incompletely understood. Rigorous interpretations of Hill coefficients that are greater and less than 1.0 are not straightforward [18]. In this paper, for the sake of simplicity, Hill coefficients greater than 1.0 will be taken to indicate positively cooperative behavior and Hill coefficients less than 1.0 will be taken to indicate negatively cooperative behavior. This, with the understanding that the kinetic mechanisms which underlie the cooperative behavior remain to be elucidated.

Experimental Procedures

Materials

$^{45}CaCl_2$ was obtained from New England Nuclear Corp. The calcium ionophore A23187 was from Calbiochem. Phospho*enol*pyruvate, ATP, triethanolamine, EGTA, β-NADH, lactate dehydrogenase, pyruvate kinase, and other salts and buffers were purchased from Sigma Chemical Corporation. Chemicals for polyacrylamide gel electrophoresis were obtained from Bio-Rad. An antiserum against GP-53 produced in sheep [19], as well as preimmune serum, was generously provided by Dr. David H. MacLennan.

Preparation of sarcoplasmic reticulum vesicles

Sarcoplasmic reticulum (SR) vesicles were prepared from thigh muscle of adult rabbits by the method of Eletr and Inesi [20]. SR was stored at −70 °C in 10 mM Mops, 30% (w/v) sucrose (pH 7.0).

Protein analysis of SR vesicles

Protein concentrations were estimated by the method of Lowry et al. [21]. Protein composition was characterized with SDS-polyacrylamide slab gel electrophoresis by the method of Laemmli [22] using 5–15% acrylamide gels, with visualization of protein bands by Coomassie blue staining.

Cholate extraction of SR vesicles

SR vesicles were extracted with cholate in the presence of low (50 mM) or high (1 M) KCl [7].

The low KCl-cholate preparation. SR vesicles were diluted to a final protein concentration of 2.5 mg/ml with 250 mM sucrose, 100 mM NaCl, 50 mM potassium phosphate (pH 7.4), 1 mM NaN_3, 0.1 mM DTT (Buffer A) and incubated on ice for 10 min. A solution of cholate, 5% (w/v), was prepared in Buffer A, but at pH 8.0. The cholate had been recrystallized three times [23]. The cholate solution was added in five equal aliquots to the SR vesicle suspension, mixing gently after each addition, to a final protein : cholate ratio of 1 : 1 (w/w). The mixture was kept on ice for 30 min and then centrifuged at $100000 \times g$ for 60 min. The supernatant was discarded. The pellet was resuspended in Buffer A, recentrifuged, then suspended in 10 mM Mops, 30% (w/v) sucrose and stored at −70 °C.

The high KCl-cholate preparation. This preparation was made in the same way as the low KCl-cholate preparation except that solid KCl was added to the solubilization mixture to a final concentration of 1 M.

Preincubation of SR with antiserum or monoclonal antibody

SR vesicles were diluted in 2 volumes of 250 mM sucrose, 20 mM Tris (pH 7.4), 200 μM PMSF. SR vesicles were preincubated for 90 min at 37 °C with anti-GP-53 antiserum, preimmune serum, or normal sheep serum (3 μl serum/4 μg SR protein). The specificities of the anti-GP-53 antiserum [8,24,25] were previously demonstrated.

Assay of Ca^{2+}-ATPase activity

Ca^{2+}-ATPase activity was estimated by an enzyme-coupled assay in which ATP is regenerated from phospho*enol*pyruvate as it is broken down with concomitant oxidation of 1 mole of NADH per mole of ATP regenerated [26]. SR vesicles (8 to 12 μg protein) were added to 1 ml of assay buffer containing 85 mM triethanolamine (pH 7.2), 100 mM KCl, 12 mM $MgCl_2$, 0.5 mM EGTA, 1.5 mM phospho*enol*pyruvate, 1 mM NaN_3, 10 units lactate dehydrogenase, 15 units pyruvate kinase, and sufficient NADH to give an optical density at 340 nm of 1 to 1.5. Background activity was determined from the decline in absorbance at 340 nm in the presence of ATP, but with no added Ca^{2+}, then 0.55 μmoles of $CaCl_2$ was added and the increased rate of oxidation

of NADH recorded. Finally, the Ca^{2+} ionophore A23187 was added to a concentration of 6.5 μM, and the rate of NADH oxidation determined again. In experiments in which [ATP] was varied, an aliquot of 0.1 M $CaCl_2$ sufficient to bring the total Ca^{2+} added to 0.55 mM was added. In experiments in which $[Ca^{2+}]$ was varied, 3 mM Na_2ATP was used. The free $[Ca^{2+}]$ was estimated as described by Fabiato [27,28] using a computer program written and kindly provided by Dr. Chi-ming Hai of Brown University. The stability constants used for the binding of Ca^{2+}, Mg^{2+}, and H^+ to EGTA and for the binding of Ca^{2+}, Mg^+, H^+, Na^+, and K^+ to ATP were those given by Fabiato [28] for 22°C and pH 7.2.

Data analysis

The maximal Ca^{2+}-ATPase activity for each experiment was obtained by extrapolating a double-reciprocal plot of the data to infinite substrate concentration. The treatment of SR with cholate, sera, or monoclonal antibodies under the conditions described here produced relatively slight changes in the maximal Ca^{2+}-ATPase activity of the SR. To quantify the cooperativity of the dependence of Ca^{2+}-ATPase on [ATP] or [Ca^2, the data was fitted to Hill's equation:

$$V/V_{max} = \alpha K[S]^n/(1 + K[S]^n)$$

where in our case S is either ATP or Ca^{2+}. Data are displayed on 'Hill plots': $\log[V/V_{max} - V)]$ vs. log[S]. Values of n, the Hill coefficient, for the dependence of Ca^{2+}-ATPase activity on [ATP] and $[Ca^{2+}]$ were estimated by fitting Hill's equation to the data by a nonlinear least-squares procedure [29] using the Gauss-Newton method.

Results

Dependence of Ca^{2+}-ATPase activity on [ATP]

Fig. 1 shows Hill plots of data for control SR, for SR treated with cholate in low K^+ (a preparation rich in GP-53), and for SR treated with cholate in high K^+ (a preparation depleted of GP-53). The data for all three preparations have Hill coefficients less than 1.0, consistent with the negative cooperative dependence on [ATP] that has been described by several other investigators. When this data is plotted on a double-reciprocal plot, a curve to which two straight lines can be fitted is obtained [12]. This suggests that ATP is hydrolyzed with a low K_m at low [ATP] and with a significantly higher K_m at higher [ATP]. When Hill's equation is fitted to such data, a value of n less than 1 is obtained, which is taken to indicate negative cooperativity in the activation by ATP of its own hydrolysis by the Ca^{2+}-ATPase of the SR; the greater the difference between 1 and n, the greater the degree of negative cooperativity.

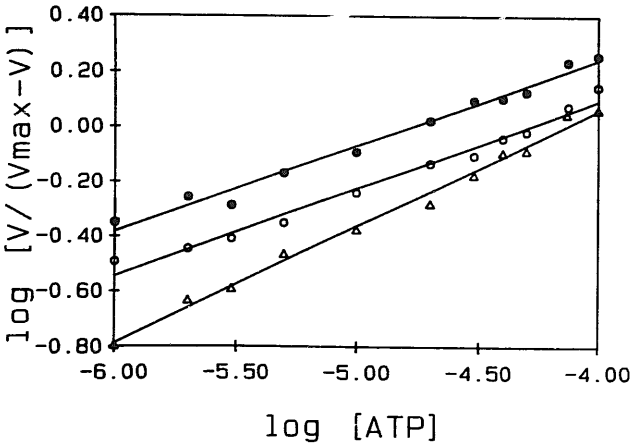

Fig. 1. Hill plots of data on the dependence of Ca^{2+}-ATPase activity on [ATP] was for untreated SR (○), for SR extracted with cholate in 50 mM KCl (●), and for SR extracted with cholate in 1 M KCl (△) as described in the text. The SR vesicles (8 to 12 μg protein) were added to 1 ml of assay buffer containing 85 mM triethanolamine (pH 7.2), 100 mM KCl, 12 mM $MgCl_2$, 0.5 mM EGTA, 1.5 mM phospho*enol*-pyruvate, 1 mM NaN_3, 10 units lactate dehydrogenase, 15 units pyruvate kinase, and sufficient NADH to give an optical density at 340 nm of 1 to 1.5, and ATP at the concentrations shown on the abscissa. Background activity was determined from the decline in absorbance at 340 nm in the presence of ATP, but with no added Ca^{2+}, then 0.55 μmoles of $CaCl_2$ was added and the increased rate of oxidation of NADH recorded. Finally, the Ca^{2+} ionophore A23187 was added to a concentration of 6.5 μM, and the rate of NADH oxidation determined again.

As shown in Fig. 1, the data for untreated SR and for SR treated with cholate at low $[K^+]$ display a similar negative cooperativity, $n = 0.364$ and $n = 0.372$, respectively. The data for the SR treated with cholate in high $[K^+]$ displays cooperativity that is less negative: $n = 0.565$.

Fig. 2 shows Hill plots of data for control SR and for SR preincubated either with normal sheep serum or with anti-GP-53 antiserum. Preincubation of SR with preimmune serum results in a Hill coefficient of 0.425 that was similar to that control SR that was not preincubated ($n = 0.364$). Preincubation with anti-GP-53 antiserum, under conditions previously shown (and reconfirmed in this case) to produce a marked decrease in active Ca^{2+} transport by SR with only a modest impairment of Ca^{2+}-ATPase activity, results in a large decrease in the degree of negative cooperativity: $n = 0.650$.

Dependence of Ca^{2+}-ATPase activity on $[Ca^{2+}]$

In Fig. 3 we present Hill plots of data for untreated SR, SR treated with cholate in low $[K^+]$, and SR treated with cholate in high [K. The data displays the positive cooperativity ($n > 1$) that has been described by many other investigators. The data for untreated SR and for SR treated with cholate in low [K display a similar degree of positive cooperativity; $n = 1.95$ and 1.97, respectively. The data for SR treated with cholate

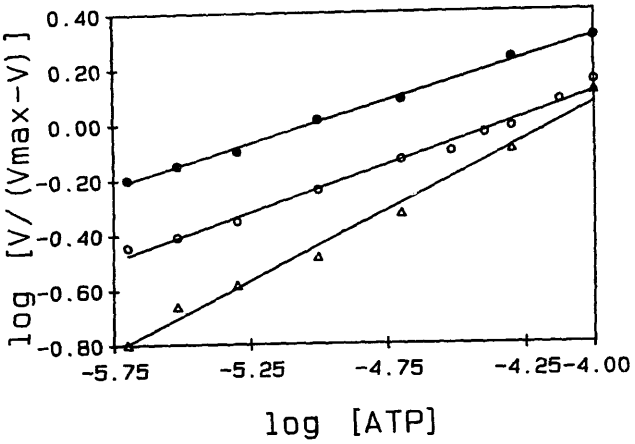

Fig. 2. Hill plots of the data on the dependence of Ca^{2+}-ATPase activity on [ATP] for untreated SR (○), for SR preincubated with normal sheep serum (●), and for SR preincubated with anti-GP-53 serum (△). SR vesicles were diluted in 2 volumes of 250 mM sucrose, 20 mM Tris (pH 7.4), 20 μM PMSF. SR vesicles were preincubated for 90 min at 37°C with anti-GP-53 antiserum or with normal sheep serum (3 μl serum/4 μg SR protein). Then the activity of Ca^{2+}-ATPase at various [ATP] was determined as described in the legend of Fig. 1.

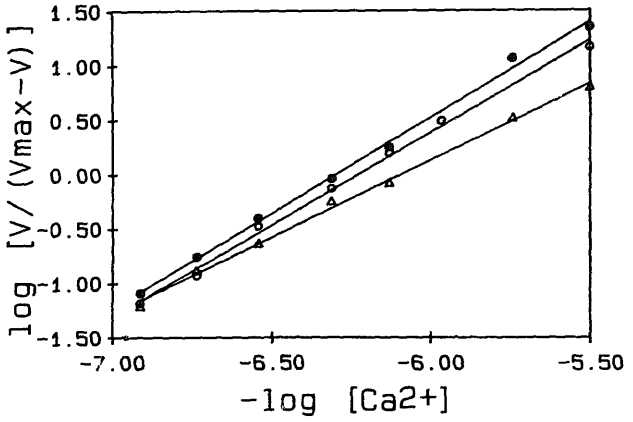

Fig. 4. Hill plots showing the effect of anti-GP-53 antiserum on the dependence of Ca^{2+}-ATPase activity on $[Ca^{2+}]_{free}$. SR vesicles were diluted in 2 volumes of 250 mM sucrose, 20 mM Tris (pH 7.4), 200 μM PMSF and then preincubated for 90 min at 37°C with preimmune serum (●) or with anti-GP-53 serum (△). Control SR (○) was not preincubated. In the preincubation mix 3 μl of preimmune serum or anti-GP-53 serum was used per 4 μg SR protein. Then the activity of Ca^{2+}-ATPase at various $[Ca^{2+}]$ was determined as described in the legend to Fig. 1 in the presence of 3 mM ATP.

in high $[K^+]$ displays significantly less positive cooperativity: $n = 1.47$.

Fig. 4 shows Hill plots of the data on the $[Ca^{2+}]$ dependence of Ca^{2+}-ATPase activity for SR preincubated for 90 min at 37°C with normal sheep serum

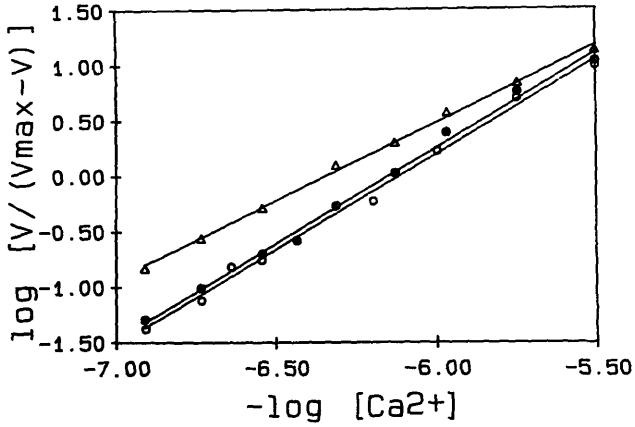

Fig. 3. Hill plots showing the dependence of Ca^{2+}-ATPase activity on $[Ca^{2+}]_{free}$ for untreated SR (○), SR extracted with cholate in 50 mM KCl (●), and for SR extracted with cholate in 1 M KCl (△) as described in the text. Ca^{2+}-ATPase activity at various $[Ca^{2+}]$ was determined as described in the legend of Fig. 1 in the presence of 3 mM ATP.

serum, with anti-GP-53 antiserum, and control SR that was not preincubated. SR preincubated with normal serum displays a somewhat decreased positive cooperativity ($n = 1.71$) compared to that of untreated SR ($n = 1.95$). SR preincubated with anti-GP-53 antiserum showed a substantial decrease in positive cooperativity with $n = 1.43$.

Estimated values of Hill coefficients

The Hill coefficient values obtained by nonlinear least-squares analysis, with estimates of their standard errors, are shown in Table I.

Discussion

We have attempted to perturb the function of GP-53 in two quite different ways: by removing most of the GP-53 present with detergent and by treating the SR with antiserum against GP-53. Both interventions led to similar effects on the cooperative behavior of the Ca^{2+}-ATPase; namely, to diminish the positively cooperative behavior with $[Ca^{2+}]$ and to decrease the negatively cooperative behavior with [ATP]. These two interventions had been previously shown to lead to diminished Ca^{2+} uptake with little effect on Ca^{2+}-ATPase activity.

TABLE I

Summary of Hill coefficient values (± standard errors) obtained for the various experimental conditions by a nonlinear-least-squares procedures [29] to fit Hill's equation to the data

Hill coeff.	Normal SR	SR plus preimm. ser.	SR plus antiserum	Lo K-cholate SR (high in GP-53)	Hi K-cholate SR (low in GP-53)
$n_{[Ca^{2+}]}$	1.95 ±0.03	1.71 ±0.02	1.43 ±0.03	1.97 ±0.03	1.47 ±0.03
$n_{[ATP]}$	0.364±0.007	0.425±0.008	0.650±0.013	0.372±0.007	0.565±0.011

Since numerous investigators [11,15,16] have found that treatment of SR with detergents diminishes cooperativity with respect to [ATP] and [Ca^{2+}], it is possible that the effects on SR treated with cholate, 1 M KCl may have been due to the effects of residual cholate, even though we attempted to remove cholate by centrifugation and resuspension of the SR, or to some permanent effect of detergent treatment, rather than to removal of GP-53. We regard as more convincing the decreased cooperativity observed in SR treated with anti-GP-53 antiserum, especially since the maximum rate of Ca^{2+}-ATPase activity was not significantly affected by this treatment.

In addition to changes in Hill coefficients in SR treated with cholate, 1 M KCl and with anti-GP-53 antiserum, there were also changes in the apparent affinity of the Ca^{2+}-ATPase for ATP and for Ca^{2+}. The [Ca^{2+}] required for half-maximal ATPase activity was similar for control SR and SR preincubated with normal serum, but higher [Ca^{2+}] was required for SR preincubated with anti-GP-53 serum. Control SR and SR treated with cholate, low KCl had similar [Ca^{2+}] required for half-maximal ATPase activity, but lower [Ca^{2+}] was required for SR treated with cholate, 1 M KCl. We could speculate on the meaning of these changes in the apparent affinity of the Ca^{2+}-ATPase for Ca^{2+}, but for reasons discussed in the next paragraph we prefer not to do so.

Changes were also observed in the [ATP] required for half-maximal ATPase activity. Preincubation with normal serum decreased the [ATP] required for half-maximal ATPase activity compared to control SR, while preincubation with anti-GP-53 antiserum slightly increased the [ATP] required. SR extracted with cholate in low KCl required lower [ATP] for half-maximal ATPase activity than control SR or SR treated with cholate, 1 M KCl. We attribute a significant portion of these variations in apparent affinity of the Ca^{2+}-ATPase for [ATP] to error. The activity of Ca^{2+}-ATPase rose quite rapidly at low [ATP], below about 20 μM, but at higher [ATP] Ca^{2+}-ATPase activity increased quite slowly with increasing [ATP]. This led to significant error in estimating V_{max}. Analysis of the linear transform of the Hill Equation shows that an error in V_{max} will result in a much greater influence on the calculated value of the [ATP] required for half-maximal velocity than on the calculated value of n. For example using the numerical values derived from the Hill plot for SR treated with cholate, low KCl, we found that a 10% alteration in V_{max} caused a 51% change in the calculated [ATP] required for half-maximal velocity, but caused only a 6% change in the computed value of n.

The results presented here, as well as the findings of Chiesi and Carafoli [6], Leonards and Kutchai [7], and Kutchai and Campbell [8], suggest that GP-53 can influence the function of the Ca^{2+}-ATPase. Some findings about GP-53 that are consistent with this interpretation, but do not bear directly on its possible function are: (i) GP-53 is located principally in the longitudinal SR, which is also the location where Ca^{2+}-ATPase is most prominent [30,31]; (ii) The biosynthesis of GP-53 and Ca^{2+}-ATPase appears to be coordinately regulated in muscle cells, both in vitro and in vivo [32,33]; (iii) GP-53 has a high-affinity nucleotide binding site [5,9]; and (iv) the existence of interactions between GP-53 and Ca^{2+}-ATPase in the SR membrane is supported by the finding that monoclonal antibodies against GP-53 can inhibit the rotational mobility of Ca^{2+}-ATPase in cytoplasmic-side-out SR vesicles [34].

There is significant evidence against the interpretation that GP-53 influences the function of Ca^{2+}-ATPase. Leberer et al. [9] expressed in COS-1 cells the cDNA that encodes Ca^{2+}-ATPase, with and without the cDNA encoding GP-53. Microsomes isolated from the transfected COS-1 cells displayed active Ca^{2+} uptake. The specific activity of Ca^{2+} uptake was the same in the presence and absence of of GP-53. It is not clear how to reconcile these findings with the results of Leonards and Kutchai [7] and Kutchai and Campbell [8]. The results of Leberer et al. [9] demonstrate that the presence of GP-53 is not required for coupling of Ca^{2+} transport to ATP hydrolysis (however, coupling ratios were not reported). Leberer et al. [9] report only the maximal rate of Ca^{2+} uptake. It is still possible that GP-53 can influence the affinity of Ca^{2+}-ATPase for Ca^{2+} as suggested by Chiesi and Carafoli [6] or modulate some other aspect of Ca^{2+}-ATPase function, such as its cooperativity, as the present data suggests. It is also possible that the influence of GP-53 on Ca^{2+}-ATPase requires the presence of another protein component of the SR that is not present in the COS-1 microsomes. A candidate for this additional protein is the 160 kDa glycoprotein (GP-160), which co-localizes with GP-53. Leberer et al. [35] have cloned and sequenced the cDNA for GP-160 and find that the C-terminal end of GP-160 is identical to GP-53 and that GP-160 has an N-terminal extension that binds 35 Ca^{2+} ions with high capacity, but low affinity [31,35].

The data presented here are consistent with the interpretation that GP-53 can influence the function of Ca^{2+}-ATPase in the SR membrane. It remains for future investigations to test this interpretation and to elucidate the mechanisms by which GP-53 influences the Ca^{2+}-ATPAse.

Acknowledgements

The idea for the experiments reported here arose from a personal communication by Dr. Patricia A. Sokolove of the University of Maryland, School of Medicine, Baltimore of her unpublished results; we are grateful for her generous collegiality. We acknowledge

the kindness of Drs. David H. MacLennan in supplying us with anti-GP-53 antiserum and preimmune serum. We are pleased to thank Dr. Chi-ming Hai for providing the computer program used to estimate free [Ca^{2+}] and Dr. Guillermo Romero for assistance in applying a nonlinear least-squares curve fitting routine to our data. This study was supported by a Grant-in-Aid from the American Heart Association, Virginia Affiliate (H.K.). K.B. and C.P.W. were supported by a training grant from the National Heart, Lung, and Blood Institute (T32 HL 07284).

References

1 Pepper, D.R., Raab, C.R. and Campbell, K.P. (1985) Biophys. J. 47, 344a
2 Timms, B.G. and Campbell, K.P. (1985) Biophys. J. 47, 344a.
3 Michalak, M., Campbell, K.P. and MacLennan, D.H. (1980) J. Biol. Chem. 255, 1317–1326.
4 Campbell, K.P. and MacLennan, D.H. (1981) J. Biol. Chem. 256, 4626–4632.
5 Campbell, K.P. and MacLennan, D.H. (1983) J. Biol. Chem. 258, 1391–1394.
6 Chiesi, M. and Carafoli, E. (1982) J. Biol. Chem. 257, 984–991.
7 Leonards, K.S. and Kutchai, H. (1985) Biochemistry 24, 4876–4884.
8 Kutchai, H. and Campbell, K.P. (1989) Biochemistry 28, 4830–4839.
9 Leberer, E., Charuk, J.H.M., Clarke, D.M., Green, N.M., Zubrzycka-Gaarn, E. and MacLennan, D.H. (1989) J. Biol. Chem. 264, 3484–3493.
10 Weber, A., Herz, R. and Reiss, L. (1966) Biochem. Z. 345, 329–369.
11 Inesi, G., Cohen, J.A. and Coan, C.R. (1976) Biochemistry 15, 5293–5298.
12 Yamamoto, T. and Tonomura, Y. (1967) J. Biochem. 62, 558–575.
13 Inesi, G., Goodman, J.J. and Watanabe, S. (1967) J. Biol. Chem. 242, 4637–4643.
14 De Meis, L. and Hasselbach, W. (1971) J. Biol. Chem. 246, 4759–4763.
15 Dean, W.L. and Tanford, C. (1978) Biochemistry 17, 1683–1690.
16 Fernandez-Belda, F., Kurzmack, M. and Inesi, G. (1984) J. Biol. Chem. 259, 9687–9698.
17 Kutchai, H., Campbell, K.P. and Froehlich, J.P. (1988) Biophys. J. 53, 133a.
18 Cornish-Bowden, A. (1976) Principles of Enzyme Kinetics, London, Butterworths.
19 Campbell, K.P. and MacLennan, D.H. (1982) J. Biol. Chem. 257, 1238–1246.
20 Eletr, S. and Inesi, G. (1972) Biochim. Biophys. Acta 282, 174–197.
21 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
22 Laemmli, U.K. (1970) Nature (London) 227, 680–685.
23 MacLennan, D.H. (1970) J. Biol. Chem. 245, 4508–4518.
24 Campbell, K.P., MacLennan, D.H., Jorgensen, A.O. and Mintzer, M.C. (1983) J. Biol. Chem. 258, 1197–1204.
25 Zubryzcka-Gaarn, E., Campbell, K.P. and MacLennan, D.H. (1983) J. Biol. Chem. 258, 4576–4581.
26 Madden, T.D., Chapman, D. and Quinn, P.J. (1979) Nature 279, 538–541.
27 Fabiato, A. and Fabiato, F. (1979) J. Physiol. (Paris) 75, 463–505.
28 Fabiato, A. (1981) J. Gen. Physiol. 78, 457–497.
29 Johnson, M.L. and Frasier, S.G. (1985) Methods Enzymol. 117, 301–342.
30 Jorgensen, A.O., Arnold, W., Pepper, D.R., Kahl, S.D. and Campbell, K.P. (1988) Cell Motil. Cytoskel. 9, 164–174.
31 Leberer, E., Timms, B.G., Campbell, K.P. and MacLennan, D.H. (1990) J. Biol. Chem. 265, 10118–10124.
32 Zubrzycka-Gaarn, E., Campbell, K.P., MacLennan, D.H. and Jorgensen, A.O. (1976) J. Biol. Chem. 258, 4576–4581.
33 Zubryzcka-Gaarn, E. and MacLennan, D.H. (1988) in Cellular Calcium in Health and Disease, pp. 133–139, Alan R. Liss, Baltimore.
34 Mahaney, J.E., Weis, C.P., Grisham, C.M. and Kutchai, H. (1991) Biochim. Biophys. Acta 1064, 55–68.
35 Leberer, E., Charuk, J.H.M., Green, N.M. and MacLennan, D.H. (1989) Proc. Natl. Acad. Sci. USA 86, 6047–6051.