J. Membrane Biol. 164, 169–175 (1998)

The Journal of
Membrane
Biology

Membrane Asymmetry in Isolated Canine Cardiac Sarcoplasmic Reticulum: Comparison with Skeletal Muscle Sarcoplasmic Reticulum

R.J. Bick[1], L.M. Buja[1], W.B. Van Winkle[1], G.E. Taffet[2]

[1]Department of Pathology and Laboratory Medicine, University of Texas-Houston Medical School, 6431 Fannin St., Houston, TX 77030, USA
[2]Section of Cardiovascular Sciences, Department of Medicine, Baylor College of Medicine, Houston, TX 77030, USA

Received: 19 December 1997/Revised: 3 April 1998

Abstract. Cardiac sarcoplasmic reticulum (CSR), isolated from dog hearts, was shown to be asymmetric in the distribution of phospholipids across the CSR bilayer. Phosphatidylethanolamine was mostly resident in the outer leaflet, phosphatidylcholine was equally distributed across both monolayers and phosphatidylserine was found primarily in the inner monolayer. This distribution of headgroups is similar to that found in fast skeletal muscle sarcoplasmic reticulum (SSR); however, the asymmetry in CSR is not as striking as that in SSR.

Phospholipids retained by the CSR calcium pump protein (CaATPase) after detergent ''stripping'' were similar to those intimate to the SSR CaATPase, although the percentages of unsaturated phospholipids and plasmalogenic phospholipids are not as great as in the skeletal system. Lipids associated with the CSR CaATPase following DFDNB cross-linking showed a preference for retention of the aminophospholipids, again similar to the SSR CaATPase. Because the nonrandom distribution of membrane lipids modifies SSR function, it is likely these membrane lipids impact *in situ* the function of the CSR.

Key words: Sarcoplasmic reticulum — Membranes — Asymmetry — Phospholipids

Introduction

In skeletal muscle sarcoplasmic reticulum (SSR) the phospholipid headgroups and fatty acyl chains comprising the bulk of the membrane are asymmetrically distributed across the bilayer (Herbette et al., 1984; Bick et al., 1987). This fact, together with our findings that: (i) unsaturated aminophospholipids are retained by the SSR CaATPase during detergent ''stripping'' procedures (Bick et al., 1991); and (ii) phosphatidylethanolamine (PE) activates delipidated SSR CaATPase (Knowles & Racker, 1975; Hidalgo, Petrucci & Vergara, 1982; Navarro, 1984; Cheng et al., 1986) led us to theorize that PE is strategically situated to allow conformational changes to occur in the transport protein due to phosphatidylethanolamine's predominantly unsaturated, and therefore fluid, nature.

Since the cardiac sarcoplasmic reticulum (CSR) calcium pump protein (CaATPAse) carries out similar transport functions to those shown by SSR, albeit more slowly, we hypothesized that phospholipid requirements and distribution might be similar. We found this to be true to some extent, but when one considers the importance of cardiac muscle, it would seem logical that every architectural aspect of the CSR membrane would be tailored to optimize the function of the CSR CaATPase. However, this does not seem the case. We here describe the asymmetric nature of the CSR membrane and compare its make-up to that of fast skeletal muscle SR and detail the intimately associated lipids following detergent stripping and 1,4-Difluoro-2,4-dinitrobenzene sulfonic acid (DFDNB) cross-linking.

Materials and Methods

Sarcoplasmic Reticulum Isolation

Hearts were removed from dogs under pentobarbital anesthesia and the left ventricle was separated and cleaned of blood, connective tissue and fat and rinsed in ice-cold normal saline. Only the left ventricle was

Correspondence to: R.J. Bick

These results were presented in part at the New York Academy of Sciences meeting entitled, ''Cardiac Sarcoplasmic Reticulum Function and Regulation of Contractility'', Washington, D.C., September, 1997

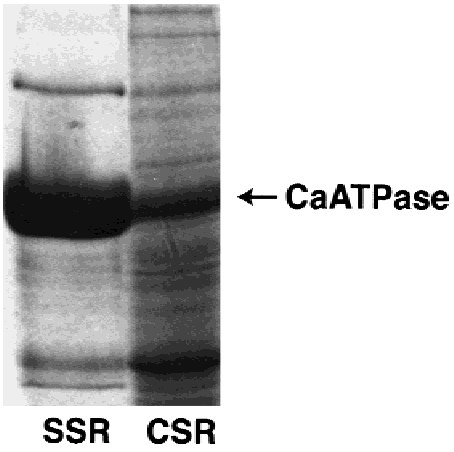

Fig. 1. One-dimensional SDS polyacrylamide (10%) gel electrophoresis of Skeletal muscle Sarcoplasmic Reticulum (SSR) and Cardiac Sarcoplasmic Reticulum (CSR) showing the most prominent proteins in the two isolates were found at approximately 100 kD, the mobility of the respective CaATPAse proteins.

used in all preparations. All procedures were carried out at 4°C. The tissue was minced with scissors in isolation buffer containing 0.29 M sucrose, 10 mM imidazole, 3 mM NaN_3, 10 mM leupeptin and 0.5 mM dithiothreitol (DTT), pH 7.0. The mixture was homogenized in a Waring blender at maximum speed for 4 × 15 sec with 15-sec pauses between bursts and then centrifuged at 5,000 × *g* (Sorvall GSA) for 15 min. The supernatant was poured through 2 layers of cheesecloth and centrifuged at 9,500 rpm for 15 min, the pellet was discarded and the supernatant spun again at 100,000 × *g* for 1 hr. This pellet was resuspended in a small volume of isolation buffer by swirling, taking care to dislodge only the tan-colored, upper region of the pellet and leave the glycogen-containing, clear, lower pellet. The final homogenate was quick-frozen in liquid nitrogen. For use, an aliquot is allowed to thaw on ice and then oxalate loaded following the procedure of Jones et al. (1979). The resulting pellet is resuspended and used for subsequent studies. The trapped oxalate runs as a white, easily distinguishable, spot on thin layer chromatography of SR lipid extracts and as an early peak via GLC.

This isolation procedure gives a mitochondrial contamination of <5% as determined by succinate dehydrogenase activity (Singer, 1974) and sarcolemmal contamination of approximately 3% as measured by ouabain inhibitable ($Na^+ + K^+$)-ATPase activity (Besch, Jones & Watanabe, 1976). One-dimensional SDS-gel electrophoresis was performed according to the method of Laemmli (1970) using 10% acrylamide and 20 μg of protein per lane. Detection was with Coomassie blue in 10% methanol and acetic acid. The predominant protein in both the CSR and SSR isolations is at approximately 100 kD, the expected size of the respective CaATPase's. (*See* Fig. 1).

Fast twitch skeletal muscle SR was prepared from rabbits by the method of Van Winkle et al. (1981).

Phospholipid Extraction

Isolated SR was extracted twice with 4 ml of 2:1 chloroform/methanol, slightly acidified by the addition of a few drops of 1N HCl. The denatured protein was removed from the extract by filtration through glass wool and the upper aqueous phase was removed. The organic extract was taken to dryness under a stream of nitrogen and the residue redissolved in chloroform/methanol and spotted on 20 × 20-cm silica gel thin layer chromatography plates. Chromatographic separation was carried out in two dimensions using the solvent system chloroform: methanol:NH_4OH:water (70:30:4:1). The dry plates were sprayed with 0.1% ninhydrin in ethanol and heated in an oven at 100°C for 5 min. Red spots were marked accordingly and the plates were then exposed to iodine vapor for 10 min to show all chromatographed lipids. These spots were scraped into test tubes and assayed for phosphate content by the method of Rouser (1966).

Plasmalogen estimations were carried out as above, except that the plates were developed in two dimensions with a 15-min exposure of the plates to HCl vapor between dimensions (Horrocks, 1968).

Fatty Acyl Chain Determinations

Membranes were extracted with two 4-ml volumes of 2:1 chloroform/methanol, once with 4-ml volume of acidified isopropanol:heptane (2:1) and finally with 5-ml volume of petroleum ether/ether (1:1). These organic extracts were combined and dried under nitrogen and 3 ml of methanolic NaOH (Supelco, Bellefontaine, PA) were added to the residues which were placed in a boiling water bath for 5 min; 3 ml of boron trifluoride (Supelco, Bellefontaine, PA) were then added and the samples heated again. After cooling, 10 ml of hexane was added and the tubes shaken vigorously for 5 min. The samples were allowed to stand until phase separation was complete, then the upper hexane layer was removed to another tube and dried under nitrogen. The dry samples were reconstituted in iso-octane and injected onto a 30-meter, 10% Silar column housed in a Hewlett-Packard 5890 Gas chromatograph. Nonadecanoic acid was run as a standard.

Protein-Associated Phospholipids

To determine the extent of the similarity between the cardiac and skeletal muscle fractions, we carried out a ''stripping'' procedure as detailed in Bick et al. (1991). Briefly, SR membranes were mixed with increasing amounts of the detergent $C_{12}E_8$ (Octaethylene glycol monododecyl ether) and the protein and associated membrane lipids were recovered by centrifugation. Both the protein pellet and supernatant were extracted as described above and examined for lipid content and type. CaATPase assays were carried out at each detergent concentration.

Inner vs. Outer Layer Phospholipids

This procedure was as described in Herbette et al. (1984); briefly, SR vesicles were incubated with phospholipase A2 and the formation of lysophospholipids (LPL) was followed by thin layer chromatography. Digestion of the outer monolayer resulted in an increasing amount of LPLs, a halt in production, then again an increase. The break in LPC production was taken as the point beyond which the inner membrane had become leaky and/or was no longer protected by the outer monolayer (*See* Fig. 2).

To further determine the CSR membrane asymmetry 10 mg of CSR protein was incubated at room temperature and in the dark with 10 mg of DFDNB. The protein was harvested by centrifugation at 100,000 × *g*, resuspended in 10 mM TES buffer, pH 6.8, containing 1 mM ethanolamine to remove unreacted DFDNB. The pellet was further washed in 10 mM TES and then extracted as above for lipid type and content.

Table 1. Comparison % of total phospholipids

	CSR	SSR
	$n = 10$	$n = 4$
PE	27.5 ± 1.9	16.4 ± 3.0**
PC	53.0 ± 3.5	67.6 ± 4.0*
PS	9.2 ± 1.9	11.01 ± 1.2
PI	0.9 ± 0.4	1.7 ± 0.3
LPC	6.4 ± 1.2	2.2 ± 0.4*
LPE	3.4 ± 0.6	0.9 ± 0.5*

* $P < 0.05$, ** $P < 0.01$

CSR-Cardiac sarcoplasmic reticulum, SSR–Fast Skeletal muscle sarcoplasmic reticulum, PE–Phosphatidylethanolamine, PC–Phosphatidylcholine, PS–Phosphatidylserine, PI–Phosphatidylinositol, LPC–Lysophosphatidylcholine, LPE–Lysophosphatidylethanolamine

Table 2. Inner *vs.* outer leaflet phospholipids (%)

	CSR	SSR
	$n = 7$	$n = 7$
Inner PE	27 ± 2.2	20 ± 1.5*
Outer PE	73 ± 3.2	80 ± 2.0
Inner PC	54 ± 3.8	52 ± 2.8
Outer PC	46 ± 3.1	48 ± 2.0
Inner PS	76 ± 4.4	84 ± 1.3
Outer PS	24 ± 3.3	16 ± 4.9

* $P < 0.05$

ATPase Activity

ATPase activity was measured by continuously monitoring the oxidation of NADH at 340 nm with a linked enzyme system as described in Van Winkle (1981). The reaction medium included (in mM): 40 tris-(hydroxymethyl)aminomethane (Tris) maleate (pH 7.4), 100 KCl, 1 $MgCl_2$, 2 phosphoenolpyruvate (PEP), 0.4 NADH, 0.02 mM $CaCl_2$ plus 8.75 and 12.5 U/ml of pyruvate kinase and lactate dehydrogenase, respectively. The concentration of protein was 10 μg/ml in a final volume of 2 ml. Sodium azide (10 mM) was included to inhibit mitochondrial ATPAse activity. Reactions were started with ATP after 5-min equilibration at 30°C; the difference between the rate measured in the presence of calcium and magnesium and the rate in the presence of 1-mM ethyleneglycol-bis(β-aminoethylether) N,N,N′,N′-tetraacetic acid (EGTA) was taken as the CaATPase activity.

Statistics

All comparisons were made by Student's *t* test with a $P < 0.05$ chosen as the criteria for statistical significance. Data are expressed as mean ± SE.

Results

Table 1 shows the content of individual phospholipids in isolated CSR and SSR. CSR contains a larger percentage of phosphatidylethanolamine (PE) than SSR, but this increase is compensated for by a reduced amount of phosphatidylcholine (PC). Phosphatidylserine (PS) and phosphatidylinositol (PI) are approximately the same percentage in both systems whereas the amount of lysophospholipids in CSR is higher than that in SSR, although this may be due to phospholipase activity during the isolation procedure, the CSR being less stable than the SSR.

When the CSR outer monolayer is compared to the inner monolayer, a striking asymmetry in the distribution of phospholipids is apparent. PE is primarily in the outer leaflet and PS in the inner leaflet. PC is distributed equally between the leaflets. The asymmetric distribution of PE is less in CSR than in SSR ($P < 0.05$, Table 2). Figure 2 shows the increasing production of lysoPC in the reaction mixture supernatant as a result of phospholipase digestion. The curves tend to flatten at certain times, indicating that the outer leaflet has been breached, an the acyl chains of the inner leaflet have been encountered by the phospholipase. In CSR this occurs between 3–5 min, in SSR, between 5–10 min.

Comparison of major fatty acids shows significant differences between CSR and SSR (Table 3). CSR has slightly more unsaturated fatty acyl chains overall. The most striking differences between CSR and SSR being in palmitic acid [16:0] (14% in CSR, 27.5% in SSR), stearic acid [18:0] (18% in CSR, 8% in SSR), linoleic acid [18:2] (13% in CSR, 19% in SSR) and arachidonic acid [20:4] (17.5% in CSR, 11.5% in SSR). Despite significant differences in individual fatty acids, the average chain length and unsaturation index were quite similar.

As shown in Table 4, the plasmalogen content of PE is approximately the same in both cardiac and skeletal fractions. However, PC plasmalogen is higher in CSR and plasmalogenic PS content is slightly lower in CSR. Overall, the plasmalogen content of CSR phospholipids is 50% compared to 40% in SSR.

In both CSR and SSR the ratio of detergent to protein yielding the highest ATP hydrolysis rates is the same at 1:1, albeit the SSR activities are much higher than those found with the cardiac fractions (Table 5). The inhibitory effects of further increases in detergent concentration are also similar for SSR and CSR.

Tables 6 and 7 detail the amounts and types of phospholipid found in native preparations and in ''stripped' preparations after detergent treatment. In both the skeletal and cardiac membranes, the types, ratios and patterns are similar in the native state, with the small differences as noted earlier. However, in the stripped preparations the pattern changes dramatically. SSR shows a loss of PC, but with only a minor removal of PE by detergent. This suggested to us the close proximity of PE to the calcium pump protein. In contrast, the CSR tends to retain a phospholipid content not greatly different from the native enzyme, with a more generalized removal of phospholipids by detergent. Only PS showed

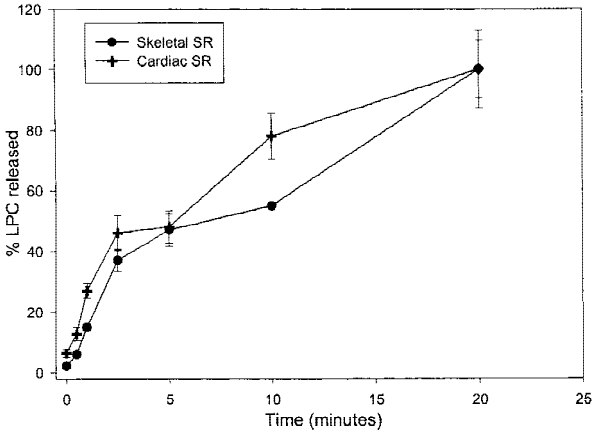

Fig. 2. Time course of phospholipase digestion of CSR and SSR, and the appearance of LPC in the supernatant of samples removed from the reaction mixture. The appearance of this lysophospholipid was used as a means to follow the digestion of the outer vs. inner leaflet of the membrane.

Table 3. Fatty Acyl chain content of CSR and SSR (%)

C#	CSR	SSR
14	2.1	1.1
14:1	1.6	1.3
16	13.8	27.5
16:1	1.6	2.0
18	18.2	8.2
18:1	18.5	21.4
18:2	12.7	19.0
18:3	1.9	Trace
20	0.3	0.3
22		0.8
22:1	1.1	1.7
20:4	17.5	11.5
20:5	2.0	2.2
22:6	2.3	2.4
Other	6.4	0.4
Saturated	34.4	37.9
Polyunsaturated	36.4	35.1
Avg. Chain Length	18.1	17.8
Unsaturation Index	1.6	1.4

any change in CSR, the amount of this particular phospholipid remaining with the protein being approximately twice that of the native fraction (17% to 10%), but this difference did not attain statistical significance. For both CSR and SSR the retained PE was enriched in plasmalogen content. The PC retained in the CSR after detergent stripping is also highly enriched in plasmalogen.

Table 8 compares the DFDNB cross-linked preparations and shows that following detergent treatment, proteins of the SSR and CSR retained aminophospholipids. The extent of the enrichment of PE in the DFDNB crosslinking for CSR is less than that of the SSR.

Table 4. Plasmalogen content of CSR and SSR phospholipids (%)

	CSR	SSR
	$n = 7$	$n = 7$
PE plasmalogen	71 ± 8	69 ± 10
PC plasmalogen	36 ± 4	26 ± 5
PS plasmalogen	52 ± 2	46 ± 7
Total plasmalogen	50 ± 6	40 ± 4

Table 5. Effects of increasing detergent on CaATPase activities

C12E8:SR protein	CSR	SSR
(mg/mg)	(μmol/mg/min) $n = 10$	(μmol/mg/min) $n = 10$
0 :1	0.130 + 0.4	0.606 + .11
0.5:1	0.133 + .04	0.627 + .08
1 :1	0.158 + .02	2.888 + .36
2.5:1	0.154 + .04	2.405 + .28
4 :1	0.085 + .03	1.433 + .18
6 :1	0.076 + .01	1.400 + .22

Table 6. Phospholipids of native and detergent treated SR (%)

	Native CSR	Stripped CSR	Native SSR	Stripped SSR
PE	28 ± 4	23 ± 3	16 ± 2	38 ± 2*
PC	53 ± 3	59 ± 4	67 ± 5	38 ± 6*
PS	10 ± 4	17 ± 3	7 ± 2	24 ± 5*
	$n = 7$			

* $P < 0.05$ *vs.* native membranes

Table 7. Plasmalogen content of native and protein associated phospholipids (%)

	Native CSR	Stripped CSR	Native SSR	Stripped SSR
PE	64 ± 2	77 ± 3**	63 ± 2	96 ± 2**
PC	58 ± 3	100 ± 4**	24 ± 1	22 ± 3
PS	52 ± 3	38 ± 2**	46 ± 4	39 ± 6
$n = 6$				

** $P < 0.01$ *vs.* native membranes

Discussion

Aminophospholipids are required for maximum activation of the SSR CaATPase and are retained preferentially during detergent treatment and DFDNB cross-linking (Bick et al., 1991). We theorized that these particular phospholipids were present due to their inherent unsaturation, therefore facilitating the conformational changes in the protein that occur with ATP hydrolysis (Blasie et al., 1990). As the cardiac calcium pump protein carried out similar functions to the skeletal enzyme, we expected

Table 8. Comparison of phospholipids from native and DFDNB labeled preparations (%)

	Native CSR	+ DFDNB	Native SSR	+DFDNB
PE	27.0	32	19	45
PC	50.4	22	71	36
PS	7.4	11	11	19
LPC		11		
LPE		12		
$n = 4$				

to find the CSR membrane to be similar in phospholipid distribution, pattern of retention, and asymmetry.

Overall, the asymmetry of the membrane tends to be the same in both the skeletal and cardiac preparations. There are differences in the extent of the asymmetry and composition of the monolayers, the CSR membrane being less asymmetric. The average fatty acyl chain length and unsaturation index were also similar though differences in the relative proportions of specific fatty acids were present. When the phospholipids retained after detergent stripping were determined, major differences between CSR and SSR were seen. SSR retains PE to a great extent and most, if not all, of the aminophospholipid is plasmalogenic in nature. In contrast, CSR does not show this preference based on head group, removal of lipid being approximately equal for each of the two major species, but the PC retained does show a preference for the plasmalogenic form. So, both systems retain plasmalogenic lipids, SSR selecting PE, CSR having less headgroup selectivity. Additionally, SSR retains PE and CSR tends to retain more PS, so both systems increase their content of aminophospholipid, possibly indicating that the amine head groups of these two phospholipids are important for optimal calcium pump activities in the two muscle systems. Preferential retention of aminophospholipids following DFDNB treatment is apparent with both enzymes, pointing to these lipids being close to, and possibly associated with, the pump protein, despite the possibility that their importance in the two systems may be somewhat different.

The differences in lipid retained, and amount of particular lipid required, may reflect the fact that the CSR activity depends upon the presence of other proteins (e.g., phospholamban) which co-isolate with the CaATPase (Warren, 1974; Van Winkle, Pitts & Entman, 1978), at least during the prepurification steps of the isolation procedure (Kranias et al., 1983). These other proteins may have their own lipid specificity and therefore appear to influence the species of phospholipid retained by the preparation during detergent stripping.

Obviously more work is needed to test for lipid selectivity and requirement by the CSR, but these studies indicate that aminophospholipids may be an important part of the protein-associated lipid membrane, either due to the unsaturation of these lipids and component fatty acyl chain lengths that allow an appropriate membrane thickness close to the pump protein (Cheah, 1981; London & Feigenson, 1981), the amino headgroup, or possibly all these factors. Long chain, unsaturated fatty acids are required for maximum CaATPAse activity (Cheah, 1981; Lee, East & Froud, 1986), and other research has shown optimal function of reconstituted sarcoplasmic reticulum CaATPase in aminophospholipid containing systems, this requirement possibly being dictated by the evolution in a low cholesterol environment (Vemuri & Philipson, 1989).

In our earlier work we detailed the asymmetric construction of the SSR membrane and theorized that these findings would impact the functions of the calcium pump protein (Herbette et al., 1984; Bick et al., 1987). With the cardiac system we expected to find a similar pattern of membrane architecture to that found in skeletal muscle and this proved to be the case. Possibly of some surprise was the fact that there was little difference between the skeletal and cardiac preparations with respect to the content of unsaturated and saturated fatty acids, if one considers the importance of the heart as a single, irreplaceable unit and its requirement for life sustaining functions. We speculated that the importance of unsaturated fatty acid containing phospholipids close to the CaATPase was to allow the pump to undergo the required conformational changes during substrate hydrolysis (Asturias, Pascolini & Blasie, 1990).

Also of interest was the fact that while the CSR contains more plasmalogenic phospholipids, those lipids close to the pump protein are different plasmalogens when compared to the similar SSR lipids. Again, we theorized in our earlier work that the plasmalogens would be more amenable to packing as required by various protein conformations. The enrichment of plasmalogen in the CSR protein-associated lipid may explain the relatively modest effects of fatty acid modification on CSR function (Taffet et al., 1993), with previous work having detailed the role of fatty acyl chains in the activation of membrane proteins (Lee et al., 1986).

With SSR we, as have others (Knowles et al., 1975; Hidalgo et al., 1982; Yeagle, 1989), have found a requirement for PE to allow the CaATPase to function at maximum capacity. Szymanska et al. (1991) showed a possible dependence of CSR CaATPase on the presence of PS and our findings generally support that conclusion. However, it is clear from our results that PE resides in the outer monolayer of both CSR and SSR and that PS is an inner membrane phospholipid. The difference in membrane location implies that the PE and PS may be fulfilling different requirements.

The CSR CaATPase has a substantially lower hydrolysis rate, enzyme phosphorylation and slower time to peak E-P formation than the SSR isozyme. This, how-

ever, is unlikely to be due to excessive inhibition by the native CSR membrane because the stimulation by the detergent $C_{12}E_8$ was meager compared to that seen for the SSR CaATPase. For the SSR CaATPase, $C_{12}E_8$ maintains a functional confirmation and allows for the uninhibited, maximal rates of ATP hydrolysis, originally detailed by le Maire, Moller & Tanford, (1976), and later confirmed as a system in which some or all of the relevant phospholipids remain associated with the ATPase (de Foresta et al., 1989). Whether $C_{12}E_8$ allows the CSR CaATPase to maximally cycle is uncertain, so the retention of PS, plasmalogenic PC and a higher saturated fatty acid amount may still contribute to the slower rate of hydrolysis of the CSR CaATPase.

We recognize that this work has a number of limitations. As possibly shown by Table 1, the activity of phospholipase may be greater in the CSR and modification of phospholipids during the isolation procedure may confound our findings. The membranes were isolated from different species and species differences may also be present. However, this research again points to the preferential requirement for specific lipid(s), most likely due to their unique composition, which satisfy the necessity for specific chain lengths, packing capabilities and headgroup. Also, the proposal of a ''unique lipid environment'' model (Blazyk, Wu & Wu, 1985), or dual lipid population membrane (Selinsky & Yeagle, 1984) is supported by these results. The lesser selectivity we noted in CSR may simply reflect the competing lipid requirements of other CSR proteins, necessary for calcium transport or other functions in the cardiac system, that also have to be satisfied.

Obviously, more detailed research is required to answer these questions regarding the possible regulatory role of specific phospholipids on CSR function, with the findings of the present study being extended by membrane phospholipid manipulations. Though previous reports have detailed the requirement of a minimum number of annular phospholipids for the SSR CaATPase (Hesketh et al., 1976), the CSR may be different. This will also require further study.

The authors wish to thank Dr. Mark L. Entman for his continuing support and guidance, Mr. Mark Snuggs, Mr. Brian Poindexter and Ms. Melissa Jacobs for their expert technical assistance and Mr. Bruce Folsom for animal care expertise. This work was supported in part by National Institutes of Health Grants AG 13251 and HL 13870.

References

Asturias, F.J., Pascolini, D., Blasie, J.K. 1990. Evidence that lipid lateral phase separation induces functionally significant structural changes in the Ca^{2+} ATPase of the sarcoplasmic reticulum. *Biophys. J.* **58:**205–217

Besch, H.R. Jr., Jones, L.R., Watanabe, A.M. 1976. Intact vesicles of canine cardiac sarcolemma: evidence from vectorial properties of Na^+, K^+-ATPase. *Circ. Res.* **39:**586–595

Bick, R.J., Van Winkle, W.B., Tate, C.A., Entman, M.L., Blasie, J.K., Herbette, M.L. 1987. Phospholipid fatty acyl chain asymmetry in the membrane bilayer of isolated skeletal muscle sarcoplasmic reticulum. *Biochemistry* **26:**4831–4836

Bick, R.J., Youker, K.A., Pownall, H.J., Van Winkle, W.B., Entman, M.L. 1991. Unsaturated aminophospholipids are preferentially retained by the fast skeletal muscle CaATPase during detergent solubilization. *Arch. Biochem. Biophys.* **286:**346–352

Blasie, J.K., Pascolini, D., Asturias, F., Herbette, L.G., Pierce, D., Scarpa, A. 1990. Large-scale structural changes in the sarcoplasmic reticulum ATPase appear essential for calcium transport. *Biophys. J.* **58:**687–693

Blazyk, J., Wu, C-J., Wu, S-C. 1985. Correlation between lipid fluidity and tryptic susceptibility of Ca^{2+}-ATPase in sarcoplasmic reticulum membranes. *J. Biol. Chem.* **260:**4845–4849

Cheah, A. 1981. Effect of long chain unsaturated fatty acids on the calcium transport of sarcoplasmic reticulum. *Biochem. Biophys. Acta.* **648:**113–119

Cheng, K-H., Lepock, J.R., Hui, S.W., Yeagle, P.L. 1986. The role of cholesterol in the activity of reconstituted Ca-ATPase vesicles containing unsaturated phosphatidylethanolamine. *J. Biol. Chem.* **261:**5081–5087

de Foresta, le Maire, M., Orlowski, S., Champeil, P., Lund, S., Moller, J.V/. Michelangeli, F., Lee, A.G. 1989. Membrane solubilization by detergent: Use of brominated phospholipids to evaluate the detergent-induced changes in Ca^{2+}-ATPase/lipid interaction. *Biochem.* **28:**2558–2567

Herbette, L., Blasie, J.K., DeFoor, P., Fleischer, S., Bick, R.J., Van Winkle, W.B., Tate, C.A., Entman, M.L. 1984. Phospholipid asymmetry in the isolated sarcoplasmic reticulum membrane. *Arch. Biochem. Biophys.* **234:**235–242

Hesketh, T.R., Smith, G.A., Houslay, M.D., McGill, K.A., Birdsall, N.J.M., Metcalfe, J.C., Warren, G.B. 1976. Annular lipids determine the ATPase activity of a calcium transport protein complexed with dipalmitoyllecithin. *Biochem.* **15:**4145–4151

Hidalgo, C., Petrucci, D.A., Vergara, C. 1982. Uncoupling of Ca^{2+} transport in sarcoplasmic reticulum as a result of labeling lipid amino groups and inhibition of Ca^{2+} ATPase activity by modification of lysine residues of the Ca^{2+}ATPase polypeptide. *J. Biol. Chem.* **254:**4224–2432

Horrocks, L.A. 1968. The alk-1-enyl group content of mammalian phosphoglycerides by quantitative two-dimensional thin-layer chromatography. *J. Lipid. Res.* **9:**469–472

Jones, L.R., Besch, H.R. Jr., Fleming, J.W., McConnaughey, M.M., Watanabe, A. 1979. Separation of vesicles of cardiac sarcolemma from vesicles of cardiac sarcoplasmic reticulum. Comparative biochemical analysis of component activities. *J. Biol Chem.* **254:**530–539

Knowles, A.F., Racker, E. 1975. Properties of a reconstituted calcium pump. *J. Biol. Chem.* **250:**3508–3544

Kranias, E.G., Nakamura, J., Schwartz, A. 1983. Phospholamban, the regulator of the cardiac sarcoplasmic reticulum calcium pump, does not copurify with the Ca^{2+}-ATPase enzyme. *Biochim. Biophys. Acta* **749:**62–68

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:**680–685

Lee, A.G., East, J.M., Froud, R.J. 1986. Are essential fatty acids essential for membrane function? *Prog. Lipid Res.* **25:**41–46

le Maire, M., Moller, J.V., Tanford, C. 1976. Retention of enzyme activity by detergent-solubilized sarcoplasmic reticulum Ca^{2+}-ATPase. *Biochem.* **15:**2336–2342

London, E., Feigenson, G.W. 1981. Fluorescence quenching in model

membranes. 2. Determination of the local lipid environment of the calcium adenosinetriphosphatase from sarcoplasmic reticulum. *Biochem.* **20:**1939–1948

Navarro, J., Toivio-Kinnucan, M., Racker, E. 1984. Effect of lipid composition on the calcium/adenosine 5′-triphosphate coupling ratio of the Ca^{2+}-ATPase of sarcoplasmic reticulum. *Biochemistry* **23:**130–136

Rouser, G., Siakotos, A.N., Fleischer, S. 1966. Quantitative analysis of phospholipids by thin-layer chromatography and phosphorus analysis of spots. *Lipids* **1:**85–86

Selinsky, B.S., Yeagle, P. 1984. Two populations of phospholipids exist in sarcoplasmic reticulum and in recombined membranes containing Ca-ATPase. *Biochem.* **23:**2281–2288

Singer, T.P. 1974. Determination of the activity of succinate, NADH, choline and alpha-glycerophosphate dehydrogenases. *Methods. Biochem. Anal.* **22:**123–154

Szymanska, G., Kim, H.W., Kranias, E.G. 1991. Reconstitution of the skeletal sarcoplasmic reticulum Ca^{2+}-pump: influence of negatively charged phospholipids. *Biochim. Biophys. Acta.* **1098:**127134

Taffet, G.E., Pham, T.T., Bick, D.L.M., Entman, M.L., Pownall, H.J., Bick, R.J. 1993. The calcium uptake of the rat heart sarcoplasmic reticulum is altered by dietary lipid. *J. Membrane BIol.* **131:**35–42

Van Winkle, W.B., Tate, C.A., Bick, R.J., Entman, M.L. 1981. Substrate utilization by cardiac and skeletal muscle sarcoplasmic reticulum? *J. Biol. Chem.* **256:**2268–2274

Van Winkle, W.B., Pitts, B.J.R., Entman, M.L. 1978. Rapid purification of canine cardiac sarcoplasmic reticulum Ca^{+}-ATPase. *J. Biol. Chem.* **253:**8671–8673

Vemuri, R., Philipson, K.D. 1989. Influence of sterols and phospholipids on sarcolemmal and sarcoplasmic reticular cation transporters. *J. Biol. Chem.* **25:**8680–8685

Warren, G.B., Toon, P.A., Birdsall, N.J., Lee, A.G., Metcalfe, J.C. 1974. Complete control of the lipid environment of membrane-bound proteins: application to a calcium transport system. *FEBS Lett.* **41(1):**122–124

Yeagle, P. 1989. Lipid regulation of cell membrane structure and function. *FASEB J.* **3:**1833–1842