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Characteristics of the Ca^{2+} pump and Ca^{2+}-ATPase in the plasma membrane of rat myometrium

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A plasma membrane-enriched fraction from rat myometrium shows ATP-Mg^{2+}-dependent active calcium uptake which is independent of the presence of oxalate and is abolished by the Ca^{2+} ionophore A23187. Ca^{2+} loaded into vesicles via the ATP-dependent Ca^{2+} uptake was released by extravesicular Na^+. This showed that the Na^+/Ca^{2+} exchange and the Ca^{2+} uptake were both occurring in plasma membrane vesicles. In a medium containing KCl, vanadate readily inhibited the Ca^{2+} uptake ($K_{1/2}$ 5 μM); when sucrose replaced KCl, 400 μM-vanadate was required for half inhibition. Only a slight stimulation of the calcium pump by calmodulin was observed in untreated membrane vesicles. Extraction of endogenous calmodulin from the membranes by EGTA decreased the activity and Ca^{2+} affinity of the calcium pump; both activity and affinity were fully restored by adding back calmodulin or by limited proteolysis. A monoclonal antibody (JA3) directed against the human erythrocyte Ca^{2+} pump reacted with the 140 kDa Ca^{2+}-pump protein of the myometrial plasma membrane. The Ca^{2+}-ATPase activity of these membranes is not specific for ATP, and is not inhibited by mercurial agents, whereas Ca^{2+} uptake has the opposite properties. Ca^{2+}-ATPase activity is also over 100 times that of calcium transport; it appears that the ATPase responsible for transport is largely masked by the presence of another Ca^{2+}-ATPase of unknown function. Measurements of total Ca^{2+}-ATPase activity are, therefore, probably not directly relevant to the question of intracellular Ca^{2+} control.

INTRODUCTION

To understand myometrial function, it is important to understand the cellular mechanisms controlling cytoplasmic free Ca^{2+}. The plasma membrane of excitable cells contains two independent mechanisms for removing Ca^{2+} from the cytosol: the plasma membrane Ca^{2+} pump and the Na^+/Ca^{2+} exchanger. The presence of the Na^+/Ca^{2+} exchanger in myometrial membrane preparations (Grover *et al.*, 1981; Grover *et al.*, 1983) has been demonstrated. ATP-Mg^{2+}-dependent Ca^{2+} uptake by a plasma membrane-enriched preparation has also been shown (Grover *et al.*, 1982), but several attempts to characterize the myometrial plasma membrane Ca^{2+} pump have been inconclusive. Reported properties of Ca^{2+}-ATPase activity of myometrial plasma-membrane preparations vary strikingly among the reports, with the half-maximal Ca^{2+} concn. varying from < 16 nM to 10 μM (Akerman & Wikstrom, 1979; Soloff & Sweet, 1982; Popescu *et al.*, 1985). The maximal activity also varied from 16 to 630 nmol/min·mg. One study (Popescu *et al.*, 1985) showed a half-maximal Ca^{2+} stimulation of 0.2 μM, calmodulin responsiveness and an activity of 16 nmol/min·mg. These properties are close to those expected of Ca^{2+}-transport ATPase, but they were measured on membranes solubilized with SDS. In another study (Soloff & Sweet, 1982), the half-maximal Ca^{2+} concentration was less than 16 nM and the maximum activity was 630 nmol/min·mg. These properties are typical of those seen for non-transport Ca^{2+}-ATPases (Lin, 1985*a*).

Another important feature of these studies is to distinguish between plasma-membrane and sarcoplasmic reticulum calcium pumps because obtaining pure plasma-membrane preparations from cells containing subcellular organelles is a difficult task. Even when well-characterized plasma membrane and sarcoplasmic reticulum fractions are prepared, a careful investigation of the plasma-membrane fraction frequently demonstrates the presence of some sarcoplasmic reticulum contamination (Raeymaekers *et al.*, 1985).

Some of the properties which distinguish plasma-membrane Ca^{2+} pumps from those of the sarcoplasmic reticulum have been studied in other types of smooth muscle (Raeymaekers *et al.*, 1983; Wuytack *et al.*, 1984; Raeymaekers *et al.*, 1985). It has been shown that the plasma-membrane Ca^{2+} pump of pig stomach smooth muscle is directly regulated by calmodulin (Wuytack *et al.*, 1980) and the enzyme was purified by calmodulin affinity chromatography (Wuytack *et al.*, 1981; Wuytack *et al.*, 1982). This pump has an M_r of 140000 (Wuytack *et al.*, 1982) and antibodies raised against it react with the Ca^{2+} pump in pig and human erythrocytes (Wuytack *et al.*, 1983).

In the present report, using the plasma membrane-enriched preparation described by Soloff & Sweet (1982), we present evidence showing the presence of an erythrocyte-type Ca^{2+} pump in the plasma membrane of rat myometrium. We used several criteria to demonstrate that the active Ca^{2+} transport of this membrane shares the properties known for the erythrocyte Ca^{2+} pump and differs from those of the sarcoplasmic reticulum. Our

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results also indicate that the Ca^{2+}-ATPase activity of this membrane is due to a different enzyme from that of the Ca^{2+} pump.

MATERIALS AND METHODS

Immature female Holtzmann rats, 22 days old, were obtained from the Holtzmann Company (Madison, WI, U.S.A.) γ-[^{32}P]ATP and $^{45}CaCl_2$ were obtained from New England Nuclear (Boston, MA, U.S.A.). Calmodulin, trypsin inhibitor and chemicals used in the enzymic assay were obtained from Sigma Chemical Company (St. Louis, MO, U.S.A.). *N*-Tosyl-L-phenylalanylchloromethane ketone-treated trypsin was purchased from Cooper Biomedical.

Preparation of plasma membrane

The rats described above were injected intraperitoneally with 20 μg of diethylstilboestrol in 0.5 ml of saline containing 1% ethanol for 6 days (20 μg of diethylstilboestrol/day/rat) and were killed on the seventh day. The uterine horns were excised and trimmed of connective tissue, fat, and endometrium. The myometrium was minced with scissors and gently homogenized in a Duall tissue grinder and then with a Potter–Elvehjem homogenizer in 100 mM-KCl/5 mM-$MgCl_2$/50 mM-Tris/HCl, pH 7.2, at 0 °C. Plasma membrane was prepared from the homogenate essentially as described by Soloff & Sweet (1982). The final pellet was gently but thoroughly resuspended in 0.25 M-sucrose in 10 mM-Tris/HCl buffer, pH 7.2, and was kept in 0.5 ml batches in liquid N_2. The membranes could be stored under these conditions without losing Ca-transport activity, whereas Ca^{2+}-ATPase activity was much more sensitive to storage. Therefore, in studies comparing ATPase with transport, fresh membrane preparations were used. Thirty rats were usually used, giving about 4 mg of membrane protein. All buffers used for the preparation of membranes contained 5 mM-benzamidine, 0.5 mM-phenylmethanesulphonyl fluoride and 50 μg of trypsin inhibitor/ml. Protein was measured by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

Ca^{2+} uptake assay

Membranes (15–20 μg) were suspended to a total vol. of 0.5 ml in the appropriate incubation medium. The reaction mixture was incubated at 37 °C for 5 min except where otherwise noted. Portions were taken and membrane vesicles were separated from incubation medium by rapid filtration through Millipore membrane filters (0.45 μm pore size, type HA). The filters were washed three times with 5 ml of 0.25 M-sucrose/20 mM-Tris/HCl, pH 7.2. Radioactivity remaining on the filters was counted in 10 ml of scintillation liquid (Safety Solv, Research Products International, IL, U.S.A.).

Ca^{2+}-ATPase assay

Ca^{2+}-ATPase activity was measured by monitoring the release of inorganic ^{32}P from γ-[^{32}P]ATP as previously described (Verma & Penniston, 1981). Calcium-stimulated activity was determined by subtracting the value in the absence of calcium from that in its presence.

Nucleotide specificity was assayed by utilizing 6 mM-ATP, -ITP, -CTP, -GTP or -UTP in the same medium as that used in Ca^{2+}-ATPase assay. After the released inorganic phosphate was extracted into the organic phase, non-radioactive inorganic phosphate was measured colorimetrically by the Martin & Doty method described by Lindberg & Ernster (1956).

Preparation of monoclonal antibody

The monoclonal antibody used was JA3, the preparation and properties of which were described by Borke *et al.* (1987).

Preparation of Western blot

SDS/polyacrylamide-gel electrophoresis was performed on 7.5% gels as described by Laemmli (1970) using Pyronin Y as a tracking dye. Proteins were transfered to nitrocellulose paper as described by Towbin *et al.* (1979). Binding of antibody to the nitrocellulose-immobilized proteins was done by the method of Burnette (1981). Binding was assessed by detection of a coloured product produced by peroxidase-conjugated goat anti-(mouse IgG) in the presence of 4-chloro-1-naphthol plus H_2O_2.

RESULTS

A plasma membrane-enriched fraction obtained by discontinuous sucrose density-gradient centrifugation was used for characterization of the myometrial plasma-membrane calcium pump. This preparation was characterized by Soloff & Sweet (1982). They showed that this material had a remarkable increase in plasma-membrane marker activities (such as 5′-nucleotidase and oxytocin binding) accompanied by a marked decrease in the sarcoplasmic reticulum marker enzyme, rotenone-insensitive NADPH–cytochrome *c* oxidoreductase. They also showed that a Ca^{2+}-ATPase, which they took to be related to Ca^{2+} transport, was highly enriched in the plasma-membrane fraction.

Fig. 1 shows that Ca^{2+} uptake, presumably by the inside-out component of these membranes, is active. This conclusion is supported by the observations that, in the absence of either ATP or Mg^{2+}, essentially no Ca^{2+} uptake occurred and the accumulated Ca^{2+} was rapidly and completely released by the divalent cation ionophore A23187. In the presence of a relatively high amount of ATP (6 mM) the uptake increased with time up to 15–20 min, whereas at lower ATP concentrations it stopped within 10 min (not shown), most probably because of the fast cleavage of ATP by the non-pump ATPase (see below). Active Ca^{2+} transport was not affected by 5 mM-oxalate or oligomycin in a concentration (20 μg/mg of protein) high enough to inhibit completely ATP-dependent calcium transport in mitochondria. EGTA caused release of Ca^{2+} at a moderate speed, persumably due to Ca^{2+} passing through channels native to these membranes.

Release by Na^+ of the Ca^{2+} accumulated by the pump is shown in Fig. 2. The vesicles were preloaded with 4 nmol of Ca/mg of protein in K^+ medium and then were diluted into a medium containing either 130 mM-NaCl or -KCl. Na^+ induced about a 50% net efflux of internal Ca^{2+}. Under the same conditions, K^+ was not effective, demonstrating that the Ca^{2+} releasing effect of Na^+ is specific. As the sarcoplasmic reticulum does not possess a Na^+/Ca^{2+} exchanger, the results show that the ATP-Mg^{2+}-dependent transport system pumps Ca^{2+} into plasma-membrane vesicles. It is worth mentioning that

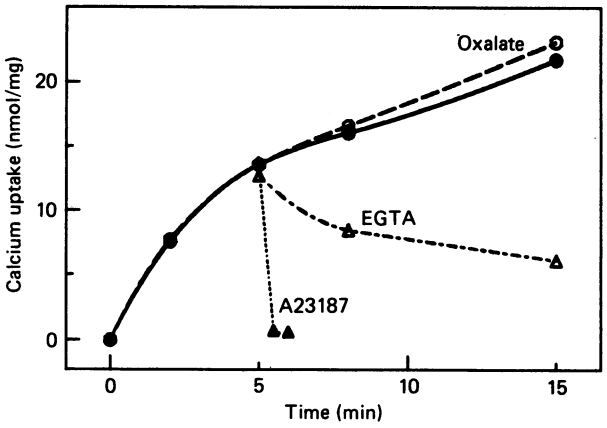

Fig. 1. Time course of ATP-dependent Ca^{2+} uptake by plasma-membrane vesicles

Ca^{2+} uptake was carried out at 37 °C, in 0.25 M-sucrose, 50 mM-Tris/HCl, pH 7.2, 0.1 mM-ouabain, 10 mM-$MgCl_2$, 6 mM-ATP, 100 μM-$^{45}CaCl_2$ and enough EGTA to produce a 1.2 μM final concn. of free Ca^{2+}. The solid line with filled circles shows Ca^{2+} uptake by the membrane vesicles in the absence, and the dashed line with open circles in the presence, of 5 mM-potassium oxalate. The dot–dash line with open triangles shows the effect of the addition (after 5 min) of EGTA (final concn. 2 mM) while the dotted line with filled triangles shows the effect of the addition of the divalent cation ionophore A23187 (final concn. 10 μg/ml). The A23187 was dissolved in dimethyl sulphoxide; this solvent alone had no effect on measured Ca^{2+} uptake.

the Na^+-induced Ca^{2+} release was not as rapid or complete as it is in brain or cardiac tissue (Caroni & Carafoli, 1980; Gill *et al.*, 1981), but the release of Ca^{2+} by Na^+ was comparable with that seen in plasma membranes from intestinal or renal epithelium (Gmaj

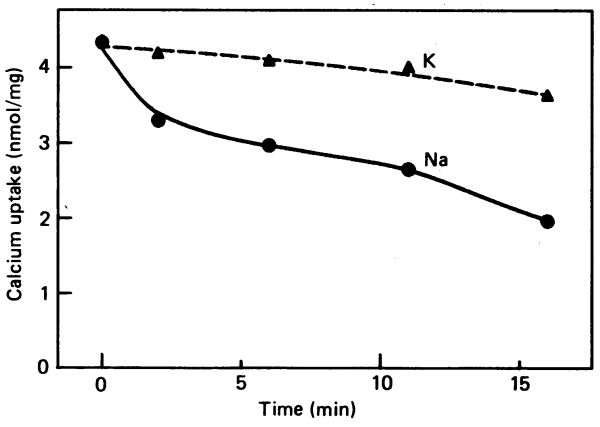

Fig. 2. Na^+-induced efflux of Ca^{2+} from plasma-membrane vesicles loaded via the ATP-dependent Ca^{2+} uptake

Membrane vesicles (50 μg) were loaded with ^{45}Ca for 10 min at room temperature in 0.5 ml of a medium containing 130 mM-KCl, 10 μM-$^{45}CaCl_2$, 2 mM-ATP and 5 mM-$MgCl_2$. Efflux was initiated by diluting 100 μl portions of the ^{45}Ca-loaded vesicles into 1 ml of either 130 mM-NaCl medium (solid line with filled circles) or 130 mM-KCl medium (dashed line with filled triangles). External media for efflux contained the same concentrations of $^{45}CaCl_2$ as during influx, but no ATP + Mg^{2+}. Efflux continued at room temperature for the times indicated.

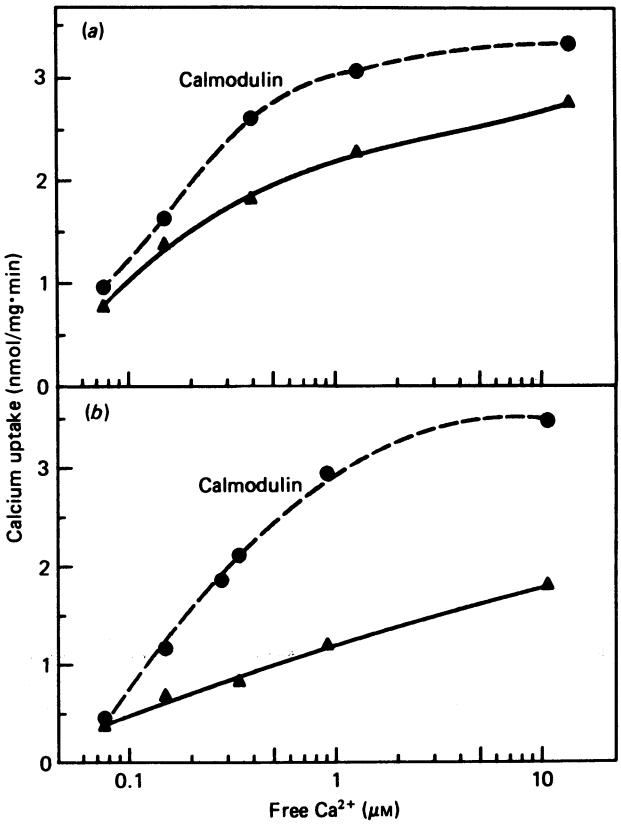

Fig. 3. Effect of calmodulin on Ca^{2+} uptake by plasma-membrane vesicles

Ca^{2+} concentration dependence of active Ca^{2+} uptake. Membrane vesicles were preincubated in the absence (*a*) or presence (*b*) of 0.2 mM-EGTA at 37 °C for 5 min in a concentration of 1 mg of membrane protein/ml. The Ca^{2+} uptake was initiated by suspending 15 μl of membrane vesicles in 0.5 ml of reaction mixture containing 0.25 M-sucrose, 10 mM-$MgCl_2$, 6 mM-ATP, 100 μM-$^{45}CaCl_2$ and variable concentrations of EGTA to attain the appropriate free Ca^{2+} concentrations (0–10 μM). The incubation was carried out at 37 °C, for 5 min. Solid line with filled triangles shows Ca^{2+} uptake in the absence, and dashed line with filled circles in the presence, of 50 μg of calmodulin/ml.

et al., 1979; Hildmann *et al.*, 1982). This relatively slow rate of efflux, with some Ca^{2+} remaining in the vesicles, may indicate a lower activity of the Na^+/Ca^{2+} exchanger combined with additional Ca^{2+} uptake due to residual ATP (theoretically 0.2 mM) in the efflux medium.

In the following experiments the characteristics of ATP-dependent Ca^{2+} uptake by the myometrial plasma-membrane vesicles are compared with those of Ca^{2+} uptake in the well-characterized erythrocyte plasma membrane. The calmodulin responsiveness of Ca^{2+} transport as a function of free Ca^{2+} concn. is shown in Fig. 3. In membranes that had not been extracted with EGTA there was a small calmodulin response in all the experiments (Fig. 3*a*). $V_{max.}$ was stimulated 20% by calmodulin while $K_{1/2}$ for Ca^{2+} did not change significantly. Maximal velocity of Ca^{2+} transport was about 3 nmol/min·mg and $K_{1/2}$ for Ca^{2+} activation was 0.25 μM. Washing the plasma membranes with 2 mM-EGTA at 0 °C did not have any drastic effect on

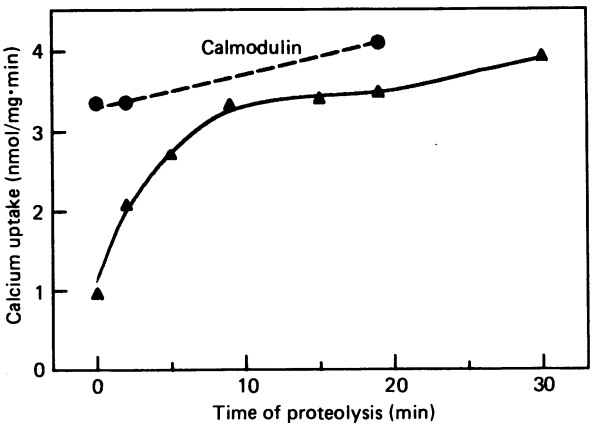

Fig. 4. Effect of limited proteolysis on Ca^{2+} uptake

Time course of tryptic digestion. Membrane vesicles were preincubated in the presence of 0.2 mM-EGTA, at 37 °C, for 5 min as described in the legend to Fig. 3. The membranes were cooled down to 0 °C and 200 μl of the membrane suspension was exposed to 10 μg of trypsin for the times indicated. The reaction was terminated by diluting 15 μg of membrane samples into the Ca^{2+} uptake media containing excess (50 μg/0.5 ml) trypsin inhibitor, 0.25 M-sucrose, 10 mM-$MgCl_2$, 6 mM-ATP, 100 μM-$^{45}CaCl_2$ and EGTA to produce 0.9 μM final concn. of free Ca^{2+}. Ca^{2+} uptake was carried out at 37 °C for 5 min and terminated as described in the Materials and methods section. The solid line with filled triangles shows Ca^{2+} uptake in the absence, and the dashed line with filled circles in the presence of 50 μg calmodulin/ml.

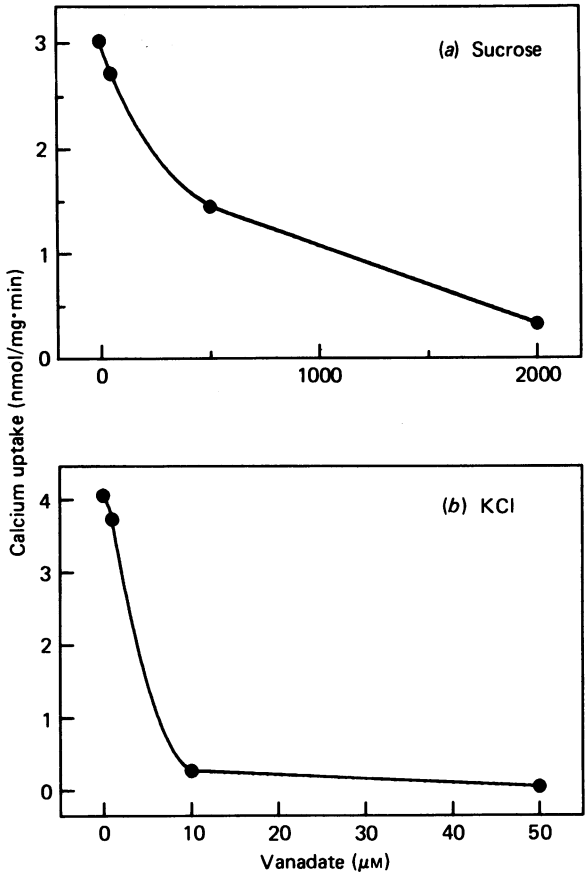

Fig. 5. Effect of vanadate on Ca^{2+} uptake

Ca^{2+} transport was measured in 0.5 ml of a medium containing either 0.25 M-sucrose (*a*) or 130 mM-KCl (*b*), 1 mM-ATP, 5 mM-$MgCl_2$, 10 μM-$^{45}CaCl_2$ and varying amounts of orthovanadate. The reaction was started by addition of 20 μg of membrane suspension and was terminated after incubation at 37 °C for 5 min.

calmodulin response; total activity of calcium uptake was reduced but stimulation by calmodulin was not affected. In order to remove tightly bound calmodulin from the membrane it was necessary to incubate the vesicles in the presence of 0.2 mM-EGTA at 37 °C for 5 min. After this treatment, both maximal velocity and Ca^{2+} affinity of the enzyme were decreased, and the high velocity and high affinity for Ca^{2+} were fully restored by addition of calmodulin (Fig. 3*b*). $V_{max.}$ was doubled by added calmodulin while $K_{1/2}$ for Ca^{2+} went from 1.5–2 μM without calmodulin to 0.25 μM with calmodulin. This behaviour is typical of a plasma-membrane Ca^{2+}-pumping ATPase.

The effect of tryptic digestion on the activity of Ca^{2+} uptake by EGTA pre-extracted membrane vesicles can be seen in Fig. 4. Trypsin treatment of the membrane proteins increased the pump activity 3-fold while the enzyme lost calmodulin sensitivity. At the end of about 10 min digestion the activity of the calcium pump was nearly equal to that obtained by maximum calmodulin stimulation. This result is consistent with that obtained for erythrocyte membrane Ca^{2+}-ATPase (Zurini *et al.*, 1984).

In contrast with sarcoplasmic reticulum Ca^{2+}-ATPase, plasma-membrane Ca^{2+} pumps are generally very sensitive to inhibition by less than 10 μM-vanadate (Niggli *et al.*, 1981). In KCl-containing medium, using 1 mM-ATP as substrate, the myometrial Ca^{2+} pump was fully inhibited by 10 μM-vanadate, with half-inhibition occurring at about 3–5 μM-vanadate (Fig. 5*b*). On the other hand, in a medium containing sucrose instead of KCl, about 400 μM-vanadate was required for half-inhibition (Fig. 5*a*). At higher ATP concentrations $K_{1/2}$ for vanadate was also shifted; in the presence of KCl and 6 mM-ATP $K_{1/2}$ was 30 μM (not shown). These results are in good agreement with observations on the erythrocyte calcium pump; the sensitivity of that enzyme to vanadate is similarly dependent on Mg^{2+}, K^+ and ATP concentrations in the incubation medium (Barrabin *et al.*, 1980; Schatzmann *et al.*, 1986).

Immunoblots of human erythrocyte ghost and myometrial plasma membrane proteins can be seen in Fig. 6. A monoclonal antibody (JA3), raised against purified erythrocyte Ca^{2+} pump, specifically cross-reacted with the 140 kDa Ca^{2+} pump in the erythrocytes and also with a protein of similar size in the myometrial plasma membrane. This result provides further evidence for the existence of an erythrocyte-type calcium pump in the myometrium.

In view of the question about the relationship between Ca^{2+}-ATPase and Ca^{2+} transport, experiments were done to compare these two activities. The effect of Mg^{2+} on Ca^{2+}-ATPase is shown in Table 1. Rather low concentrations of Mg^{2+} increased substantially the ATPase observed in the absence of Ca^{2+}, while having little effect on ATPase in the presence of Ca^{2+}. As a result, the increment in ATPase due to Ca^{2+} diminished four-fold

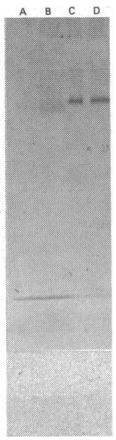

Fig. 6. Western blot of erythrocyte and myometrial plasma-membrane proteins treated with monoclonal antibody (JA3) raised against purified human erythrocyte Ca^{2+} pump

Lanes A and B, 50 and 100 μg of myometrial plasma-membrane proteins. Lanes C and D, 50 and 100 μg of protein of human erythrocyte ghost.

Table 1. Effect of Mg^{2+} on Ca^{2+}-ATPase

ATPase activity was measured at 37 °C for 2.5 min in 50 mM-Tes/triethanolamine, pH 7.0, 0.25 M-sucrose, 0.1 mM-ouabain, 5 mM-NaN_3, 6 mM-[γ-^{32}P]ATP (specific activity 0.2 Ci/mol), enough $CaCl_2$ to make 30 μM free Ca^{2+}, and Mg^{2+} as indicated. The blank contained the same ingredients without protein.

Free Mg (μM)	ATPase activity (nmol/min·mg)		
	No Ca	Plus Ca	ΔCa
0	−4	837	841
10	362	854	492
50	760	962	202

Table 2. Nucleoside triphosphate specificity of Ca^{2+} uptake

The data shown are ±S.D. of both Ca uptake and Ca^{2+}-ATPase activities assayed in triplicate. Calcium uptake was measured after 5 min of incubation at 37 °C in 50 mM-Tris/HCl, pH 7.2, 0.25 M-sucrose, 0.1 mM-ouabain, antimycin A (1 μg/mg of membrane), 10 mM-$MgCl_2$, 6 mM-nucleoside triphosphate, and 10 μM-$^{45}CaCl_2$ (free Ca is 6.6 μM). For nucleoside triphosphatase (XTPase) activity, the conditions were the same except that $MgCl_2$ was 0.8 mM (11 μM free Mg^{2+}), total $CaCl_2$ was 55 μM (2.2 μM free Ca^{2+}) and 0.1 μM-A23187 was present.

Triphosphate	Ca^{2+} uptake (nmol/min·mg)	XTPase (nmol/min·mg)
ATP	2.52±0.077	410±40
GTP	0.015±0.035	310±100
UTP	−0.066±0.031	320±50
ITP	−0.026±0.03	410±110
CTP	−0.064±0.025	300±50

Table 3. Sensitivity of Ca^{2+} uptake and Ca^{2+}-ATPase to mercurials

The data shown are means±S.D. of both Ca uptake and Ca-ATPase activity assayed in triplicate. *p*-Chloromercuribenzoic acid (PCMB) was dissolved in dimethyl sulphoxide. The amount of dimethyl sulphoxide added to the reaction mixture did not affect the enzyme activity. Calcium uptake was measured at 37 °C for 5 min in 50 mM-Tris/HCl, pH 7.2, 0.25 M-sucrose, 0.1 mM-ouabain, antimycin A (1 μg/mg of membrane), 10 mM-$MgCl_2$, 6 mM-ATP, and 10 μM-$^{45}CaCl_2$. Reaction mixtures containing these mercurials were preincubated at 37 °C for 5 min. For Ca^{2+}-ATPase, the reaction conditions were the same as those for Ca^{2+} uptake, except that total $MgCl_2$ was 0.3 mM (4 μM free Mg^{2+}), total $CaCl_2$ was 55 μM (2.0 μM free Ca^{2+}) and 0.1 μM-A23187 was present. PCMPS = *p*-chloromercuriphenylsulphonic acid.

Mercurial		Ca^{2+} uptake (nmol/min·mg)	ATPase activity (nmol/min·mg)
None		2.07±0.13	250±17
PCMB	10 μM	0.06±0.013	160±29
	100 μM	−0.012±0.001	170±8
PCMPS	10 μM	0.06±0.003	176±30
	100 μM	0.058±0.023	157±22
$HgCl_2$	10 μM	−0.062±0.007	149±11
	100 μM	−0.025±0.005	122±12

between 0 and 50 μM free magnesium. At higher free Mg^{2+} concentrations, Ca^{2+}-ATPase was essentially undetectable, so it was impossible to assay Ca^{2+}-ATPase under conditions which were optimal for Ca^{2+} transport. This apparent inhibition of Ca^{2+}-ATPase by Mg^{2+} is in striking contrast with the absolute Mg^{2+} requirement of Ca^{2+} transport.

Table 2 compares nucleoside triphosphate specificity of Ca^{2+} uptake with nucleoside triphosphatase. It is immediately apparent from this Table that only ATP supported any significant Ca^{2+} uptake, while all five nucleotides tested supported a comparable amount of Ca^{2+}-dependent nucleoside triphosphatase. This Table also shows that nucleoside triphosphatase activity was more than 100-fold greater than the maximal Ca^{2+} uptake observed.

Comparison of the sensitivity of Ca^{2+} uptake and Ca^{2+}-ATPase activity to Hg-containing reagents is shown in Table 3. Once again ATPase activity is more than 100 times the Ca^{2+} uptake, and only a portion of the ATPase activity is inhibited by any of the three mercurials, whereas Ca^{2+} uptake is completely inhibited even at the lowest concentrations of these agents. In addition, Ca^{2+}-ATPase activity of this membrane was easily inactivated during storage, whereas the Ca pump was not; the latter was stable for months when the membranes were kept under liquid N_2.

DISCUSSION

As was discussed in the Introduction, many of the data so far presented on myometrial plasma-membrane Ca^{2+} pumps have been difficult to interpret. Difficulties arose because of the assumption that Ca^{2+}-ATPase activity

was due to the pump and because no attempt was made to distinguish between the properties of the plasma-membrane and sarcoplasmic reticulum Ca^{2+} pumps. It is evident from this study that Ca^{2+}-ATPase present in the myometrial plasma membrane is largely due to enzymes not involved in Ca^{2+} transport. The Ca^{2+} transporter must also have Ca^{2+}-ATPase activity, but this is masked by the non-pump Ca^{2+}-ATPase with unknown function. This situation has been observed in liver (Lin, 1985*a*,*b*) and in corpus luteum (Minami & Penniston, 1987). Most pertinently to the present study, similar observations have also been made in stomach (Grover *et al.*, 1984) and in dog aorta (Kwan *et al.*, 1986).

On the other hand, ATP-powered Ca^{2+} transport studied here has all the properties that characterize a calcium transport system as a plasma-membrane Ca^{2+} pump. Its molecular mass (140 kDa), cross-reactivity with a monoclonal antibody raised against the human erythrocyte Ca^{2+} pump, sensitivity to vanadate inhibition and dispositions to calmodulin and tryptic digestion are all diagnostic of a plasma membrane pump, and would appear to differentiate it from the sarcoplasmic reticulum Ca^{2+}-pumping ATPase.

In summary, the results suggest that the myometrial plasma membrane possesses two distinct mechanisms for Ca^{2+} extrusion from the cytosol: an erythrocyte-type Ca^{2+} pump and the Na^+/Ca^{2+} exchange. As was reported by Grover and co-workers (1983), the Na^+/Ca^{2+} exchange seems to be much less efficient than it is in brain or cardiac tissue. These findings enhance the relative importance of the plasma-membrane Ca^{2+} pump in regulating intracellular Ca^{2+} in the myometrium.

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REFERENCES

Akerman, K. E. O. & Wikstrom, M. K. F. (1979) FEBS Lett. **97**, 283–285
Barrabin, H., Garrahan, P. J. & Rega, A. F. (1980) Biochim. Biophys. Acta **500**, 796–804
Borke, J. L., Minami, J., Verma, A., Penniston, J. T. & Kumar, R. (1987) J. Clin. Invest. **80**, 1225–1231
Burnette, W. N. (1981) Anal. Biochem. **112**, 195–203
Caroni, P. & Carafoli, E. (1980) Nature (London) **283**, 765–767
Gill, D. L., Grollman, E. F. & Kohn, L. D. (1981) J. Biol. Chem. **256**, 184–192
Gmaj, P., Murer, H. & Kinne, R. (1979) Biochem. J. **179**, 549–557
Grover, A. K., Kwan, C. Y. & Daniel, E. E. (1981) Am. J. Physiol. **240**, C175–C182
Grover, A. K., Kwan, C. Y. & Daniel, E. E. (1982) Am. J. Physiol. **242**, C278–C282
Grover, A. K., Kwan, C. Y., Rangachari, P. K. & Daniel, E. E. (1983) Am. J. Physiol. **244**, C158–C165
Grover, A. K., Kwan, C. Y. & Oakes, P. (1984) Fed. Proc. Fed. Am. Soc. Exp. Biol. **43**, 521
Hildmann, B., Schmidt, A. & Murer, H. (1982) J. Membr. Biol. **65**, 55–62
Kwan, C. Y., Kostka, P., Grover, A. K., Law, J. S. & Daniel, E. E. (1986) Blood Vessels **23**, 22–33
Laemmli, U. K. (1970) Nature (London) **227**, 680–685
Lin, S. H. (1985*a*) J. Biol. Chem. **260**, 10976–10980
Lin, S. H. (1985*b*) J. Biol. Chem. **260**, 7850–7856
Lindberg, O. & Ernster, L. (1956) in Methods of Biochemical Analysis (Glick, D., ed.), vol. 3, pp. 1–22, Interscience, New York
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275
Minami, J. & Penniston, J. T. (1987) Biochem. J. **242**, 889–894
Niggli, V., Adunyah, E. S., Penniston, J. T. & Carafoli, E. (1981) J. Biol. Chem. **256**, 395–401
Popescu, L. M., Nuto, O. & Panoiu, C. (1985) Biosci. Rep. **5**, 21–28
Raeymaekers, L., Wuytack, F., Eggermont, J. & deSchutter, G. (1983) Biochem. J. **210**, 315–322
Raeymaekers, L., Wuytack, F. & Casteels, R. (1985) Biochim. Biophys. Acta **815**, 441–454
Schatzmann, H. J., Luterbacher, S., Stieger, J. & Wuthrich, A. (1986) J. Cardiovasc. Pharmacol. Suppl. **8**, 833–837
Soloff, M. S. & Sweet, P. (1982) J. Biol. Chem. **257**, 10687–10693
Towbin, H., Stahelin, T. & Gordon, J. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 4350–4354
Verma, A. K. & Penniston, J. T. (1981) J. Biol. Chem. **256**, 1269–1275
Wuytack, F., deSchutter, G. & Casteels, R. (1980) Biochem. J. **198**, 827–831
Wuytack, F., deSchutter, G. & Casteels, R. (1981) FEBS Lett. **129**, 297–300
Wuytack, F., Raeymaekers, L., deSchutter, G. & Casteels, R. (1982) Biochim. Biophys. Acta **693**, 45–52
Wuytack, F., deSchutter, G., Verbist, J. & Casteels, R. (1983) FEBS Lett. **154**, 191–195
Wuytack, F., Raeymaekers, L., Verbist, J., DeSmedt, H. & Casteels, R. (1984) Biochem. J. **224**, 445–451
Zurini, M., Krebs, J., Penniston, J. T. & Carafoli, E. (1984) J. Biol. Chem. **259**, 618–627

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