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Ca PUMPS IN RABBIT STOMACH SMOOTH MUSCLE PLASMA MEMBRANE AND ENDOPLASMIC RETICULUM

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ABSTRACT

Ca uptake, Ca-dependent phosphorylation and reactivity to monoclonal antibodies against skeletal and cardiac sarcoplasmic reticulum (SR) Ca pump were studied using subcellular membranes obtained from rabbit stomach smooth muscle. The ATP-dependent azide insensitive Ca uptake by the subcellular fractions was studied in three modes: no additives, oxalate-stimulated and phosphate-stimulated. The overall recoveries of Ca uptake in all the three modes were quantitative. Without oxalate or phosphate, the membrane fraction F1 showed the highest Ca uptake. The ratio of oxalate- to phosphate-stimulated Ca uptake was highest in F3 and lowest in F1- Phosphorylation was examined by autoradiography and sodium dodecyl sulfate polyacrylamide slab gel electrophoresis. Both the fractions showed Ca-dependent phosphorylation in the presence and absence of 50 uM $LaCl_3$. In the absence of $LaCl_3$, F1 showed very low phosphorylation and F3 gave a very intense band at 100 kDa. The presence of $LaCl_3$ reduced the intensity of this band and showed a band at 130 kDa. Thus the ratio of intensities of the 130 kDa to 100 kDa bands was high in F1 and low in F3. F3 but not F1 also showed a band at 100 kDa which reacted with the monoclonal antibody against the dog cardiac SR Ca pump. This band reacted very poorly with monoclonal antibodies against skeletal muscle SR Ca pump. The membrane fraction F1 was enriched in the plasma membrane (PM) marker 5'-nucleotidase but poor in the inner mitochondrial marker cytochrome c oxidase and the putative endoplasmic reticulum (ER) marker rotenone insensitive NADH:cytochrome c reductase. however fraction F3 was poor in 5'-nucleotidase but enriched in the other two markers. Electron microscopy revealed that F1 contained mainly smooth surface vesicles but F3 contained smooth surface vesicles, some ribosomes, broken mitochondria and a large amount of a ground substance. We conclude that the rabbit stomach smooth muscle contains two Ca pumps - one in PM and another in ER. The ER Ca pump from stomach resembles more the SR Ca pump of the cardiac than the skeletal muscle.

Ca-pumps play an important role in the regulation of cytosolic Ca concentration which is a major determinant in the contractility of smooth muscle [1]. From experiments on skinned smooth muscle cells it is clear that there are two Ca pumps in smooth muscle - one in plasma membrane (PM) and another in endoplasmic reticulum (ER). Using isolated membrane fractions it has been shown that the phosphoenzyme intermediate of the Ca pump from pig stomach smooth muscle PM has a subunit molecular weight of 130 kDa, and that this pump resembles the Ca pump in pig erythrocytes in several properties including reaction to antibodies [7-9,11,12]. The 130 kDa phosphoenzyme intermediate of the PM Ca pump, however, decomposes very rapidly. $LaCl_3$ inhibits the PM Ca^{2+}-pump by preventing the decomposition of the phosphoenzyme intermediate. This has allowed detection of very low concentrations of the

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Ca^{2+}-pump in PM of erythrocytes and pig stomach and coronary artery (7, 9, 12). The oxalate-stimulated Ca pump has been shown to correlate with phosphoenzyme formation of a subunit molecular weight of 100 kDa [10]. The subunit molecular weight of the phosphoenzyme subunits of Ca pump in the cardiac and skeletal muscle sarcoplasmic reticulum (SR) is also 100 kDa [13,14]. However it has been reported that the antibodies against the skeletal muscle SR Ca pump do not react with the smooth muscle ER Ca pump [15]. Here we have used monoclonal antibodies against Ca pumps from cardiac and skeletal muscle SR and shown that the smooth muscle ER Ca pump resembles the cardiac muscle SR Ca pump in this respect.

In the previous studies the oxalate-stimulated Ca uptake was inactivated during the subcellular membrane fractionation process [2-6]. In this study we report a subcellular fractionation procedure in which quantitative recoveries of the oxalate-stimulated and -independent modes of Ca uptake were obtained. Thus this quantitative recovery of the oxalate-stimulated Ca uptake which is attributed to the smooth muscle ER and the homology of this ER pump with the cardiac but not skeletal muscle SR pump are the two new observations in this communication.

EXPERIMENTAL PROCEDURES

Subcellular Fractionation: Stomachs were removed from euthenized New Zealand rabbits weighing 1 to 2 kg and immediately placed in ice-cold homogenization buffer containing 250 mM sucrose, 20 mM morpholinopropane sulfonate-NaOH pH 7.0, 1 mM phenylmethylsulfonyl fluoride and 2 mM dithiothreitol. From these stomachs mucosa, submucosa and serosa were removed as described previously [16] and the remaining tissue was gently blotted and frozen. The tissue was thawed minced with scissors, passed through 1 mm and then 0.3 mm sieves, homogenized in 10 volumes of the homogenization buffer once with a loose teflon-glass motor driven homogenizer, and then two times for 5 sec using a Polytron PT20 The homogenate was centrifuged at 1,000 g for 10 min. The supernatant from this step was passed through 4 layers of cheese cloth and designated as postnuclear supernatant (PNS). PNS was centrifuged at 10,000 g for 10 min separating a mitochondnal pellet (MIT) from a supernatant which was treated with 0.7 M KCl for 15 mm and then centrifuged at 140,000 g for 2 h. The pellet from this high speed centrifugation was designated as microsomes (MIC) and the supernatant as the soluble (SOL) fraction. MIC was suspended in a solution containing 50 % sucrose (w/v), 0.7 M KCl, 1 mM phenylmethylsulfonyl fluoride and 2 mM dithiothreitol and 4 ml of the suspension was layered at the bottom of a gradient tube. On top were layered 2, 3, 2 and 2 ml each of 43, 33, 28 and 15 % sucrose (w/v) respectively and the tubes were centrifuged in a swinging bucket rotor overnight at 100,000 g. From the gradients, the fractions were collected as follows: F1 contained 15 % sucrose and the 15-28 % interphase, F2 contained 28-33 % interphase, F3 contained 33-43 % interphase and F4 contained the remainder.

Microsomes were also prepared as described above using fresh cardiac ventricular muscle from rabbit and dog and skeletal muscle from hind leg of rabbit. Rabbit erythrocyte ghosts were prepared according to Niggli et. al. [21] .

Enzyme Assays: The assays for 5'-nucleotidase. cytochrome c oxidase, NADPH:cytochrome c reductase, and rotenone-insensitive NADH:cytochrome c reductase were carried out as described in the literature [17,18].

Ca uptake: Ca uptake was carried out at 37°C as described previously [16,19] in a reaction mixture containing 250 mM sucrose, 50 mM imidazole-HCl pH 7.1 (pH at

37°C was 6.8), 5 mM $MgCl_2$, 5 mM Na-ATP pH 7.1, 5 mM Na azide, and 100 uM $CaCl_2$ containing trace amounts of radioactive Ca. In some samples potassium oxalate (5 mH) or phosphate (25 mM, pH 7.1) was also included. Routinely the Ca uptake was carried out for 20 nin and then terminated by filtration. The following definitions of different modes of Ca uptake were used. Azide insensitive Ca uptake was the uptake in presence of 5 mM Ma azide and the azide sensitive uptake was the uptake in absence of azide minus the value in its presence. Passive Ca binding was the uptake in the absence of any ATP. The control ATP-dependent Ca uptake was that obtained in the presence of ATP minus uptake in its absence when oxalate and phosphate were not added- Oxalate-stimulated uptake was the uptake in presence of oxalate and ATP minus the uptake in the absence of oxalate but presence of ATP. The phosphate-stimulated uptake was defined similarly to the oxalate-stimulated uptake.

Electron Microscopy: Thin sections of membrane pellets were processed as. described previously [17]. To view the membranes by negative staining 5 ul of membrane suspension was placed on a pariodian filmed grid, blotted with a filter paper and then placed on a drop of aqueous saturated uranyl acetate for 2 min. The stained preparation was again blotted, air dried and examined in a Philip 301 electron microscope.

Polyacrylamide slab gel electrophoresis: Polyacrylamide slab gel electrophoresis was carried out routinely by the method of Laemmli [20] in media containing sodium dodecyi sulfate (SDS) using 10 % acrylamide. Proteins were detected either by coomassie blue or silver staining [24].

Immunoblotting and antibody reactivity: SDS-polyacrylamide slab gels containing 10 % acrylamide were run as described above and electroblotted according to Towbin et. al. [23]. The blots were quenched with Blotto (5 % non fat dry milk powder in phosphate buffer saline containing 50 mM Na-phosphate pH 7.4 and 0.9 % NaCl) and then treated with the primary antibodies diluted 250- to 1000-fold in Blotto. The blots were then washed extensively and treated with the horseradish peroxidase conjugated antimouse IgG diluted 500-fold. Again the blots were washed extensively in the phosphate buffer saline and then stained with o-dianisidine. The preparation and characteristics of the antirabbit skeletal muscle SR monoclonal antibodies A20, A25 and A52, and the antidog cardiac SR antibody IID8 have been described elsewhere [25,26].

Phosphoenzyme detection: The phosphoenzyme intermediate was formed and analyzed according to Wuytack et. al. [9] in media containing 30 mM imidazole-HCl pH 6.8, 100 mM KCl, 5 uM Na_2ATP and trace amounts of gamma-^{32}-ATP (3000 Ci/mmol). The media also contained one of the following: (a) 5 mM EGTA, (b) 50 uM $CaCl_2$ and (c) 50 uM $CaCl_2$ plus 50 uM lanthanum chloride. The phosphoenzyme formation was carried out at 0°C for 20 sec and the reaction terminated by adding ice cold solution containing 10 % trichloroacefcic acid, 1 mM ATP and 50 mM phosphoric acid. After removing the unbound label by repeated washes in this quenching solution, the samples were suspended in a solution containing 3 % SDS, 0.1 % methyl green, 10 % sucrose, 10 mM morpholinopropane sulfonate -NaOH pH 5.5 and 20 mM dithiothreitol. These samples were electrophoresed using 7.5 % SDS polyacrylamide slab gels at 12°C prepared according to Lichtner and Wolf [22] except that the gel pH was 4.0. The dye front moved to the end of the gels in about 2 h but the run was continued for another 2 h. The gels were dried and ^{32}P-bands detected by autoradiography.

Results

Distribution of Enzyme markers in Subcellular Fractions: In initial preliminary experiments, the effects of freezing and thawing the tissues,

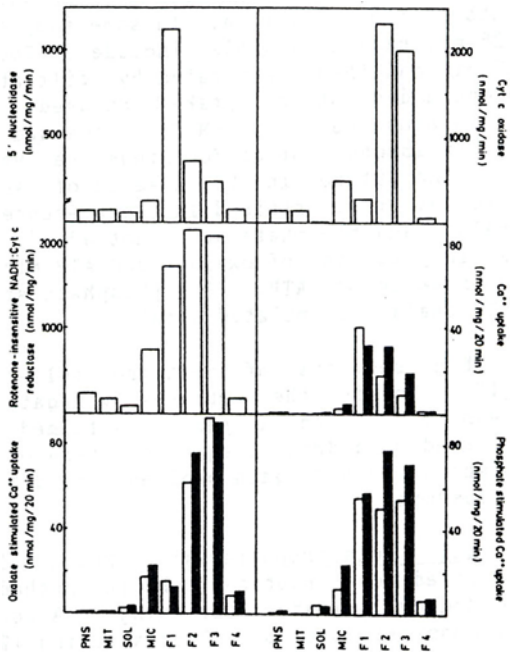

Figure 1. Subcellular fractionation of dog stomach smooth muscle: The tissue was fractionated as described in the Experimental Methods to obtain the differential centrifugation fractions PNS, MIT, SOL and MIC, and the gradient fractions F1 to F4 as designated in the Experimental Methods. The specific activities of three membrane enzymes and Ca uptake in absence (solid bars) and presence (hollow bars) of azide are shown. The distribution of the ATP-dependent Ca uptake without added oxalate or phosphate (Ca^{++} uptake) and the oxalate-stimulated and the phosphate-stimulated $Ca+^{+}$ uptake are presented.

Table 1. Recoveries of biochemical activities during subcellular fractionation

Fraction	Protein	5'-nucleotidase	Cyt. c oxidase	R.I. NADH Cyt. c Reductase	ATP-dependent azide insensitive Ca uptake Stimulation None	oxalate	Phosphate
	Recovery	% PNS					
MIT	31.4	20.4	35.0	18.9	22.6	8.7	8.3
SOL	51.0	29.3	3.6	8.1	34.5	27.5	39.7
MIC	4.7	25.9	42.0	37.1	37.2	60.8	59.4
	*87.1	75.6	80.6	64.1	94.3	97.0	107.4
	Recovery	% MIC					
F1	5.2	15.4	1.1	4.1	59.2	2.3	9.4
F2	9.6	13.5	14.2	12.9	21.3	12.2	12.6
F3	41.5	32.3	68.7	46.3	48.1	63.0	46.7
F4	18.4	13.6	5.6	11.3	5.6	21.0	18.4
	**74.7	74.8	89.6	74.6	135.4	98.5	87.1

Note: The designations for the fractions are as in Fig. 1. These values are from one membrane preparation although similar recoveries were obtained routinely. The protein yield in PNS in 9 such preparations was 25.9+-3.8 mg/g tissue.

* Total of % recoveries in MIT, SOL and MIC

** Total of % recoveries in Fl, F2, F3 and F4.

different modes of homogenization, inclusion and exclusion of KCl and/or $MgCl_2$ in the buffers on the subcellular fractionation were examined, and continuous sucrose density gradient centrifugation performed. Thus after attempting a variety of procedures described previously [7,16,27], the one described in the Experimental Methods was arrived at and was used in all the subsequent experiments. The distribution of subcellular markers and the Ca uptake in a typical preparation are shown in Fig. 1. The specific activity of the PM marker 5'-nucleotidase was highest in the fraction F1, and this represented a 17.4 -fold increase over PMS. It was expected that the specific activity of the inner mitochondrial marker cytochrome c oxidase would be highest in the MIT fraction. Instead it was highest in the fraction F2. Rotenone insensitive NADH:cytochrome c reductase has been used as an ER marker [7,28]. Fig. 1 shows that the fractions F2 and F3 were enriched in this marker. The specific activity of NADPH:cytochrome c oxidase, another putative ER marker [18], was also distributed similarly (data not shown). Table 1 shows that the recoveries of protein, 5'-nucleotidase, cytochrome c oxidase and the rotenone insensitive NADH:cytochrome c reductase ranged from 65 to 87 % in the differential centrifugation steps and from 75 to 90 % in the sucrose density gradient centrifugation.

Distribution of Ca uptake in Subcellular Fractions: A time course for the azide insensitive Ca uptake by F1 and F3 fractions is compared in Fig. 2. The passive Ca binding and the ATP-dependent Ca uptake without oxalate or phosphate occurred mainly in the first 5 min. However, the oxalate-stimulated or the phosphate-stimulated Ca uptake increased linearly with time for up to 20 min. For distribution studies, the Ca uptake in 20 min was studied. This uptake then represents an initial velocity for the oxalate- or the phosphate-stimulated Ca uptake but a pseudosteady state value in the absence of any added Ca-precipitating agents. The distribution of the Ca uptake in the absence (solid bar) and presence (hollow bar) of 5 mM Na azide which is known to inhibit the mitochondrial Ca uptake is presented. Thus the difference between the hollow and solid bars in Fig. 1 is the azide sensitive or the mitochondrial Ca uptake. In the absence of added Ca precipitating agents the azide sensitive Ca uptake was maximal in the fraction F2. This was also the case when the oxalate- or the phosphate-stimulated Ca uptake was considered. This distribution of the mitochondrial Ca uptake is thus consistent with the cytochrome c oxidase distribution.

The distribution of the azide insensitive Ca uptake is also shown in Fig. 1. In the absence of any added Ca precipitating agents, this activity was maximal in F1. The phosphate stimulated Ca uptake showed somewhat similar enrichment in fractions F1, F2 and F3 but the oxalate-enrichment was very high in F3 and poor in F1. These data are qualitatively but not quantitatively consistent with the hypothesis that the azide insensitive oxalate-stimulated Ca uptake is due to ER and the phosphate-stimulated uptake in the PM or ER. The recoveries of the azide insensitive Ca uptake in the subcellular fractions are shown in Table 1. The recoveries of the three modes of Ca uptake ranged from 94 to 107 % in the differential centrifugation steps, and 87 to 135 % in the gradient centrifugation step.

Electron Microscopy of Subcellular Fractions: Fig. 3 shows electron micrographs of thin sections of the F1 and F3 fractions. The fraction F1 contained mainly smooth surface vesicles but F3 shows smooth surface vesicles, a large amount of ground substance perhaps actomyosin, darkly staining small particles which may be ribosomes and some mitochondria. The diameters of the vesicles in these thin sections for F1 (0.15 ± 0.02 nm, mean ± SEM) and for F3 (0.19 ± 0.03 nm) did not differ significantly. The vesicle size was also examined by negative staining and the conclusion was similar.

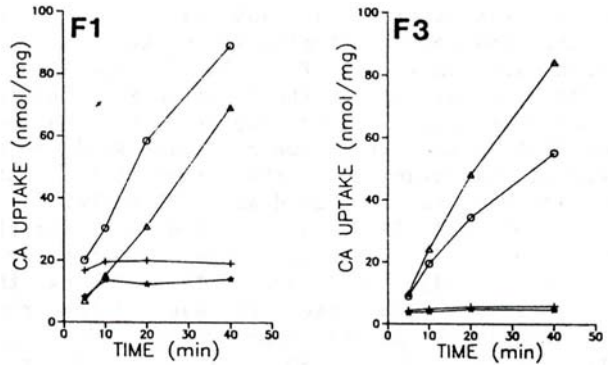

Figure 2. Time course of azide insensitive Ca uptake by F1 and F3 fractions: Ca uptake was described in the Experimental Methods but the reaction was stopped after the specified times. Passive Ca binding (*), ATP-dependent uptake without oxalate or phosphate (+), oxalate-stimulated uptake (△) and the phosphate-stimulated uptake (○) are shown.

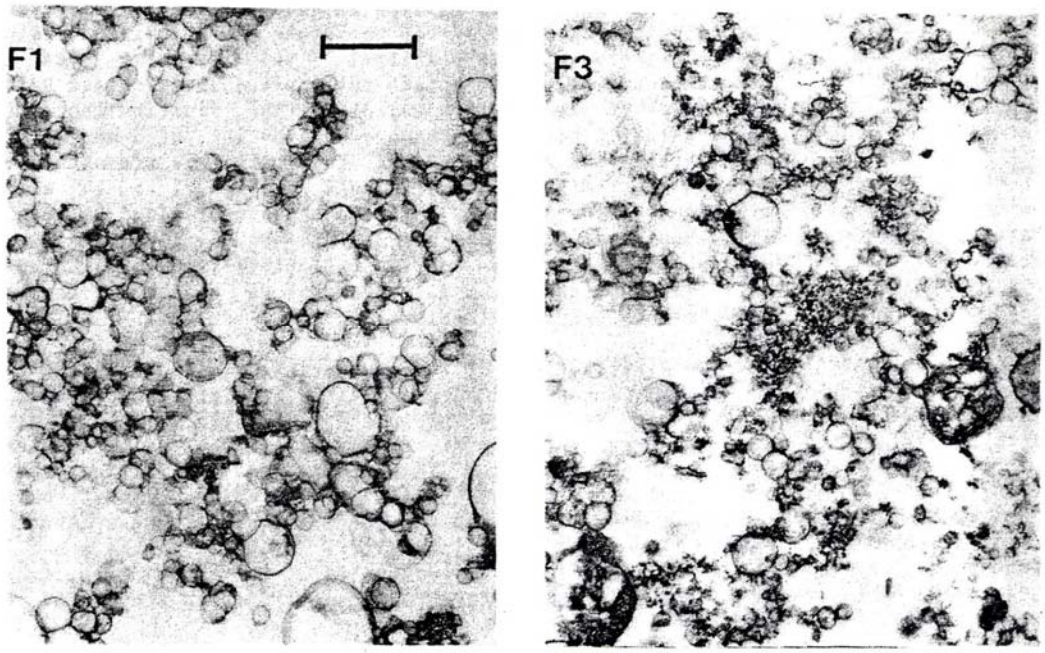

Figure 3. Electron micrograph of thin sections of F1 and F3. The bar =0.5 um.

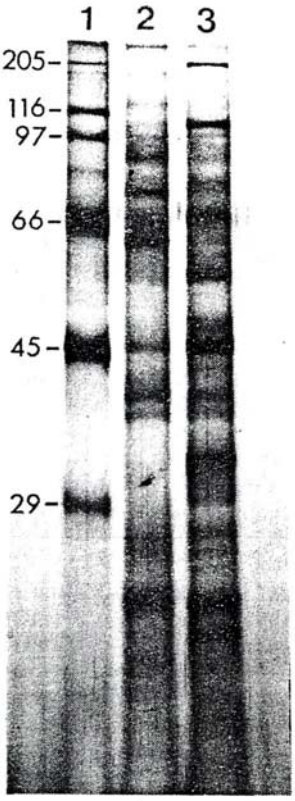

Fig. 4

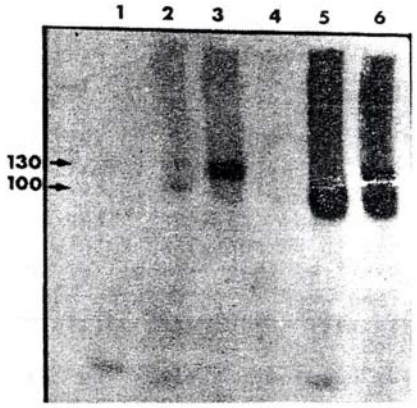

Fig. 5

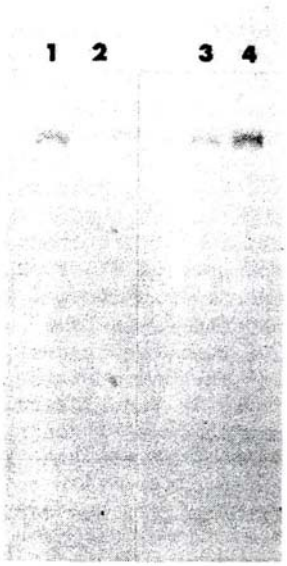

Fig. 6

Figure 4. Silver stained SDS-polyacrylamide gels: on lane 1 was applied 1 ug of a mixture of the following molecular weight standards - myosin (205 kDa), B-galactosidase (116 kDa), phosphorylase b (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa) and carbonic anhydrase (29 kDa), on lane 2 - 1 ug of Fl and on lane 3 - 1 ug F3.

Figure 5. Autoradiogram of phosphoenzyme SDS-slab gel electrophoresis: 22.2 ug of Fl was electrophoresed in lanes 1 to 3 and 21.6 ug of F3 in lanes 4 to 6. Phosphoenzyme formation was carried out in 5 mM EGTA for lanes 1 and 4, in 50 uM $CaCl_2$ for lanes 2 and 5 and in 50 uM $CaCl_2$ plus 50 uM $LaCl_3$ for lanes 3 and 6. The arrows indicate the position of phosphoenzyme intermediates of rabbit erythrocyte ghosts (130 kDa) and skeletal muscle SR (100 kDa) which were used as markers.

Figure 6. Western blot using the anticardiac SR monoclonal antibody IID8: 100 ug of F3 was electrophoresed in lane 1, 100 ug of Fl in lane 2, 5 ug of rabbit cardiac microsomes in lane 3 and 5 ug of dog cardiac microsomes in lane 4.

SDS-Polyacrylamide Gel Electrophoresis: The fractions F1 and F3 were examined by SDS-polyacrylamide gel electrophoresis for protein bands present, Ca-dependent phosphoenzyme intermediate formation and reactivity to monoclonal antibodies against cardiac SR Ca-pumps. The protein band pattern obtained on silver staining shown in Fig. 4 indicates that (a) there were no strong protein bands at 130 or 100 kDa and thus the Ca-pumps in these membranes were only relatively low in abundance, (b) bands corresponding to 93, 77, 63, 41 and 36 kDa were stronger in F1, and (c) those corresponding to 205, 109, 68, 56, 31 and 20 kDa were stronger in F3.

Fig. 5 shows that (a) in the absence of Ca neither F1 nor F3 showed any significant acid stable phosphoenzyme formation at 100 or 130 kDa, (b) in the absence of lanthanum the Ca-dependent phosphoenzyne formation of 130 kDa band occurred in F1 at extremely low intensity which could be observed only on extremely prolonged exposures of the autoradiograms (not shown) but in F3 the phosphoenzyme band for 100 kDa was dominant; (c) lanthanum inhibited the 100 kDa band phosphoenzyme formation but stabilized another band at 130 kDa, and (d) the ratio of the intensities of 130 to 100 kDa bands was high in F1 and low in F3. These data are thus consistent with the hypothesis that F1 which was enriched in PM contained mainly the Ca-pump similar in subunit molecular weight (130 kDa) to that in the erythrocyte PM and F3 which was enriched in ER also contained another pump which had the same subunit molecular weight (100 kDa) as the skeletal and cardiac muscle SR.

The homology between the Ca pumps in the fractions F1 and F3 and those in the skeletal and the cardiac muscle SR was explored further using monoclonal antibodies. Three monoclonal antibodies (A20, A25, A52) produced against the rabbit skeletal muscle SR showed extremely weak to no reaction against up to 100 ug of F1 or F3 on Western blots although in the same experiments 0.1 to 0.5 ug of the rabbit skeletal auscle microsomes gave very intense reaction at 100 kDa (data not shown). However, IID8, a monoclonal antibody produced against dog cardiac muscle SR did react against thestomach membranes as shown in Fig. 6. Examin ation of Fig. 6 shows that: (a) this monoclonal antibody reacted more strongly against 5 ug of dog cardiac muscle microsomes than against 5 ug of rabbit cardiac muscle microsomes, (b) 100 ug of F3 gave a reaction comparable to 5 ug of crude microsomes from rabbit cardiac muscle, (c) 100 ug of F1 gave a very faint reaction compared to F3, and (d) the antibody is specific for the 100 kDa band in that it did not cross react with any other protein in the blots including the 130 kDa pump. These data are thus consistent with the hypothesis that the F3 fraction which was enriched in oxalate-stimulated Ca uptake contained a Ca pump which resembled more closely the Ca pump in the SR of the cardiac muscle than that in the skeletal muscle.

DISCUSSION

The Results show that by the methods used in this communication it is possible to fractionate cell organelles without appreciable loss of oxalate stimulated Ca uptake, and that there are two Ca-pumps in these fractions. One pump is not stimulated by oxalate, forms an acid stable phosphoenzyme intermediate of subunit molecular weight of 130 kDa and is localized in the PM. The other pump is stimulated by oxalate, shows a phosphoenzyme subunit of 100 kDa which also cross reacts more strongly with a monoclonal antibody against cardiac than with monoclonals against skeletal muscle SR and may be localized in the ER. The Discussion will focus on the expected and observed correlations between activities in the subcellular fractions, the possible pitfalls in these observations, and on the possible relationships between the Ca pumps present in

various organelles of different tissue types.

The data presented here are qualitatively but not quantitatively consistent with the hypothesis that there are two Ca pumps - one in PM and another in ER. It has previously been shown in pig stomach and in pig coronary artery that there is a strong correlation between the MADPH: or rotenone insensitive NADH: cytochrome c reductases and the oxalate - stimulated but not the oxalate- independent azide sensitive ATP-dependent Ca uptake (7,10,12,19,28). In the present study, this correlation was observed in general But it could have been weakened somewhat by the presence of mitochondrial membranes in the F2 and F3 fractions since NADH:cytochrome c reductase has also been reported to be located in the outer mitochondrial membranes (18). There was also a good correlation between the oxalate -stimulated Ca uptake and the level of 100 kDa phosphoenzyme formed and its reactivity with the monoclonal antibody against dog cardiac muscle SR, and the oxalate- independent Ca uptake and the level of the 130 kDa phosphoenzyme band. However, it must be pointed out that there are possible pitfalls in such a study. Firstly, it is known that the stomach also contains nerves, blood vessels and connective tissue in addition to the smooth muscle which is the major tissue left after the dissection. It can be argued that some of these minor components may be very rich in the pump studied here. Immunohistochemical studies using strongly and selectively binding antibodies are needed to answer this question. The reliability of the markers, especially for ER is also questionable even though in recent studies these two markers have correlated with the 100 kDa Ca pump (7,28). Another complication is that Ca-uptake is a composite manifestation of punp density, vesicle size, membrane sidedness, and membrane leakiness and Ca-Mg-ATPase measurement is marred by the presence of a very active Mg-ATPase. The vesicle size in Fl and F3 was similar. Furthermore, the phosphoenzyme intermediate formation coupled to electrophoresis and reactivity to an antibody on western blots have also been shown here to correlate suitably, thus making some of the above objections less justified.

Whether the PM and the ER Ca pumps are structurally related to each other and to those in the PM and the SR of other tissues has not been fully investigated. The Ca pump present in the PM of erythrocytes, and smooth muscle have a phosphorylating subunit of molecular weight 130 kDa and the Ca pump present in the ER of smooth muscle as well as in the SR of cardiac and skeletal muscle SR has a phosphorylating subunit of 100 kDa. Using antibodies, Wuytack et. al. have shown that in pig there is a close homology between the erythrocyte and the smooth muscle PM Ca pumps (7,8,10.11). Chiesi et. al. (15) reported the lack of reactivity between the bovine vascular smooth muscle membranes and the polyclonal antibodies against skeletal muscle SR. Zubrzycka-Gaarn et. al. (25) reported that ER prepared by oxalate-loading from rabbit stomach smooth muscle reacted very weakly with the monoclonal antibodies against the skeletal muscle SR. In this communication, we confirm these findings and also report a stronger reaction between the smooth muscle ER Ca pump and a monoclonal antibody against cardiac muscle SR. The strength of the antibody reaction may reflect concentration as well as nature of the Ca pump present. In this study oxalate-stimulated Ca uptake values up to 100 nmol/20 min/mg were observed whereas in the cardiac SR rates as high as 2800 nmol/min/mg have been reported (31). This antibody reacts with the Ca pumps from the slow skeletal and the cardiac muscles (26) which may also be similar according to the cloning studies of MacLennan et. al (29). Thus it is clear that there are different degrees of homology between the 100 kDa Ca pumps in different tissues. There may also be species differences. The monoclonal against dog cardiac SR reacted more strongly against the cardiac muscle of dog from that of rabbit. Verma et. al. (30) reported differences in reactivity of

the erythrocyte Ca-Mg-ATPases of various species to the polyclonal antibodies against human erythrocyte Ca-Mg-ATPase. Levistsky et. al. (32) have also reported differences in the reactivity of dog, human, rat, guinea pig and rabbit hearts with monoclonal antibodies prepared against dog heart SR Ca pump.

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