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Identification and Characterization of Proteins in Sarcoplasmic Reticulum from Normal and Failing Human Left Ventricles

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M. A. MOVSESIAN, C. LEVEILLE, J. KRALL, J. COLYER. J. H. WANG AND K. P. CAMPBELL. Identification and Characterization of Proteins in Sarcoplasmic Reticulum from Normal and Failing Human Left Ventricles. *Journal of Molecular and Cellular Cardiology* (1990) **22**, 1477-1485. Monoclonal and polyclonal antibodies to the major Sarcoplasmic reticulum proteins of rabbit skeletal and canine cardiac muscle have been used to identify and characterize the corresponding components of human cardiac Sarcoplasmic reticulum. The Ca^{2+}-transporting ATPase of human cardiac Sarcoplasmic reticulum was identified as a 105000-Ua protein antigenically distinct from its rabbit skeletal muscle counterpart. Human cardiac Sarcoplasmic reticulum also contained 53000- 155000- and 165000-Da glycoproteins antigenically related to the low and high molecular weight glycoproteins of canine cardiac and rabbit skeletal muscle Sarcoplasmic reticulum. The ryanodine-sensitive Ca^{2+} channel of human cardiac Sarcoplasmic reticulum was identified as a 400 000-Da protein antigenically related to its counterparts in canine cardiac and rabbit skeletal muscle. Human cardiac calsequestrin was identified as a 52 000-Da protein. Human phospholamban was identified as a 29 000-Da substrate for phosphorylation by cAMP-dependent protein kinase.

Immunoblots of Sarcoplasmic reticulum from the normal left ventricles of four unmatched organ donors and the excised failing left ventricles of nine patients with idiopathic dilated cardiomyopathy were compared in search of qualitative differences in the protein patterns of the failing hearts. No such differences were found with respect to the Ca^{2+} ATPase, the 53 000-Da glycoprotein, the ryanodine-sensitive Ca^{2+} channel, calsequestrin or phospholamban. In contrast, the 165000-Da glycoprotein band, present in all four preparations from non-failing hearts, was absent from three of nine preparations from failing hearts, and staining of the 55000-Da glycoprotein in these three preparations appeared to be relatively increased. The absence of the 155 000-Da glycoprotein band may identify or reflect a pathogenetic mechanism in a subset of patients with idiopathic dilated cardiomyopathy.

KEY WORDS: Sarcoplasmic reticulum; Cardiomyopathy; Ca^{2+} ATPase; Ryanodine-sensitive Ca^{2+} channel; Calsequestrin; Glycoproteins; Phospholamban; Heart failure; Cardiomyopathy.

Introduction

Heart failure is accompanied by abnormal intracellular Ca^{2+} handling in human myocardium (Gwathmey *et al.*, 1987), raising the possibility that alterations in the molecular mechanisms of Ca^{2+} uptake, binding and release by the sarcoplasmic reticulum might be involved (either directly or indirectly) in the pathophysiology of this condition, Until now, however, the identification and characterization of the proteins involved in these processes and the mechanisms through which these processes occur has been carried out in sarcoplasmic reticulum prepared from non-human sources, and the extent to which observations made in these preparations apply to humans is unclear. These considerations seem particularly important in view of recent studies demonstrating significant differences in the biochemical characteristics of dilated cardiomyopathy in humans and in animal models. In the latter, decreased Ca^{2+} uptake by cardiac sarcoplasmic reticulum has been observed in both crude tissue homogenates

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and isolated microsomal preparations (Whitmeretal., 1988). In humans, in contrast, Ca^{2+} uptake is decreased in crude tissue homogenates but not in isolated microsomal preparations (Limas *et al.*, 1987; Movsesian *et al.*, 1989; Movsesian *et al.*, 1990). These differences suggest that the human and animal diseases result from separate pathogenic mechanisms, and make clear the need for direct studies using human tissue.

The increase in the number of cardiac transplantations performed in recent years has made it possible to carry out such studies. In this paper, we describe our use of monoclonal and polyclonal antibodies that bind to the major proteins of rabbit skeletal and canine cardiac muscle sarcoplasmic reticulum to identify and characterize the corresponding protein constituents of human cardiac sarcoplasmic reticulum. In addition, we have used these antibodies to compare protein patterns in sarcoplasmic reticulum prepared from normal human left ventricles and from the left ventricles of patients with idiopathic dilated cardiomyopathy.

Materials and Methods

Preparation of sarcoplasmic reticulum

Skeletal muscle triads were isolated from adult rabbit muscle according to Mitchell *et al.* (1983), as modified by Sharp *et al.* (1987), in the presence ofaprotinin (76.8 nM), benzamidine (0.83 mM), iodoacetamide (1 mM), leupeptin (1.1 (μM), pepstatin A (0.7 μM), and phenylmethylsulfonyl fluoride (PMSF, 0.23 mM). Canine cardiac microsomes were prepared according to Jones and Cala (1981) in the presence of protease inhibitors. Bovine cardiac microsomes were prepared according to Suzuki and Wang (1986). Membranes were stored at –135°C in 0.25 M sucrose, 10 mm histidine (pH 7.4), 0.83 mM benzamidine, 1 mM iodoacetamide and 58 μM PMSF.

Human cardiac sarcoplasmic reticulum was prepared from normal and failing left ventricular free wall tissue according to Movsesian *et al.* (1989). Normal left ventricular tissue was obtained from kidney donors aged 17-40 years with no evidence of cardiac disease who had suffered trauma-related brain death and had been maintained on ventilators for 2-5 days prior to tissue procurement. No suitable recipients for these hearts were identified. Failing left ventricular tissue was obtained from the excised hearts of transplant recipients with Class IV heart failure resulting from idiopathic dilated cardiomyopathy. The β-adrenergic receptor density in crude sarcolemmal preparations was 110.0 ± 15.3 fmol/mg in the normal hearts and 52.1 ± 4.5 fmol/mg in the failing hearts. Hearts were excised following infusion of cold cardioplegia solution into the root of the cross-clamped aorta and maintained at 4°C until preparation of microsomes. The time between cardiac excision and homogenization was less than 2.5 h in the case of normal hearts procured at distant locations and less than 0.5 h in the case of normal and failing hearts procured locally (all failing hearts were procured locally). Preparations from normal and failing hearts had been compared with respect to Ca^{2+} uptake activity and phospholipid: protein ratios as previously described (Movsesian *et al.*, 1989). values for V_{max}, $K_{0.5}$ and nniii in the preparations from normal ventricles were 593 nmol/mg-min, 0.68 μM, and 1.7, respectively. The corresponding values in the preparations from failing ventricles were 593 nmol/mg-min, 0.63 μM and 1.6. The phospholipid:protein ratio (nmol phosphate per mg protein) was 35.3 ± 4.0 in preparations from normal hearts and 34.1 ± 2.4 in preparations from failing hearts. Studies with monoclonal antibodies have shown no differences between the two sets of preparations with regard to phospholamban-mediated stimulation of Ca^{2+} uptake (Movsesian *et al.*, 1990).

Preparation of antibodies

Monoclonal antibodies (IID8, Al) against the canine cardiac sarcoplasmic reticulum ATPase and phospholamban were prepared as previously described (Jorgensen *et al.*, 1988; Suzuki and Wang, 1986). Monoclonal antibody (XIIC4) to rabbit skeletal muscle sarcoplasmic reticulum glycoproteins was prepared using the procedures described for the preparation of anti-ryanodine-sensitive Ca^{2+} channel antibodies from mice immunized with sarcoplasmic reticulum vesicles (Campbell and MacLennan, 1981). Polyclonal antibodies (GP–3) to the purified ryanodine receptor have been previously prepared (Hoff-

man *et al.*, 1987) using guinea pigs immunized with gel slices of the purified 450 000-Da rabbit skeletal muscle ryanodine receptor according to the method of Tung (1983). Polyclonal antibodies (rabbit A) to calsequestrin have been previously prepared using rabbits immunized with gel slices of the purified canine cardiac calsequestrin (Campbell *et al.*, 1983; Jorgensen and Campbell, 1984). The specificity of the various antibodies was determined by immunodot, immunoblot and immunoprecipitation assays as described (Campbell *et al.*, 1987; Leung *et al.*, 1988).

Immunostaining of Western blots

Sarcoplasmic reticulum membranes (5-60 μg) were solubilized in sample buffer (130 mM Tris(hydroxymethyl)aminomethane HCl (Tris-HCl, pH 6.8), 1 M sucrose, 6% sodium dodecyi sulfate (SDS), 0.002% bromophenol blue and 200 mM DTT) and electrophoresed on 3-12% polyacrylamide gels (with 3% stacking gels) as described by Laemmli (1970). Following electrophoresis for 15 h at 50 V, proteins were transferred electrophoretically to nitrocellulose paper in 25 mM Tris, 195 mM glycine, and 20% methanol at 100 V for 90 min at 4°C according to the method of Towbin *et al.* (1979). The nitrocellulose blots were first dried and then incubated in buffer containing 10 mM Tris-HCl (pH 7.4, 25°C), 0.5 M NaCl, 0.05% Tween-20, and 0.5% nonfat dry milk ("buffer A") for 1 h to block excess binding sites. Blots were then incubated in buffer A in the presence of 1:1000 dilutions of monoclonal or polyclonal antibodies to Ca^{2+} ATPase, calsequestrin, ryanodine-sensitive Ca^{2+} channel and 53 000-Da and 165 000-Da glycoproteins at room temperature under gentle rocking for 16 h. Blots were washed twice for 10 min in buffer A to remove excess unbound antibody and then incubated for 1 h in buffer A containing the appropriate secondary antibody (goat anti-mouse and anti-rabbit IgG-peroxidase conjugates or rabbit and anti-guinea-pig IgG peroxidase conjugate) at room temperature for 1 h. Blots were washed twice with buffer A again and then developed using 4-chloro-l-naphthol as the substrate.

These procedures were modified in immunostains ofphospholamban. Gradient gels were 10-20% polyacrylamide, and electrophoretic transfer was performed at 25 V for 6.5 h. The concentration of anti-phospholamban antibody used in staining was 2.5 μg/ml.

Endo-β-N-acetylglucosaminidase H digestion of cardiac sarcoplasmic reticulum

High mannose oligosaccharide chains were removed from cardiac sarcoplasmic reticulum glycoproteins with endo-β-N-acetylglucosaminidase H (endo H) as previously described (Campbell *et al.*, 1983). Cardiac sarcoplasmic reticulum (25 μg) was solubilized with 5 μl of 10% SDS. The SDS-solubilized samples were boiled for 30 s and then cooled and diluted with 100 μl of 100 mM sodium citrate (pH 5.5). Proteolytic activity was inhibited by the addition of 5 μl of PMSF (0.2 M). Samples were incubated in the presence and absence of 10 mu endo H for four hours at 37°C and dissolved in SDS buffer (Laemmli, 1970) for 1 min at 95°C prior to electrophoresis.

Results

Identification and characterisation of proteins in human cardiac sarcoplasmic reticulum using monoclonal and poly clonal antibodies

Immunoblot staining of human cardiac sarcoplasmic reticulum with a monoclonal antibody to the Ca^{2+}-transporting ATPase of canine cardiac sarcoplasmic reticulum demonstrated the presence of an antigenically-related protein in human preparations (Fig. 1). The apparent molecular weight of the human protein was 105 000-Da, slightly larger than that of the canine form. The antibody did not cross-react with the Ca^{2+}-transporting ATPase of skeletal muscle, a separate gene product. Phospholamban was identified in human cardiac sarcoplasmic reticulum using a monoclonal antibody to its canine and bovine forms (Fig. 1). When solubilized at room temperature, the human protein migrated on SDS polyacrylamide gels predominantly with an apparent molecular weight of 29 000-Da, slightly higher than that of the canine form. Another band migrating with an apparent molecular weight of 7 000-Da was also present in both human and canine preparations. When human or canine sarcoplasmic reticulum was solubilized at

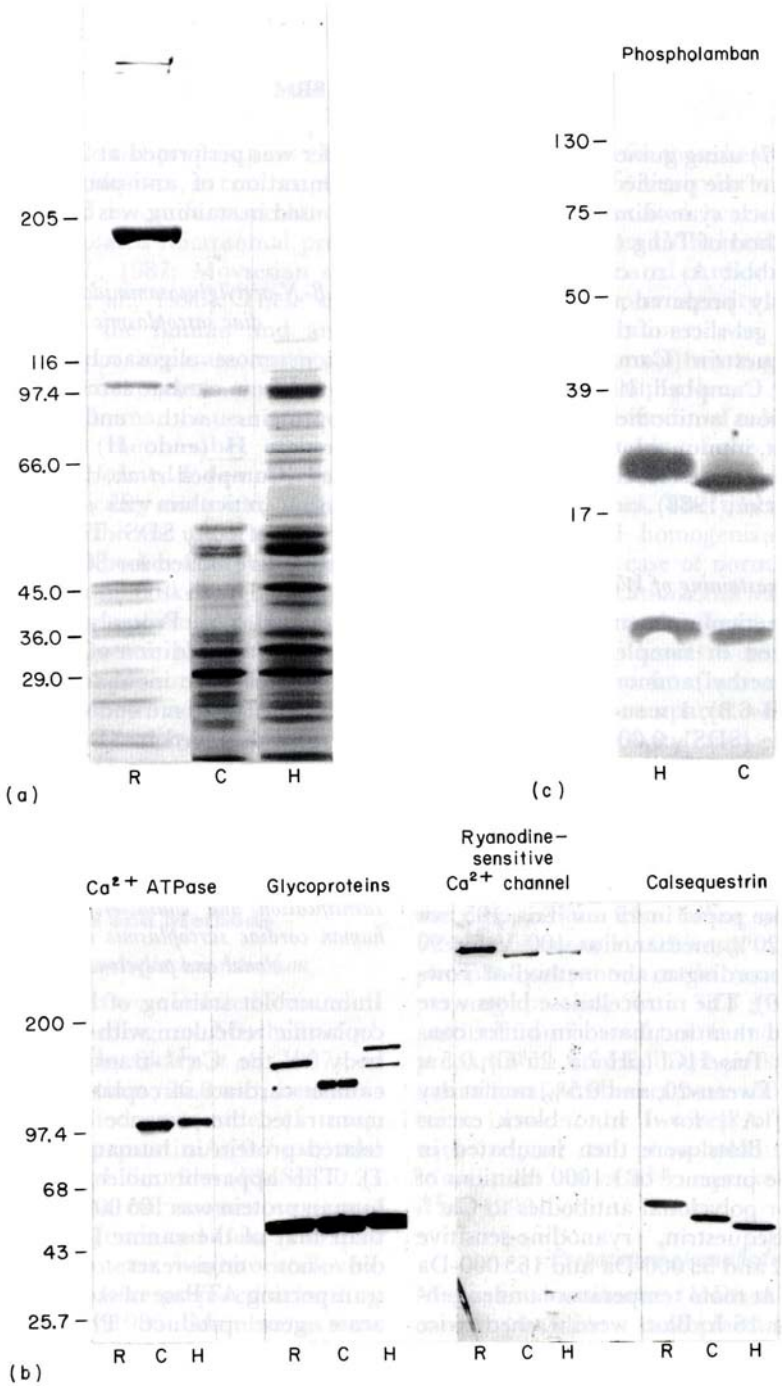

FIGURE 1. Comparison of rabbit skeletal, canine cardiac, and human cardiac sarcoplasmic reticulum. (a) Protein samples (20 μg/lane) from rabbit skeletal muscle triads (R), canine cardiac muscle triads (C), and human cardiac microsomes (H) were electrophoresed through SDS-polyacrylamide gradient gels (3-12%) and stained with Coomassie blue. Molecular weights ($\times 10^{-3}$) are indicated at left; (b) Proteins electrophoresed through SDS-polyacrylamide gels were transferred electrophoretically to nitrocellulose and immunoperoxidase-stained using monoclonal antibody IID8 (anti-Ca^{2+} ATPase), monoclonal antibody XIIC4 (anti-glycoprotein), polyclonal antibody GP-3 (anti-ryanodine-sensitive Ca^{2+} channel), and polyclonal antibody rabbit A (anti-calsequestrin). Proteins per lane were:

	Rabbit	Canine	Human
anti-Ca^{2+} ATPase	5 μg	5 μg	5 μg
anti-glycoproteins	10 μg	10 μg	30 μg
anti-Ca^{2+} channel	5 μg	60 μg	60 μg
anti-calsequestrin	5 μg	5 μg	5 μg

(c) 20 μg samples of human and canine cardiac sarcoplasmic reticulum were electrophoresed, transferred electrophoretically to nitrocellulose and immunoperoxidase-stained using monoclonal antibody Al (anti-phospholamban).

95°C, however, only the 7000-Da form was present (data not shown). These findings are consistent with the heat-induced pentamer-monomer shift of phospholamban.

A monoclonal antibody to the 53 000- and 160 000-Da glycoproteins of rabbit skeletal muscle sarcoplasmic reticulum and the 53 000- and 130 000-Da glycoproteins of canine cardiac sarcoplasmic reticulum also cross-reacted with proteins in human cardiac preparations (Fig. 1). The major species identified in human cardiac sarcoplasmic reticulum migrated with an apparent molecular weight of 53 000-Da, indistinguishable from that of its counterparts in rabbit skeletal and canine cardiac sarcoplasmic reticulum. The antibody also cross-reacted with two higher molecular weight species in human cardiac sarcoplasmic reticulum, one of which migrated with an apparent molecular weight of 165 000-Da and the other with an apparent molecular weight of 155 000-Da. To establish that the species identified by this antibody in human cardiac sarcoplasmic reticulum were glycoproteins, vesicle preparations were treated with endo H prior to electrophoresis, transfer and immunostaining (Fig 2). The apparent molecular weights of the three glycoprotein bands were all reduced by 4 000-5 000-Da by this treatment. This effect is comparable to that seen in rabbit skeletal and canine cardiac sarcoplasmic reticulum, and confirms that the immunostained bands in human cardiac sarcoplasmic reticulum are glycoproteins with mannose-containing oligosaccharide side chains (Campbell and MacLennan, 1981; Campbell *et al.*, 1983). The magnitude of the mobility shifts also made clear that the 155 000-Da band in human cardiac sarcoplasmic reticulum was *not* generated by cleavage of the glyco-peptide bond of the 165 000-Da glycoprotein.

Finally, polyclonal antibodies were used to identify and characterize the ryanodine-sensitive Ca^{2+} channel and calsequestrin in human cardiac sarcoplasmic reticulum (Fig. 1). The apparent molecular weight of the human cardiac ryanodine receptor was approximately 400 000-Da, slightly less than that of the rabbit skeletal muscle form but essentially identical to that of the canine cardiac form. Human cardiac calsequestrin migrated with an apparent molecular weight of 52000-Da, lower than those of the rabbit skeletal (63 000-Da) or canine cardiac (55 000-Da) forms.

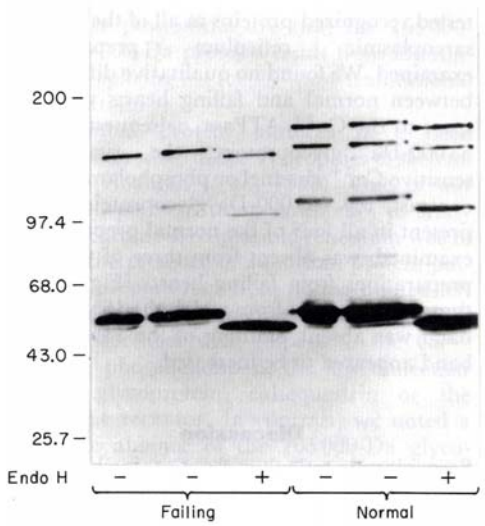

FIGURE 2. Effect of endo H treatment on failing and normal human heart sarcoplasmic reticulum glycoproteins. Samples of human cardiac sarcoplasmic reticulum were subjected to endo H treatment as described in "Methods". Samples were then subjected to electrophoresis on a 3-12% polyacrylamide gel, transferred electrophoretically to nitrocellulose, and stained with monoclonal anti-glycoprotein antibody (XIIC4). The three lanes at left contain sample from one of the patients with idiopathic dilated cardiomyopathy in whom the 165000-Da band was absent (see below in "Results"), and the three lanes at right contain one of the normal samples. In each set, the first lane contains original sample, the second control-treated sample and the third endo H-treated sample. Each lane contains 25 μg of protein. Molecular weights ($\times 10^{-3}$) are indicated on the left.

Comparison of protein patterns in normal and failing human heart

To determine whether qualitative differences might exist between normal and failing human hearts with respect to their protein constituents, we compared immunoblots of left ventricular sarcoplasmic reticulum prepared from the normal left ventricles of unmatched organ donors and the excised failing left ventricles of patients with idiopathic dilated cardiomyopathy. The antibodies

tested recognized proteins in all of the human sarcoplasmic reticulum preparations examined. We found no qualitative differences between normal and failing hearts with respect to the Ca^{2+} ATPase, calsequestrin, the 53000-Da glycoprotein, the ryanodine-sensitive Ca^{2+} channel or phospholamban. In contrast, the 165000-Da glycoprotein band, present in all four of the normal preparations examined, was absent from three of the nine preparations from failing hearts (Fig. 3). In those preparations from which the 165 000-Da band was absent, staining of the 155 000-Da band appeared to be increased.

Discussion

Sarcoplasmic reticulum from canine heart has been studied extensively, and several of its protein constituents have been identified and characterized. Among these are a 105 000-Da Ca^{2+}-transporting ATPase (Brandl *et al.*, 1986, 1987); phospholamban, a 27000-29 000-Da pentamer whose phosphorylation is associated with stimulation of Ca^{2+} uptake (Kirchberger *et al.*, 1974; Tada *et al.*, 1974); calsequestrin, a 55 000-Da protein that binds Ca^{2+} within the sarcoplasmic reticulum with low affinity and high capacity (Campbell *et al.*, 1983); two glycoproteins, 53000-Da and 130 000-Da, whose functions remain unknown but which may be involved in intraluminal Ca^{2+}-binding and regulation of Ca^{2+} uptake (Campbell *et al.*, 1983; Campbell and MacLennon 1981, Campbell and MacLennan, 1983; Pepper *et al.*, 1985; Helmke and Howard, 1987; Leberer *et al.*, 1989a; Leberer *et al.*, 1989b; Kutchai and Campbell, 1989); and a 400000-Da ryanodine-sensitive Ca^{2+} channel (Lai *et al.*, 1988; Innui *et al.*, 1987; Campbell *et al.*, 1987; Smith *et al.*, 1988). The Ca^{2+} -transporting ATPase of cardiac sarcoplasmic reticulum, like phospholamban, is the product of a gene expressed in cardiac and slow-twitch skeletal muscle but not in fast-twitch skeletal muscle (whose sarcoplasmic reticulum Ca^{2+} ATPase is the product of a separate gene; Brandl *et al.*, 1986). Calsequestrin is also the product of two separate genes, one of which predominates in fast- and slow-twitch skeletal muscle and the other of which predominates in cardiac muscle (Fliegel *et al.*, 1989).

Less is known regarding the genetics of the other sarcoplasmic reticulum proteins. The 53 000- and 160 000-Da glycoproteins of rabbit fast-twitch skeletal muscle sarcoplasmic reticulum have been shown to be alternate-splice products of the same gene (Leberer *et al.*, 1989b). Whether the same applies to the corresponding glycoprotein of cardiac muscle sarcoplasmic reticulum is uncertain, but the

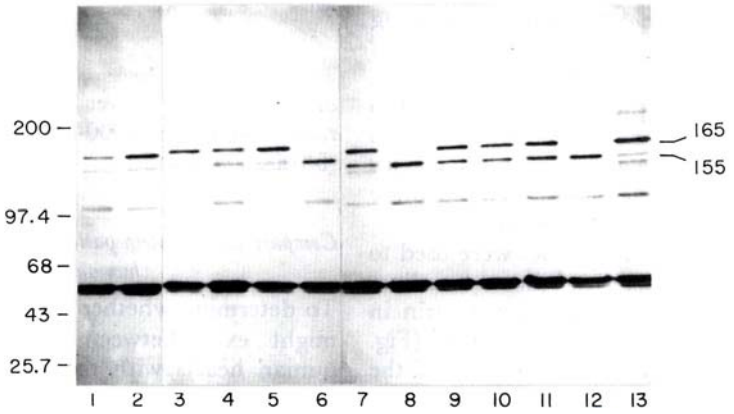

FIGURE 3. Identification of proteins in sarcoplasmic reticulum from normal and failing heart. Samples of sareoplasmic reticulum were electrophoresed through SDS-polyacrylamide gels (3-12%) and transferred electrophoretically to nitrocellulose. Nitrocellulose transfers were stained with monoclonal anti-glycoprotein (XIIC4). Molecular weights ($\times 10^{-3}$) are indicated on the left. Lanes 1-4 contained samples from normal hearts and lanes 5-13 contained samples from failing hearts.

cross-reactivity of the monoclonal antibody used in these experiments would make this seem likely. The genetics of the ryanodine-sensitive Ca^{2+} channel, whose cardiac and skeletal muscle forms are similar in apparent molecular weight and channel properties (Lai *et al.*, 1988; Inui *et al.*, 1987; Anderson *et al.*, 1989), remain undefined. Significant differences in the ryanodine-binding properties of cardiac and skeletal muscle sarcoplasmic reticulum have been noted, raising the possibility that the cardiac and skeletal muscle proteins are products of separate genes or are alternate-splice or post-translationally modified products of the same gene. These differences might just as plausibly result from differences in the molecular environments of the ryanodine receptor in cardiac and skeletal muscle sarcoplasmic reticulum. Our results do not exclude any of these possibilities.

In the experiments described in this paper, we have used immunochemical methods to characterize the major proteins of human cardiac sarcoplasmic reticulum. The apparent molecular weights of the human cardiac forms of the Ca^{2+} ATPase, ryanodine-sensitive Ca^{2+} channel, calsequestrin, phospholamban and the low molecular weight glycoprotein were similar to those of their canine counterparts. More striking differences were seen with respect to the high molecular weight glycoproteins. Two high molecuair weight glycoprotein bands were identified in humans, whereas only one high molecular weight glycoprotein band was identified in canine cardiac preparations. In addition, the human cardiac glycoproteins had apparent molecular weights 25 000- to 35 000-Da higher than that of the corresponding canine cardiac glycoprotein. The reason for these differences is not obvious. One possibility is that the 155 000-Da protein, whose staining appeared increased in samples from which the 165 000-Da band was absent, might be a shorter proteolytic derivative of the 165 000-Da glycoprotein (as shown in Fig. 2, it did not result from cleavage of the glycopeptide bond of the 165 000-Da glycoprotein). The high molecular weight glycoprotein of skeletal muscle is an intralumenal protein resistant to proteolysis in intact vesicles, however. If this property is shared by its human cardiac counterparts, proteolysis of the 165 000-Da glycoprotein would seem less likely. Other possibilities are that the 165000- and 155000-Da proteins result from alternative splicing or post-translational modification.

Abnormal protein patterns have been identified in some of the dystrophies of skeletal muscle (Hoffman *et al.*, 1988). One of our major purposes in using antibodies to study human cardiac sarcoplasmic reticulum was to test for the presence of abnormal protein patterns in preparations from failing dilated left ventricles. Our experiments revealed no qualitative differences with respect to the Ca^{2+} ATPase, phospholamban, the low molecular weight glycoprotein, calsequestrin or the ryanodine receptor. In contrast, we noted a complete absence of the 165 000-Da glycoprotein band and an accompanying increase in the 155 000-Da glycoprotein band in three of the nine preparations from failing hearts. The absence of the 165000-Da band might therefore reflect increased proteolysis in affected hearts. None of the other proteins examined showed any qualitative differences in susceptibility to proteolysis in preparations from normal and failing heart, however. For this reason, and for the reasons noted earlier, we regard this explanation as less likely.

Does the absence of the 165000-Da glycoprotein band identify or reflect a pathogenetic mechanism in a subset of humans with idiopathic dilated cardiomyopathy? The possibility is intriguing, but until more is learned about the 165 000-Da and 155 000-Da glycoproteins consideration must remain speculative. The high molecular weight glycoprotein of skeletal muscle has recently been shown to be a Ca^{2+} binding protein localized to the lumen of the longitudinal sarcoplasmic reticulum. Abnormalities involving this protein might therefore be expected to affect Ca^{2+} sequestration *in vivo*. The reason for the absence of this protein in three of the nine failing ventricles is not certain, and it would be difficult to propose altered genetic mechanisms involving a gene that has not been characterized in cardiac muscle. Finally, the number of preparations examined was too small to allow predictions of incidence. Improvements in the donor-recipient matching efficiency of organ transplantation networks have resulted in a decrease in the availability of normal human myocardium in quantities

sufficient for sarcoplasmic reticulum preparations, making it unlikely that the scope of experiments using this methodology can be expanded to any great degree. Further answers may have to wait until 155000- and 165 000-Da glycoprotein messenger RNA levels can be measured in normal and failing human hearts.

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References

ANDERSON, K., LAI, F. A., LIU, Q.-Y., ROUSSEAU, E., ERICKSON, H. P., MEISSNER, G. (1989) Structural and functional characterization of the purified cardiac ryanodine receptor-Ca^{2+} channel complex. J Biol Chem **264**: 1329-1335.

BRANDL, C.J., GREEN, N. M., KORCZAK, B., MAXLENNAN, D. H. (1986) Two Ca^{2+} ATPase genes: homologies and mechanistic implication of deduced amino acid sequences. Cell **44**: 597-607.

BRANDL, C. J., DE LEON, S., MARTIN, D. R., MACLENNAN, D. H. (1987) Adult forms of the Ca^{2+} ATPase of sarcoplasmic reticulum. J Biol Chem **262**: 3768-3774.

CAMPBELL, K. P., MACLENNAN, D. H. (1981) Purification and characterization of the 53000 Dalton glycoprotein from the sarcoplasmic reticulum. J Biol Chcm **256**: 4626-4632.

CAMPBELL, K. P., MACLENNAN, D. H. (1983) Labeling of high-affinity ATP binding sites on the 53000 and 160000 Dalton glycoproteins of the sarcoplasmic reticulum with the photoaffinity probe 8-N_3(α-^{32}P)-ATP.J Biol Chem **258**: 1391-1394.

CAMPBELL, K. P., MACLENNAN, D. H.,JORGENSEN, A. O., MINTZER, M. C. (1983) Purification and characterization of calsequestrin from canine cardiac sarcoplasmic reticulum and identification of the 53 000 dalton glycoprotein. J Biol Chem **258**: 1197-1204.

CAMPBELL, K. P., KNUDSON, C. M., IMAGAWA, T., LEUNG, A. T., SUTKO, J. L., KAHL, S. D., RAAB, C. R., MADSON, L. (1987) Identification and characterization of the high affinity [^{3}H]Ryanodine receptor of the junctional sarcoplasmic reticulum Ca^{2+} channel. J Biol Chem **262**: 6460-6463.

FUEGEL, L., LEBERER, E., GREEN, N. M., MACLENNAN, D. H. (1989) The fast-twitch muscle calsequestrin isoform predominates in rabbit slow-twitch soleus muscle. FEBS Lett **242**: 297-300.

GWATHMEY, J. K., COPELAS, L., MACKINNON, R., SCHOEN, F. J., FELDMAN, M. D., GROSSMAN, W., MORGAN, J. P. (1987) Abnormal intracellular calcium handling in myocardium from patients with end-stage heart failure. Circ Res **61**: 70-76.

HELMKE, S. M., HOWARD, B. D. (1987) Fractionation and reconstitution of the sarcoplasmic reticulum Ca^{2+} pump solubilized and stabilized by CHAPS/lipid micelles. Membr Biochem **7**: 1-22.

HOFFMAN, E. P., KNUDSON, C. M., CAMPBELL, K. P., KUNKEL, L. M. (1987) Subcellular fractionation of dystrophin as a component of triads. Nature **330**: 754-758.

HOFFMAN, E. P., FISCHBECK, K. H., BROWN, R. H., JOHNSON, M., MEDORI, R., LOIKE, J. D., HARRIS, J. B., WATERSTON, R., BROOKE, M., SPECHT, L., KUPSKY, W., CHAMBERLAIN, J., CASKEY, C. T., SHAPIRO, F., KUNKEL, L. M. (1988) Characterization of dystrophin in muscle-biopsy specimens from patients with Duchenne's or Becker's muscular dystrophy. N Engl J Med **318**: 1363-1368.

INUI, M., SAITO, A., FLEISCHER, S. (1987) Isolation of the ryanodine receptor from cardiac sarcoplasmic reticulum and identity with the feet structures. J Biol Chem **262**: 15637-15642.

JONES, L. R., GALA, S. E. (1981) Biochemical evidence for functional heterogeneity of cardiac sarcoplasmic reticulum vesicles. J Biol Chem **256**: 11809-11818.

JORGENSEN, A. O., CAMPBELL, K. P. (1984) Evidence for the presence of calsequestrin in two structurally different regions of myocardial sarcoplasmic reticulum. J Cell Biol **98**: 1597-1602.

JORGENSEN, A. O., ARNOLD, W., PEPPER, R., KAHL, S. D., CAMPBELL, K. P. (1984) A monoclonal antibody to the Ca^{2+}-ATPase of cardiac sarcoplasmic reticulum crossreacts with type I (slow) but not type II (fast) skeletal muscle fibers. Cell Motility and the Cytoskeleton **9**: 164-174.

KIRCHBERGER, M. A., TADA, M., KATZ, A. M. Adenosine (1974) 3':5'-monophosphate-dependent protein kinase-catalyzed phosphorylation reaction and its relationship to calcium transport in cardiac sarcoplasmic reticulum. J Biol Chem **249**: 6166-6173.

KUTCHAI, H., CAMPBELL, K. P. (1989) Calcium transport by sarcoplasmic reticulum of skeletal muscle is inhibited by antibodies against the 53 KDa glycoprotein of the sarcoplasmic reticulum membrane. Biochemistry **28**: 4830-4839.

LAEMMLI, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond) **227**: 680-685.

LAI, F. A., ANDERSON, K., ROUSSEAU, E., LIU, Q.-Y., MEISSNER, G. (1988) Evidence for a Ca^{2+} channel within the ryanodine receptor complex from cardiac sarcoplasmic reticulum. Biochem Biophys Res Commun 151: 441–449 (1988).
LEBERER, E., CHARUK, J. H. M., CLARKE, D. M., GREEN, N. M., Zubrzycka-Gaarn, E., MacLennan, D. H. (1989a) Molecular cloning and expression of cDNA encoding the 53000-dalton glycoprotein of rabbit skeletal muscle sarcoplasmic reticulum. J Biol Chem 264: 3484–3493.
LEBERER, E., CHARUK, J. H. M., GREEN, N. M., MACLENNEN, D. H. (1989b) Molecular cloning and expression of cDNA encoding a lumenal calcium binding glycoprotein from sarcoplasmic reticulum. Proc Natl Acad Sci **86**: 6047–6051.
LEUNG, A. T., IMAGAWA, T., BLOCK, B., FRANZINI-ARMSTRONG, C., CAMPBELL, K. P. (1988) Biochemical and ultrastructural characterization of the 1-4-dihydropyridine receptor from rabbit skeletal muscle: evidence for a 52000-Da subunit. J Biol Chem **263**: 944–1001 .
LIMAS, C. J., OLIVARI, M. T., GOLDENBERG, I. F., LEVINE, T. B., BENDITIT, D. G., SIMON, A. (1987) Calcium uptake by cardiac sarcoplasmic reticulum in human dilated cardiomyopathy. Cardiovasc Res **21**: 601–605.
MITCHELL, R. D., PALADE, P., FLEISCHER, S. (1983) Purification of morphologically intact triad structures from skeletal muscle. J Cell Biol **96**: 1008–1016.
MOVSESIAN, M. A., BRISTOW, M. R., KRALL, J. (1989) Calcium uptake by cardiac sarcoplasmic reticulum from patients with idoiopathic dilated cardiomyopathy. Circ. Res **65**: 1141–1144.
MOVSESIAN, M. A., COLYER, J., WANG, J. H., KRALL, J. (1990) Phospholamban-mediated stimulation of Ca^{2+} uptake in cardiac sarcoplasmic reticulum from normal and failing human hearts. J Clin Invest **85**: 1698–1702.
PEPPER, D. R., RAAB, C. R., CAMPBELL, K. P. (1985) Identification and characterization of 53 000 Da and 160000 Da glycoproteins and the Ca^{2+}-ATPase of sarcoplasmic reticulum in red and white skeletal, smooth and cardiac muscles. Biophys J **47**: 344a.
SHARP, A. H., IMAGAWA, T., LEUNG, A. T., CAMPBELL, K. P. (1987) Identification and characterization of the dihydropyridine-binding subunit of the skeletal muscle dihydropyridine receptor. J Biol Chem **262**: 12309–12315.
SMITH, J. S., IMAGAWA, T., MA, J., FILL, M., CAMPBELL, K. P., CORONADO, R. (1988) Purified ryanodine receptor from rabbit skeletal muscle is the calcium channel of sarcoplasmic reticulum. J Gen Physiol **92**: 1–26.
SUZUKI, T., WANG, J. H. (1986) Stimulation of bovine cardiac sarcoplasmic rericulum Ca^{2+} pump and blocking of phospholamban phosphorylation and dephosphorylarion by a phospholamban monoclonal antibody. J Biol Chem **261**: 7018–7023.
TADA, M., KIRCHBERGER, M. A., REPKE, D. I., KATZ, A. M. (1974) The stimulation of calcium transport in cardiac sarcoplasmic rericulum by adenosine 3':5'-monophosphate-dependent protein kinase.J Biol Chem **249**: 6174–6180.
TOWBIN, H., STAEHUN, T., BORDON, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci **76**: 4350–4354.
TUNG, A. S. (1983) Production of large amounts of antibodies, nonspecific immunoglobins, and other serum proteins in asicric fluids of individual mice and guinea pigs. Methods Enzymol **93**: 12–23.
WHITMER, J. T., KUMAR, P., SOLARO, R.J. (1988) Calcium transport properties of cardiac sarcoplasmic rericulum from cardiomyopathic Syrian hamsters (BIO 53.58 and 14.6): evidence for a quantitative defect in dilated myopathic hearts not evident in hypertrophic hearts. Circ Res **62**: 81-85.