Biochimica et Biophysica Acta 899 (1987) 151–158
Elsevier

BBA 73546

Smooth-muscle endoplasmic reticulum contains a cardiac-like form of calsequestrin

Frank Wuytack [a], Luc Raeymaekers [a], Jan Verbist [a], Larry R. Jones [b] and Rik Casteels [a]

[a] *Laboratorium voor Fysiologie K.U. Leuven, Leuven (Belgium), and* [b] *The Krannert Institute of Cardiology, Indianapolis, IN (U.S.A.)*

(Received 5 November 1986)

Key words: Calcium ion transport; Endoplasmic reticulum; Calsequestrin; (Pig stomach smooth muscle)

It is proposed that smooth-muscle endoplasmic reticulum contains calsequestrin and that this protein in smooth muscle resembles cardiac calsequestrin more than the skeletal-muscle form. This proposal is based on seven similarities between the smooth-muscle protein and cardiac calsequestrin. 1. Proteins with an M_r of 55000 can be extracted from the membranes of smooth muscle and of cardiac muscle using 100 mM Na_2CO_3. 2. The protein from smooth muscle binds to phenyl-Sepharose in the absence of Ca^{2+} and is released by 10 mM $CaCl_2$, as has been observed for cardiac calsequestrin. 3. The protein from smooth muscle comigrates with the cardiac calsequestrin on Laemmli-type SDS-polyacrylamide gel electrophoresis. 4. The protein of M_r 55000 from smooth muscle and cardiac calsequestrin both strain blue with the carbocyanine dye Stains-all. 5. Both proteins present similar one-dimensional Cleveland peptide maps although minor differences might exist. 6. From an analysis of subcellular membranes separated by sucrose gradient centrifugation it is concluded that the protein with M_r 55000 from the smooth muscle is confined to the endoplasmic reticulum, the same subcellular structure from which, in heart muscle, calsequestrin can be isolated. 7. Antibodies raised against canine cardiac calsequestrin bind to a protein of similar M_r in smooth-muscle endoplasmic reticulum. In addition to the calsequestrin, three other extrinsic proteins with an M_r of 130000, 100000 and 63000, stain blue with Stains-all and occur in the endoplasmic reticulum of smooth muscle.

Introduction

Ca^{2+} plays an important role in the excitation-contraction coupling in smooth muscle. The relative contribution of extracellular and intracellular Ca^{2+} for excitation may differ according to the mode of stimulation and is still a matter of dispute. Recently, a better insight has been obtained into some of the proteins engaged in the translocation of Ca^{2+} across smooth-muscle membranes. A calmodulin-binding Ca^{2+}-transport ATPase has been purified from smooth muscle [1–3] and has been shown to reside in the plasma membrane [4]. More recent experiments have also revealed the presence of the phosphoprotein intermediate of a second and different type of Ca^{2+}-transport ATPase in the endoplasmic reticulum of smooth muscle [5–7]. The Ca^{2+}-transport proteins in smooth muscle are similar to the Ca^{2+}-transport proteins in cardiac and in skeletal muscle both in their nature and localization. This prompted us to explore whether calsequestrin, a Ca^{2+}-binding protein hitherto shown to be present in the lumen

Correspondence: F. Wuytack, Laboratorium voor Fysiologie, K.U. Leuven, Campus Gasthuisberg, B-3000 Leuven, Belgium.

of the sarcoplasmic reticulum of skeletal [8] and cardiac muscle [9] was also present in smooth muscle. Calsequestrin could, in analogy to its proposed role in the striated muscles, act as a Ca^{2+} store in the endoplasmic reticulum of smooth muscle. The presence of calsequestrin within the lumen of the junctional elements of the endoplasmic reticulum of the rabbit portal vein has been proposed on morphological grounds by Somlyo and Franzini-Armstrong [10]. These authors compared, in skeletal-muscle and smooth-muscle endoplasmic reticulum, the appearance of granular strands, which are presumed to correspond to calsequestrin.

The calsequestrins from skeletal and cardiac muscle have several unique properties that, taken together, can be used to identify these proteins. One of these properties is the specific reaction of these polypeptides with the cationic carbocyanine dye Stains-all. This reaction results in a blue or purple staining of the polypeptide bands, whereas most other polypeptides stain orange or red [11]. A second property of the calsequestrins is their Ca^{2+}-dependent conformational change. This characteristic was exploited in a recently published method for the purification of calsequestrin from the fragmented sarcoplasmic reticulum of cardiac or skeletal muscle by Ca^{2+}-dependent elution of the protein from phenyl-Sepharose [12]. In alkaline media, the skeletal and cardiac form of calsequestrin differ remarkably in their electrophoretic mobility. In the Laemmli-type electrophoresis, the skeletal-muscle form of calsequestrin migrates with an apparent M_r of 63000, whereas for the cardiac form a value of 55000 is found. We report here that the smooth muscle of the pig stomach contains a protein that with seven independent characteristics corresponds to the cardiac-muscle form of calsequestrin.

Methods

Preparation of the membrane. Membranes were prepared from smooth muscle of the pig stomach antrum according to the method of Raeymaekers et al. [4]. Briefly, after removal of the mucosa about 400 g of muscle were passed through a tissue press and homogenized in 3 vol. of 0.25 M sucrose/1 mM dithiothreitol/0.05 mM phenylmethylsulfonyl fluoride by the combined action of a Waring Blendor and an Ultra-Turrax (Janke and Kunkel, K.G., Staufen, F.R.G.). The homogenate was centrifuged in a Sorvall GSA rotor at 13000 $\times g_{max}$ for 30 min. To 1200 ml of the supernatant 120 mg digitonin were added followed by solid sucrose and KCl up to 50% (w/w) and 0.6 M, respectively. This material was loaded into a Beckman Ti 15 or Kontron TZT 32.1650 zonal rotor below 250 ml of a 15–45% (w/w, linear with respect to volume) sucrose gradient containing 0.6 M KCl and 1 mM dithiothreitol. After isopycnic equilibration at 105000 $\times g_{max}$ for 20 h, fixed volume fractions were collected. The fractions were diluted 3-fold with 0.6 M KCl/1 mM dithiothreitol, pelleted in a Kontron TFT 45.94 rotor at 140000 $\times g_{max}$ for 1 h, and the pellets were resuspended in 0.25 M sucrose. The endoplasmic reticulum was collected between 18 and 25% sucrose. Sarcoplasmic reticulum was prepared from pig skeletal muscle and from pig cardiac ventricle by the method of Jones and Cala [13].

Purification of calsequestrin. Calsequestrin was purified from smooth-muscle membranes according to the slightly modified method of Cala and Jones [12]. A mixture of smooth-muscle membranes containing approximately 150 mg of protein, obtained from the gradient was centrifuged in a Beckman Ti 75 rotor at 224000 $\times g_{max}$ for 30 min. The pellets were resuspended in 100 mM Na_2CO_3 at a protein concentration of 2 mg/ml and incubated on ice for 30 min. The extracted proteins were separated from the insoluble residue by a 30 min centrifugation in a Ti 75 rotor at 224000 $\times g_{max}$. The supernatant was brought to 500 mM NaCl/50 mM 4-morpholinethanesulphonic acid (Mops)/1 mM dithiothreitol by adding the solid reagents. An adequate amount of a solution of 100 mM EGTA (pH 7.0) was added to obtain a final concentration of 0.5 mM EGTA. The pH of the mixture was then adjusted to 7.0 using concentrated HCl. This mixture (60 ml) was then incubated in an end-over-end mixer with 15 ml phenyl-Sepharose which had been equilibrated with buffer A (10 mM Mops/0.5 mM EGTA/500 mM NaCl/1 mM dithiothreitol/0.02% NaN_3 (pH 7.0)). After 1 h mixing, the gel was separated from the solution by vacuum filtration on a fritted glass disk. Thereupon the gel was washed with 3 volumes

of buffer A before transferring it to a small column of 1 cm inner diameter and further washing it with buffer A at 2 ml/min. As soon as the baseline of the ultraviolet monitor ($\lambda = 280$ nm) was stabilized, calsequestrin was eluted by addition of 10 mM $CaCl_2$ to buffer A. Finally another protein peak was eluted by 10 mM Mops/1 mM dithiothreitol/0.02% NaN_3 (pH 7.0).

SDS-polyacrylamide gel electrophoresis and Stains-all staining. Electrophoresis was performed according to the method of Laemmli [14] with 0.75 mm-thick gels and at a resolving gel concentration of 7.5% acrylamide. The proteins in the Na_2CO_3 extracts and the proteins eluted from the phenyl-Sepharose column were concentrated and the excessive amounts of salts were removed by precipitation of the proteins with 12% trichloroacetic acid. After a centrifugation at $10000 \times g_{max}$ for 5 min, the pellets were dissolved in sample buffer and the pH was readjusted to 6.8 with 2 M Tris base solution.

The gels were stained with Stains-all according to a method modified after King and Morrison [15] and Campbell et al. [11]. After fixing the gels in a solution of methanol/acetic acid/water (50 : 7 : 43, by vol.) they were repeatedly washed extensively in propan-2-ol/water (25 : 75, by vol.). Staining was done in a solution prepared by mixing 5 mg Stains-all dissolved in 25 ml formamide with 100 ml propan-2-ol/275 ml water/0.5 mg Tris base. The pH of this mixture was adjusted to 8.8 with 1 M HCl. Gels were stained for 16 h in the dark.

Cleveland peptide maps. Cleveland peptide maps were made [9] to compare the M_r 55000 band from smooth muscle with cardiac calsequestrin. First a Laemmli-type gel electrophoresis of the two proteins of M_r 55000 purified by phenyl-Sepharose chromatography was done in 0.75 mm-thick gels with a 7.5% acrylamide concentration in the separating gel. Thereupon the gels were quickly stained with Coomassie brilliant blue and immediately destained. The M_r 55000 bands from smooth muscle and cardiac muscle were excised from the gels and equilibrated for 30 min at room temperature in solution A, containing 0.125 M Tris-HCl (pH 6.8)/0.1% SDS/1 mM EDTA and frozen at $-85\,^{\circ}C$. After thawing a series of identical gel pieces each containing either of the two protein bands were incubated at room temperature for 45 min in solution A. The gel pieces were transferred into 10 mm slots of a new 0.75 mm-thick Laemmli-type gel with a separating gel of 15% acrylamide; subsequently, 10 μl of solution A containing 20% glycerol and 10 μl of solution A containing 10% glycerol, together with 0, 5, 20 or 100 ng *Staphylococcus aureus* V8 proteinase were added. The electrophoresis was immediately started and interrupted for 30 min as soon as the tracking dye reached the junction between the stacking and the separating gel. The gels were stained with the silver stain kit from Bio-Rad.

In order to compare the M_r 63000 protein from smooth muscle with skeletal-muscle calsequestrin, the fractions used in the first electrophoresis were: the Na_2CO_3-extract of smooth-muscle endoplasmic reticulum and the sarcoplasmic reticulum of pig skeletal muscle. The M_r 63000 bands were excised from the gels and treated as above. These peptide maps were stained with Stains-all.

Immunoblot techniques. Electrophoresis and electroblotting were conducted as described by Jones et al. [16]. Binding of rabbit antibodies against canine cardiac calsequestrin purified by the phenyl-Sepharose method was studied using ^{125}I-labelled protein A [16].

Materials. Phenyl-Sepharose CL-4B was from Pharmacia, Fine Chemicals AB Uppsala, Sweden, Stains-all from Eastman Kodak Co., Rochester, NY, U.S.A., *Staphylococcus aureus* V8 proteinase was from Pierce Chemical Co., Rockford, IL, U.S.A., the Silver-Stain kit was from Bio-Rad, Richmond, CA, U.S.A.

Results

That pig stomach smooth muscle contains a cardiac-like form of calsequestrin which differs from the fast skeletal muscle type is indicated by seven independent points of correspondence. (1) Both proteins are loosely associated with membranes and can be extracted with 100 mM Na_2CO_3. (2) They both bind to phenyl-Sepharose in the absence of Ca^{2+} and are released by 10 mM $CaCl_2$ (3). The two proteins have a similar apparent M_r of 55000 in Laemmli-type SDS-polyacrylamide gel electrophoresis. (4) Both proteins stain blue with Stains-all. (5) The proteins are

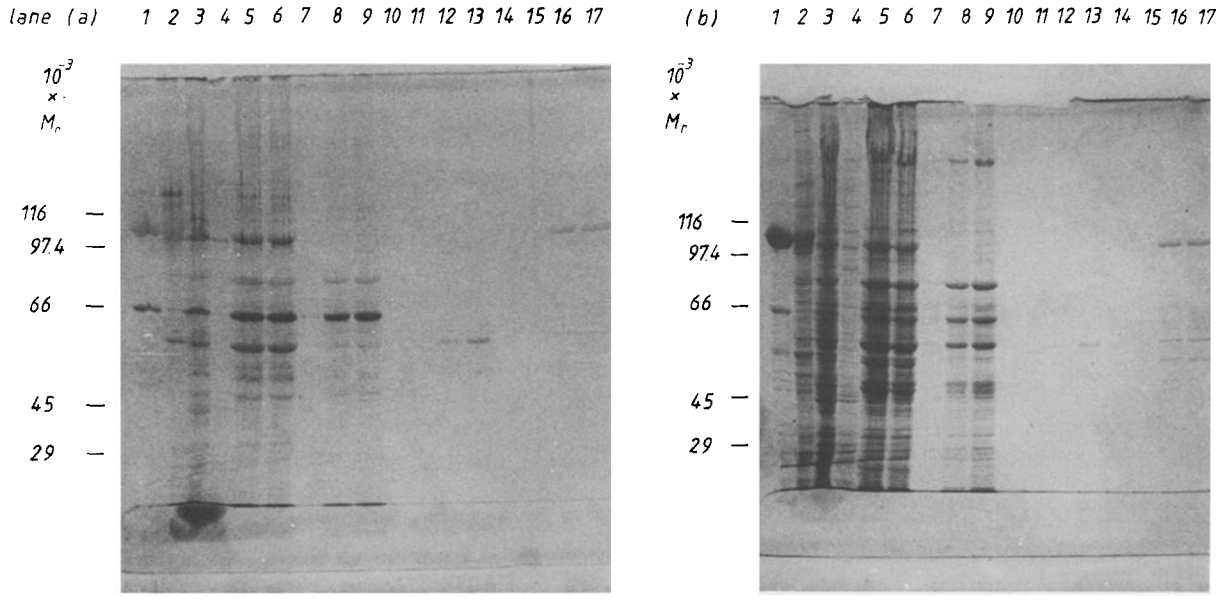

Fig. 1. Coomassie brilliant blue and Stains-all staining of porcine proteins in different membrane fractions. Two identical Laemmli-type gels with an acrylamide concentration of 7.5% in the separating gel were stained with Stains-all (a) or with Coomassie brilliant blue (b). Lanes: 1. pig skeletal-muscle sarcoplasmic reticulum; 2, pig cardiac-muscle sarcoplasmic reticulum; 3, pig antrum smooth-muscle endoplasmic reticulum; 4, Na_2CO_3-extracted smooth-muscle endoplasmic reticulum; 5 and 6, the corresponding Na_2CO_3 extract; 7, blanco; 8 and 9, samples of the proteins not bound to phenyl-Sepharose gel; 10–5, successive fractions released from the phenyl-Sepharose by 10 mM $CaCl_2$ (of these 12 and 13 show the smooth-muscle calsequestrin), 16 and 17, the M_r 100000 protein released from the phenyl-Sepharose by a low ionic strength wash.

confined to the endo(sarco)plasmic reticulum. (6) The two proteins present a similar one-dimensional Cleveland peptide map. (7) Western-type immunoblots indicate that antibodies raised against canine cardiac calsequestrin bind to the canine cardiac calsequestrin, as well as to a protein with a similar M_r present in smooth-muscle endoplasmic reticulum. Properties 1–4 are illustrated in Fig. 1, property 5 in Figs. 3 and 4, property 6 in fig. 2 and property 7 in Fig. 3.

Interestingly, we found in the smooth-muscle membranes also a polypeptide with M_r 63000, i.e., with the same M_r as the skeletal-muscle form of calsequestrin. This protein which is present in larger quantities than the M_r 55000 one can also be removed from the membranes by extraction in Na_2CO_3. It is in fact the most prominent blue-staining band on our gels of the Na_2CO_3-extract stained with Stains-all. With these properties it resembles the skeletal-muscle form of calsequestrin, but in contrast to the M_r 55000 polypeptide, and the M_r 63000 proteins in the sarcoplasmic reticulum of pig or rabbit skeletal muscle, it does not bind to phenyl-Sepharose in the absence of Ca^{2+}. The difference between the M_r 63000 band from smooth muscle and skeletal muscle has been further substantiated by comparing the Cleveland peptide maps of these proteins. These data presented in Fig. 2B do not indicate that the proteins are related. Even in the absence of *Staphylococcus* proteinase, the protein from smooth muscle migrates in the polyacrylamide gel (15% acrylamide) used to resolve the Cleveland proteolytic fragments, not as a single band but as a complex of five or six closely spaced bands. Probably some hydrolysis has taken place in the acid medium used to stain and destain the original gel. In contrast, calsequestrin from skeletal muscle does not show this characteristic.

A third extrinsic protein in smooth-muscle membranes that stains blue with Stains-all, has an M_r of 100000 and binds to the phenyl-Sepharose in the presence of 500 mM NaCl/0.5 mM EGTA. However, it is only released by lowering the ionic

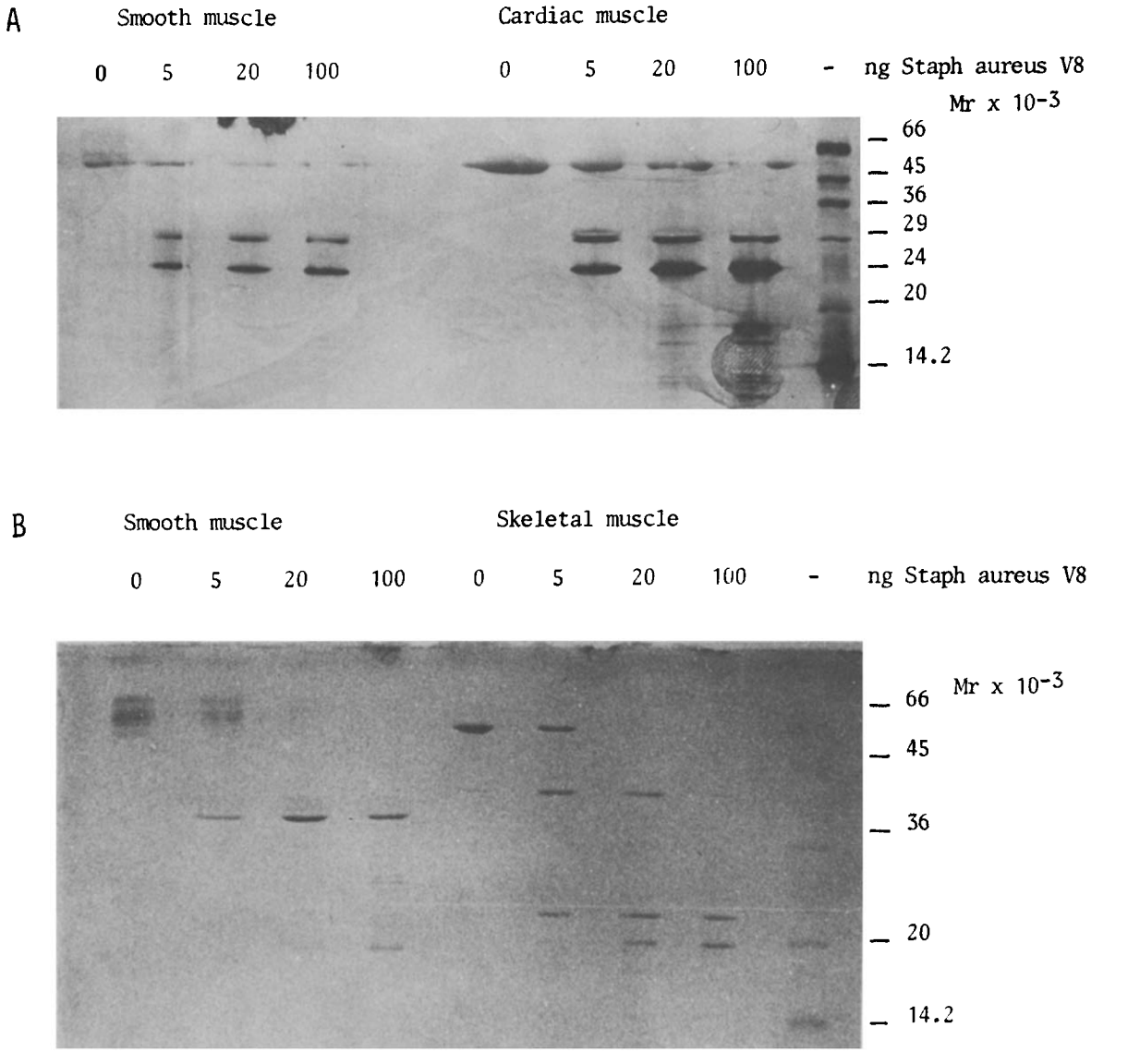

Fig. 2. Cleveland peptide maps of the M_r 55000 protein of smooth-muscle and cardiac-muscle calsequestrin (A) and of the M_r 63000 protein of smooth muscle and the skeletal-muscle calsequestrin (B). Proteolytic degradation of the two proteins by *Staphylococcus aureus* V8 proteinase is compared. The gel in A is silver-stained, that in B is stained by Stains-all. Both are Laemmli-type gels with an acrylamide concentration of 15% in the separating gel. The amount of proteinase (ng) is indicated above the lanes. The numbers to the right represent the $M_r \times 10^{-3}$ of the molecular weight standards in the lane at the right.

strength by omission of the NaCl from the buffer and not by eluting with 10 mM $CaCl_2$.

In addition to the blue-staining proteins of M_r 100000, 63000 and 55000, three other protein bands also faintly stain ultramarine blue with Stains-all. They have M_r values of, respectively, 130000, 120000 and 47000 and are most easily observed in the Na_2CO_3 extract (Fig. 1, lanes 5 and 6).

In order to specify further the similarity between calsequestrin from cardiac and smooth muscle we have examined whether antibodies raised against canine cardiac calsequestrin cross-react with the protein of M_r 55000 in smooth-

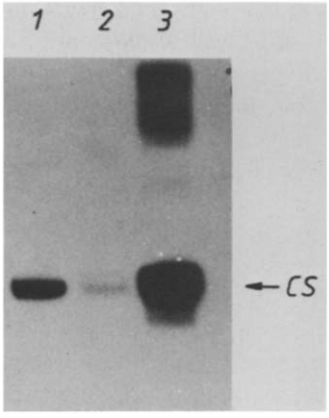

Fig. 3. Binding of anti-cardiac calsequestrin antibodies to smooth-muscle calsequestrin. 20 μg of proteins were electrophoresed and electroblotted [16] for each of the three lanes. The blot was treated with rabbit antibodies against canine cardiac calsequestrin and then with ^{125}I-labelled protein A. Endoplasmic reticulum and plasma membranes were prepared as indicated in Fig .4 (see also Ref. 4). 1, endoplasmic reticulum from pig antrum smooth muscle. 2, plasma membranes from pig antrum smooth muscle 3, dog cardiac sarcoplasmic reticulum. CS indicates the position of calsequestrin. The top of lane 3 shows also a reaction of the antibodies with a high M_r fraction copurifying with calsequestrin.

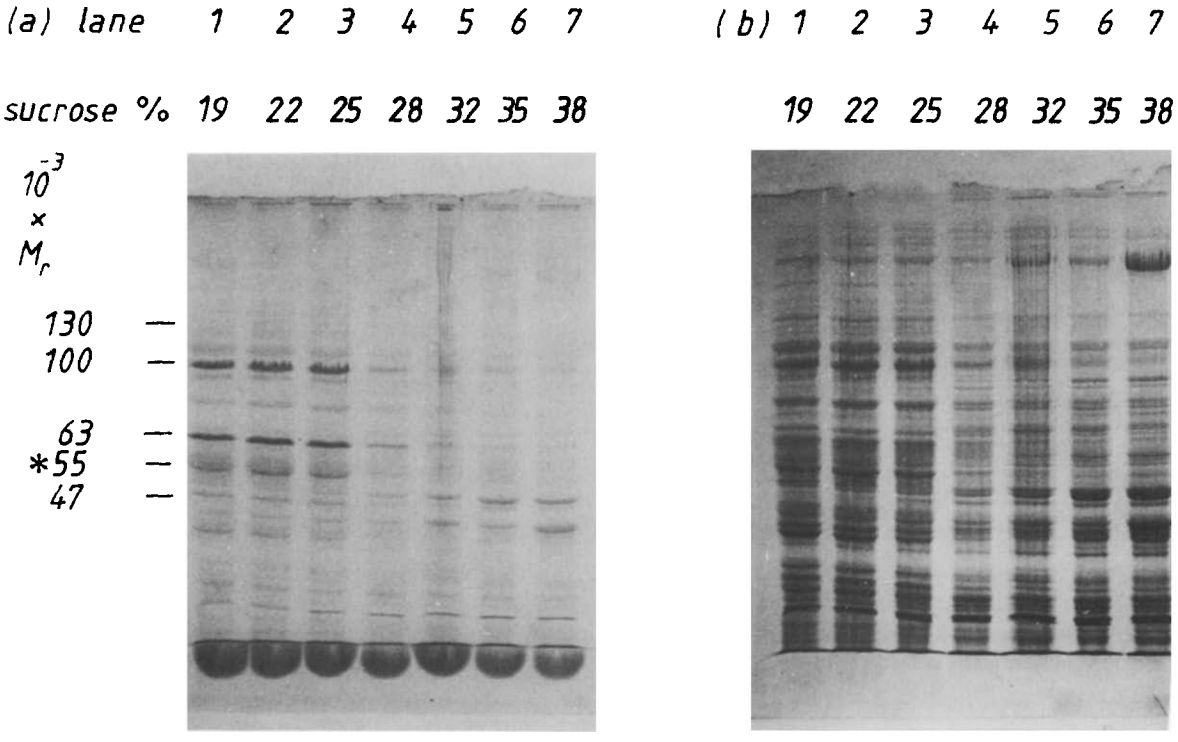

Fig. 4. The distribution of the proteins in the sucrose density gradients. Two identical Laemmli-type gels with an acrylamide concentration of 7.5% in the separating gel are shown, (a) stained with Stains-all, (b) with Coomassie brilliant blue. The numbers to the left indicate the $M_r \times 10^{-3}$ values for the blue-staining proteins. Due to the low amounts of some of these proteins and to technical difficulties in photographing the light-sensitive Stains-all pattern, not all of these blue bands are clearly reproduced. The smooth-muscle calsequestrin of M_r 55000 is indicated by the asterisk. The membranes were first treated with digitonin and separated by isopycnic equilibration on a sucrose density gradient containing 0.6 M KCl [4]. For each lane 0.1 mg of membrane protein was added. The mean concentrations of sucrose (in %, w/w) in the gradient at which the membranes equilibrated are also given. Fractions 1–3 represent the endoplasmic reticulum, 5–7 the plasma membranes.

muscle membranes. This cross-reaction is represented in Fig. 3. By using the different membrane fractions prepared by the method of Raeymaekers et al. [4] it is also shown that in smooth muscle this protein is a component of the endoplasmic reticulum.

This confinement in smooth muscle of the M_r 55 000 band staining blue with Stains-all to the endoplasmic reticulum is confirmed in Fig. 4. However, this 55 000 band is only faintly visible on the figure because of its very small amount. From the same figure it can also be concluded that the blue-staining proteins of M_r 130 000, 100 000, 63 000 and 47 000 are all copurifying with the endoplasmic reticulum.

Discussion

Smooth-muscle membranes contain a protein that presents a number of characteristics of the cardiac-muscle form of calsequestrin. We have demonstrated that this protein is found in membranes that after isopycnic equilibration on sucrose gradients seems to correspond to the endoplasmic-reticulum fragments. The total amount of this calsequestrin in smooth muscle is low. This is not surprising because also the amount of the Ca^{2+}-transport ATPase with an M_r 100 000 is much lower in antral smooth muscle than in cardiac muscle and certainly lower than in fast skeletal muscle. Even with the best techniques presently available it is difficult to prepare a few milligram of proteins of endoplasmic reticular membranes out of 100 g of antral smooth-muscle tissue. Moreover, this smooth-muscle endoplasmic reticulum contains approximately 100-fold less of the M_r 100 000 ($Ca^{2+} + Mg^{2+}$)-ATPase than the sarcoplasmic reticulum of the striated muscles [4], as can be judged from its specific activity, from its Ca^{2+}-dependent phosphoprotein intermediate [17] and from the corresponding Coomassie brilliant blue-staining polypeptides. Considering that even in cardiac muscle Campbell et al. [9] report for calsequestrin only a yield of approximately 1.0 μg/mg protein of sarcoplasmic reticulum vesicles, it is clear that not enough material could be prepared to do Ca^{2+}-binding studies or to make an amino acid analysis. In the present work we can therefore only demonstrate that smooth muscle, like skeletal and cardiac muscle, also contains calsequestrin in its endoplasmic reticulum and that the smooth-muscle form of calsequestrin resembles cardiac calsequestrin more than the skeletal-muscle form. Calsequestrin from smooth muscle has the same M_r as the cardiac-muscle form (55 000) and differs in this respect from the skeletal-muscle form (M_r = 63 000).

The present findings on the presence of the cardiac-like form of calsequestrin in smooth muscle again demonstrate the biochemical similarity of smooth muscle to cardiac muscle rather than to skeletal muscle. Further similarities between these two tissues have been proposed. Rabbit antibodies against porcine myocardial sarcoplasmic reticulum Ca^{2+}-transport ATPase bind to a polypeptide of similar M_r in porcine stomach smooth muscle (Wuytack and Gietzen, unpublished observation). Such a binding was not observed with rabbit antibodies against the Ca^{2+}-transport ATPase of porcine fast skeletal-muscle sarcoplasmic reticulum [18]. Furthermore, phospholamban was detected in cardiac muscle and in smooth-muscle sarcoplasmic reticulum [19] but not in fast skeletal muscle [20,21].

The nature of the M_r 63 000 extrinsic protein in smooth-muscle membranes which stains blue with Stains-all remains elusive. The similarity of its M_r to the skeletal-muscle form of calsequestrin is coincidental. This M_r 63 000 protein is not a component of the plasma membranes or of the mitochondria of smooth muscle because these organelles distribute in the sucrose density gradient at densities above 30% sucrose in which the concentration of this protein decreases. This density-gradient centrifugation of the membranes and the following wash were performed in the presence of 0.6 M KCl, whereby the electrostatic adsorption of proteins to the membranes is minimized. A comparative study of our M_r 63 000 protein and an acidic high affinity Ca^{2+}-binding protein of M_r 63 000 occurring in a number of tissues including smooth muscle [22] should be undertaken.

By applying the method of Maruyama et al. [23], which allows the visualization of ^{45}Ca binding to electrophoretically separated peptides on Western electroblots we could show Ca^{2+}-binding, not only to the M_r 55 000 band, but also to the M_r 63 000 band and to a lower extent to some other bands.

This observation indicates that the M_r 55000 calsequestrin of smooth muscle may not be the only protein involved in Ca^{2+}-binding in the endoplasmic reticulum. A further examination of Ca^{2+}-binding proteins in smooth-muscle endoplasmic reticulum may therefore be worthwhile.

Another protein of interest extracted by Na_2CO_3 from the smooth-muscle endoplasmic reticulum is the M_r 130000 Stains-all blue protein. Cala and Jones [12] have demonstrated by the same extraction procedure that canine cardiac muscle contains a Stains-all blue glycoprotein with the same M_r value, whereas the sarcoplasmic reticulum from rabbit fast skeletal muscle yields a Stains-all blue protein with M_r 160000. We could confirm their observations on rabbit fast skeletal muscle but found that in porcine skeletal muscle only a protein of M_r 130000 stains blue with Stains-all (results not shown). Both the sarcoplasmic reticulum from porcine skeletal and cardiac muscle seem to contain a similar M_r 130000 Stains-all blue protein, while the sarcoplasmic reticulum of rabbit skeletal muscle presents a protein with an M_r of 160000. This protein, which is a glycoprotein [12,24] may therefore be another common constituent of the sarco(endo)plasmic reticulum in different forms of muscle besides calsequestrin.

Acknowledgements

This work was supported by grant No. 3.0042.83 from the F.G.W.O. (Fonds voor Geneeskundig Wetenschappelijk Onderzoek) (Belgium). J.V. is a research assistant of the N.F.W.O. (Nationaal Fonds voor Wetenschappelijk Onderzoek) (Belgium).

References

1 Wuytack, F., De Schutter, G. and Casteels, R. (1981) FEBS Lett. 129, 297–300
2 De Schutter, G., Wuytack, F., Verbist, J. and Casteels, R. (1984) Biochim. Biophys. Acta 773, 1–10
3 Furukawa, K.-I. and Nakamura, H. (1984) J. Biochem. 96, 1343–1350
4 Raeymaekers, L., Wuytack, F. and Casteels, R. (1985) Biochim. Biophys. Acta 815, 441–454
5 Wuytack, F., Raeymaekers, L., Verbist, J., De Smedt, H. and Casteels, R. (1984) Biochem. J. 224, 445–451
6 Chiesi, M., Gasser, J. and Carafoli, E. (1984) Biochem. Biophys. Res. Commun .124, 797–806
7 Carsten, M.E. and Miller, J.D. (1984) Arch. Biochem. Biophys. 232, 616–623
8 MacLennan, D. and Wong, P.T.S. (1971) Proc. Natl. Acad. Sci. USA 68, 1231–1235
9 Campbell, K.P., MacLennan, D.H., Jorgensen, A.O. and Mintzer, M.C. (1983) J. Biol. Chem. 258, 1197–1204
10 Somlyo, A.V. and Franzini-Armstrong, C. (1985) Experientia 41, 841–856
11 Campbell, K.P., MacLennan, D.H. and Jorgensen, A.O. (1983) J. Biol. Chem. 258, 11267–11273
12 Cala, S.E. and Jones, L.R. (1983) J. Biol. Chem. 258, 11932–11936
13 Jones, L.R. and Cala, S.E. (1981) J. Biol. Chem. 256, 11809–11818
14 Laemmli, U.K. (1970) Nature (Lond.) 227, 670–685
15 King, L.E. and Morrison, M. (1976) Anal. Biochem. 71, 223–230
16 Jones, L.R., Simmerman, H.K.B., Wilson, W.W., Gurd, F.R.N. and Wegener, A.D. (1985) J. Biol. Chem. 260, 7721–7730
17 Wuytack, F., Raeymaekers, L., De Schutter, G. and Casteels, R. (1982) Biochim. Biophys. Acta 693, 45–52
18 Wuytack, F., Raeymaekers, L. and Casteels, R. (1985) Experientia 41, 900–905
19 Raeymaekers, L. and Jones, L.R. (1986) Biochim. Biophys. Acta 882, 258–265
20 Kirchberger, M.A. and Tada, M. (1976) J. Biol. Chem. 251, 725–729
21 Jorgensen, A.O. and Jones, L.R. (1986) J. Biol. Chem. 261, 3775–3781
22 Waisman, D.M., Salimath, B.P. and Anderson, M.J. (1985) J. Biol. Chem. 260, 1652–1660
23 Maruyama, K., Mikawa, T. and Ebashi, S. (1984) J. Biochem. 95, 511–519
24 Campbell, K.P. and MacLennan, D.H. (1981) J. Biol. Chem. 256, 4626–4632