Inhibitory antibodies to plasmalemmal Ca^{2+} -transporting ATPases

Their use in subcellular localization of $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -dependent ATPase activity in smooth muscle

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Antibodies directed against the purified calmodulin-binding $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase [$(\text{Ca}^{2+} + \text{Mg}^{2+})$ -dependent ATPase] from pig erythrocytes and from smooth muscle of pig stomach (antral part) were raised in rabbits. Both the IgGs against the erythrocyte $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase and against the smooth-muscle $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase inhibited the activity of the purified calmodulin-binding $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase from smooth muscle. Up to 85% of the total $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase activity in a preparation of KCl-extracted smooth-muscle membranes was inhibited by these antibodies. The $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase activity and the Ca^{2+} uptake in a plasma-membrane-enriched fraction from this smooth muscle were inhibited to the same extent, whereas in an endoplasmic-reticulum-enriched membrane fraction the $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase activity was inhibited by only 25% and no effect was observed on the oxalate-stimulated Ca^{2+} uptake. This supports the hypothesis that, in pig stomach smooth muscle, two separate types of Ca^{2+} -transport ATPase exist: a calmodulin-binding ATPase located in the plasma membrane and a calmodulin-independent one present in the endoplasmic reticulum. The antibodies did not affect the stimulation of the $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase activity by calmodulin.

INTRODUCTION

The cytoplasmic Ca^{2+} concentration in smooth-muscle cells can be lowered by two types of non-mitochondrial ATP-dependent Ca^{2+} -transport mechanisms: one present in the endoplasmic reticulum and the other one in the plasma membrane. Some plasma membrane $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPases have been shown to bind to, and to be regulated by, calmodulin (Carafoli & Zurini, 1982). This property has been used to purify the plasmalemmal $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase of human erythrocytes (Niggli *et al.*, 1979; Gietzen *et al.*, 1980), of heart (Caroni & Carafoli, 1981) and of smooth muscle (Wuytack *et al.*, 1981; De Schutter *et al.*, 1984). The purified calmodulin-binding $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase from smooth muscle has several properties in common with the $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase of erythrocytes and of the heart sarcolemma, e.g. its stimulation by calmodulin, by acidic phospholipids and by controlled proteolysis (De Schutter *et al.*, 1984), and the formation of a hydroxylamine-sensitive and alkali-labile phosphoprotein with an M_r of 130 000 (Wuytack *et al.*, 1982). This $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase mediates an active transport of Ca^{2+} in a reconstituted system at a Ca^{2+} /ATP ratio of 1 (Verbist *et al.*, 1984), as has been observed for the erythrocyte $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase (Clark & Carafoli, 1983) and for the heart sarcolemmal $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase (Carafoli & Zurini, 1982). In addition, antibodies against the calmodulin-binding $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase from smooth muscle cross-react with the $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase from erythrocytes (Wuytack *et al.*, 1983). The sarcoplasmic reticulum from both skeletal (Martonosi & Halpin, 1971) and heart

muscle (Suko & Hasselbach, 1976) contains a Ca^{2+} -transporting ATPase that differs from the calmodulin-binding ATPase: it does not interact with calmodulin and it has an M_r of 100 000. Antibodies against the calmodulin-binding $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase from smooth muscle do not bind to this $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase from sarcoplasmic reticulum of pig skeletal muscle (Wuytack *et al.*, 1983), and antibodies against human erythrocyte ATPase do not cross-react with the $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase from the sarcoplasmic reticulum of rabbit skeletal muscle (Verma *et al.*, 1982).

Recently a $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase was demonstrated in microsomes (microsomal fractions) of pig stomach smooth muscle which does not bind calmodulin and which has several properties in common with the Ca^{2+} -transporting ATPase in sarcoplasmic reticulum from skeletal muscle. It has the same M_r of 100 000, shows a similar proteolytic degradation pattern, and its Ca^{2+} -dependent phosphorylation is slightly decreased by La^{3+} , whereas the phosphorylation level of the calmodulin-binding $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase is increased by La^{3+} (Wuytack *et al.*, 1982, 1984). A similar phosphoprotein intermediate has been described in microsomes from uterine smooth muscle (Carsten & Miller, 1984) and vascular smooth muscle (Chiesi *et al.*, 1984). Raeymaekers *et al.* (1985) developed a method to purify endoplasmic reticulum from pig stomach smooth muscle without calcium oxalate loading. This endoplasmic-reticulum fraction contained a maximum of 10–20% contaminating plasma membranes. The concomitantly prepared plasma-membrane fraction reached a purity of at least 70%.

The analysis of the phosphorylated intermediates of the

Abbreviation used: $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase, $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -dependent ATPase; SDS, sodium dodecyl sulphate.

($\text{Ca}^{2+} + \text{Mg}^{2+}$)-ATPases in these fractions revealed two intermediates, with M_r values of 130 000 and 100 000 respectively. The 130 000- M_r enzyme was predominant in the fractions enriched in plasma membrane, whereas the distribution of the 100 000- M_r protein was correlated with the endoplasmic reticulum.

We now report the effects of antibodies raised in rabbits against the calmodulin-binding ($\text{Ca}^{2+} + \text{Mg}^{2+}$)-ATPase on the ($\text{Ca}^{2+} + \text{Mg}^{2+}$)-ATPase activity and on the accompanying Ca^{2+} uptake by crude microsomes from pig antrum smooth muscle, by a plasma-membrane-enriched fraction and by an endoplasmic-reticulum-enriched fraction from the same tissue.

EXPERIMENTAL

Preparation of smooth-muscle microsomes

Smooth-muscle microsomes were prepared from the antral part of the pig stomach as described by Wuytack *et al.* (1981).

Preparation of erythrocyte inside-out vesicles

Pig erythrocyte inside-out vesicles were prepared as described by Steck & Kant (1974), but the density-gradient step was omitted.

Purification of the calmodulin-binding ($\text{Ca}^{2+} + \text{Mg}^{2+}$)-ATPase

The calmodulin-binding ($\text{Ca}^{2+} + \text{Mg}^{2+}$)-ATPase from smooth muscle or erythrocytes was purified as described by De Schutter *et al.* (1984).

Subfractionation of smooth-muscle membrane vesicles

Membrane vesicles enriched in endoplasmic reticulum or plasma membrane from pig stomach smooth muscle were prepared by using a sucrose density gradient as described by Raeymaekers *et al.* (1985). Briefly, smooth-muscle homogenates were centrifuged in a Sorvall GSA rotor at 13 000 g_{max} for 30 min. Digitonin was added to the supernatant, which was applied below a 15–45% (w/w) sucrose gradient containing 0.6 M-KCl in a Beckman Ti 15 zonal rotor. Centrifugation was performed at 105 000 g_{max} for 20 h at 4 °C.

The endoplasmic-reticulum membranes were recovered between 18 and 25% (w/w) sucrose, and the plasma membranes between 30 and 35%. The activity of the plasma-membrane markers ($\text{Na}^+ + \text{K}^+$)-dependent ATPase and 5'-nucleotidase in the endoplasmic reticulum was about 5–10 times lower than in the plasma-membrane fraction. The activity of NADPH:cytochrome *c* reductase was about 4 times lower in the plasma-membrane than in the endoplasmic-reticulum fraction. These results suggest that the endoplasmic-reticulum fraction contains 10–20% plasma membranes, whereas the plasma-membrane fraction would contain, at most, 30% endoplasmic reticulum.

Production of antisera

The calmodulin-binding ($\text{Ca}^{2+} + \text{Mg}^{2+}$)-ATPase purified from smooth-muscle microsomes or from red-blood-cell vesicles was freeze-dried and redissolved in water at a protein concentration of 0.3 mg/ml. Rabbits were immunized by a subcutaneous injection of 0.5 ml of this enzyme preparation mixed with an equal volume of Freund's complete adjuvant, followed by booster injections of the same amount of enzyme in incomplete

Freund's adjuvant after 14 and 28 days. The rabbits were bled 5 days after the last injection. The blood was allowed to clot at 37 °C for a few hours, incubated for 16 h at 4 °C, and the serum was separated from the retracted clot by centrifugation. The serum obtained was stored at –70 °C.

Purification of immune and non-immune IgG

The IgG fractions from non-immune and immune rabbit serum were purified by $(\text{NH}_4)_2\text{SO}_4$ precipitation after the precipitation of most non-IgG proteins by octanoic acid as described by Steinbuch & Audran (1969).

Determination of ($\text{Ca}^{2+} + \text{Mg}^{2+}$)-ATPase activity

The ATPase activity was measured spectrophotometrically at 37 °C by using a coupled enzyme assay (Wuytack & Casteels, 1980). The reaction mixture, in a final volume of 1 ml contained 100 mM-KCl, 30 mM-imidazole/HCl (pH 6.8), 5 mM- NaN_3 , 1 mM- MgCl_2 , 0.02 mM- CaCl_2 , 0.26 mM-NADH, 1.5 mM-phosphoenolpyruvate and 40 units of pyruvate kinase and lactate dehydrogenase, 5 μg of ionophore A23187 and 5 μg of the purified calmodulin-dependent ($\text{Ca}^{2+} + \text{Mg}^{2+}$)-ATPase or 20 μg of vesicular membrane protein. The ATPase activity was measured in the presence or absence of 0.6 μM -calmodulin. This reaction mixture was preincubated with different amounts of IgG at room temperature for 30 min. The reaction was started by adding 0.5 mM- $\text{K}_2\text{-ATP}$, pH 6.8. The ($\text{Na}^+ + \text{K}^+$)-ATPase activity was inhibited by 0.1 mM-ouabain. In control experiments the effect of non-immune rabbit IgG on the ($\text{Ca}^{2+} + \text{Mg}^{2+}$)-ATPase activity has also been determined.

Determination of Ca^{2+} uptake in the vesicles

The Ca^{2+} uptake was measured at 37 °C in a medium containing 150 mM-KCl, 5 mM- NaN_3 , 30 mM-imidazole HCl, pH 6.9, 5 mM-Tris/ATP, 6 mM- MgCl_2 , 1 mM- $^{45}\text{CaCl}_2$ and 1 mM-EGTA. The Ca^{2+} uptake by the vesicle preparation designated 'endoplasmic reticulum' was measured with or without 5 mM-potassium oxalate, and that by the plasmalemmal-vesicles preparation with or without 50 mM-potassium phosphate. The protein concentration was 25 $\mu\text{g}/\text{ml}$.

The effect of IgG on the Ca^{2+} uptake was measured after a 30 min preincubation with 1 mg of IgG/ml at room temperature. The Ca^{2+} uptake was started by adding 5 mM-Tris/ATP, pH 6.8. Controls were incubated without ATP to correct for passive Ca^{2+} binding. The vesicles were separated from the solution by Millipore filtration. Thereupon the filters were rinsed and the $^{45}\text{Ca}^{2+}$ radioactivity remaining on them was counted. Controls were done with equal concentrations of non-immune rabbit IgG and without IgG.

Electrophoresis and electroblot techniques

Laemmli-type SDS/polyacrylamide gel electrophoresis (Laemmli, 1970) was done in 10% (w/v)-polyacrylamide slab gels of 0.75 mm thickness. Proteins, at a concentration of 1 mg/ml, were dissolved in 2% (w/v) SDS/10% (v/v) glycerol/62.5 mM-Tris/HCl (pH 6.8)/1% (v/v) mercaptoethanol/0.03% Bromophenol Blue. After heating at 60 °C for 4 min, 40 μl of this mixture was applied to the gels. Electroblotting on to nitrocellulose was performed as described previously (Wuytack *et al.*, 1983).

Fig. 1. Effect of antibodies on $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase activities

The effects of IgG on $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase activity of (a) purified calmodulin-dependent $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase, (b) KCl-extracted crude microsomes, (c) a plasmalemma-enriched membrane fraction and (d) an endoplasmic-reticulum-enriched membrane fraction, all prepared from pig stomach (antrum) smooth muscle, are shown. The NADH-coupled enzyme assay was used to quantify inhibition of $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase activity as described in the Experimental section. A $5 \mu\text{g}$ portion of the purified calmodulin-dependent $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase or $20 \mu\text{g}$ of membrane proteins were incubated in the reaction medium containing different concentrations of IgG for 30 min at room temperature. The reaction was started by adding $0.5 \text{ mM-K}_2\text{-ATP}$. ■, Non-immune IgG; ●, anti-(smooth-muscle ATPase) antibodies; ○, anti-(erythrocyte ATPase) antibodies. Each point represents the average of six separate determinations ($\pm$ S.E.M.). Maximal $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase activity (100%) was measured without the addition of IgG.

Detection of proteins on the nitrocellulose membrane

After blotting, the remaining protein-binding sites on the nitrocellulose were saturated by incubating the blots for 3 h at room temperature in Tris-buffered saline (0.9% NaCl/10 mM-Tris/HCl, pH 7.5) containing 0.1% Tween-20. The blot was further incubated for 2 h in

Tris-buffered saline added with 0.1% Tween-20 and IgG (0.4 mg/ml) against erythrocyte $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase. Thereupon the nitrocellulose membrane was washed four times with Tris-buffered saline containing 0.05% Tween-20 and incubated with a 1:100 dilution of peroxidase-conjugated pig anti-rabbit immunoglobulins in Tris-buffered saline added with 0.1%

Tween-20. The blots were then washed in the same way as after the incubation with the primary antibody, and the peroxidase activity was detected by exposing the nitrocellulose membrane to a solution containing 0.005% H_2O_2 and 4-chloro-1-naphthol (0.5 mg/ml) in Tris-buffered saline, pH 7.5.

Materials

Calmodulin was prepared as described by Gopalakrishna & Anderson (1982). Calmodulin-Sepharose 4B was prepared by coupling calmodulin (2 mg/ml) to CNBr-activated Sepharose 4B (Pharmacia), the manufacturer's instructions being followed. Phenylmethanesulphonyl fluoride, pyruvate kinase and lactate dehydrogenase were obtained from Boehringer. Asolectin was from Associated Concentrates, Woodside, NY, U.S.A. The Ca^{2+} -ionophore A23187 was supplied by Calbiochem, and M_r standards for gel electrophoresis were obtained from Sigma. Nitrocellulose membranes were from Millipore, and the peroxidase-conjugated pig anti-rabbit immunoglobulins were supplied by DAKO-immunoglobulins AS, Copenhagen, Denmark.

RESULTS

Effect of immune IgG on the $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase activity

Fig. 1(a) shows the effect of different concentrations of immune IgG raised against the calmodulin-binding $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase, from both pig stomach smooth muscle and pig erythrocytes, on the activity of the

$(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase purified by calmodulin affinity chromatography from pig stomach smooth muscle. Immune IgG fractions against the enzyme from both sources inhibited the ATPase activity maximally by 80–85% at an IgG concentration of 2.0 mg/ml. The percentage inhibition was the same for measurements performed in the presence or the absence of $0.6 \mu\text{M}$ -calmodulin.

Effect of the antibodies on the $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase activity in different smooth-muscle membrane preparations

Figs. 1(b), 1(c) and 1(d) represent the action of antibodies against smooth-muscle and erythrocyte ATPase on the $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase activity in three different membrane preparations obtained from pig stomach: (1) KCl-extracted smooth-muscle microsomes, (2) a plasma-membrane-enriched fraction, and (3) a membrane preparation enriched in endoplasmic reticulum. The $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase of both the microsomes and the plasma-membrane fraction is inhibited to the same extent as the purified calmodulin-binding $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase.

In contrast, the inhibition of $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase activity of the endoplasmic-reticulum preparation is much less and reaches a maximum value of 25%.

Effect of the antibodies on Ca^{2+} transport in different smooth-muscle membrane preparations

The immune IgG strongly decreased the rate of Ca^{2+} uptake by the plasmalemmal preparation, both in the presence and in the absence of phosphate (Fig. 2a).

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

The effect of IgG on the time course of the Ca^{2+} uptake by a membrane fraction enriched in plasma membrane (a) and in endoplasmic reticulum (b) is shown. Ca^{2+} uptake was measured as described in the Experimental section. Continuous lines indicate the uptake in the presence of Ca^{2+} -precipitating anions (phosphate in a or oxalate in b); the broken lines indicate uptake without precipitating anions. Vesicle protein (25 $\mu\text{g}/\text{ml}$) was preincubated for 30 min at room temperature with IgG (1 mg/ml). The reaction was started by adding 0.5 mM-Tris/ATP, pH 6.8. $\square$, Control without IgG; $\blacksquare$, non-immune IgG; $\circ$, IgG against erythrocyte $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase.

Fig. 3. Binding of antibodies against erythrocyte $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase to proteins in the subcellular fractions of smooth muscle

An SDS/polyacrylamide-gel electrophoretogram stained with Coomassie Brilliant Blue (a) and a nitrocellulose electroblot of an identical gel treated with anti-[erythrocyte $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase] IgG (b) are shown. The binding of these primary antibodies was detected by using peroxidase-conjugated pig anti-rabbit IgG as secondary antibody. Staining for peroxidase activity was done with 4-chloro-1-naphthol. Lanes 1–6 represent different membrane fractions from pig stomach smooth muscle subfractionated on a sucrose density gradient. Lanes 4–6 correspond to the plasma membranes and lanes 1–3 to the endoplasmic reticulum; lane 7 corresponds to the purified calmodulin-binding $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase from pig stomach smooth muscle and lane 8 to the M_r standards.

However, antibodies against erythrocyte ATPase did not slow down the Ca^{2+} uptake by the endoplasmic reticulum in the presence of oxalate. In the absence of IgG or after treatment with non-immune IgG, a similar time course of Ca^{2+} uptake was observed (Fig. 2b).

Binding of the antibodies to the $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase protein in different membrane fractions

An SDS/polyacrylamide-gel electrophoretogram (left) and an electroblot on to nitrocellulose of an identical gel (right) are represented in Fig. 3. The gel was stained with Coomassie Brilliant Blue, and the blot was treated with antibodies against erythrocyte $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase. Lane 7 on the blot represents the binding of these antibodies to the purified $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase from smooth muscle. A positive reaction is also observed at the same M_r of 140000 in the membrane fractions from the sucrose gradient (lanes 1–6). The intensity of this reaction is clearly much higher in the plasma-membrane fractions (lanes 4–6) than in the endoplasmic-reticulum fractions (lanes 1–3). The weak staining in these low-density fractions suggests that the endoplasmic-reticulum fraction still contains a small amount of plasma membranes, as has been reported by Raeymaekers *et al.* (1985).

DISCUSSION

The present results indicate that the antibodies which we have obtained are directed against parts of the calmodulin-binding Ca^{2+} -transport ATPase involved in the expression of enzymic activity. The antigenic similarities of the calmodulin-binding $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPases from smooth muscle and erythrocytes (Wuytack *et al.*, 1983, 1984) is confirmed by the present observation that antibodies against these two $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPases have the same inhibitory effect on the

plasmalemmal ATPase activity of the smooth-muscle enzyme.

Our antibodies against the calmodulin-binding $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase inhibit the $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase activity by 80% and significantly slow down the rate of Ca^{2+} uptake in a plasma-membrane-enriched vesicle preparation, whereas, in a membrane fraction enriched in endoplasmic reticulum, they inhibit the $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase by 25% and do not affect the oxalate-dependent Ca^{2+} uptake. These observations support the assumption that two different Ca^{2+} -transport ATPases exist in smooth muscle, namely one that binds calmodulin and is located in the plasma membrane and another enzyme that is confined to the endoplasmic reticulum (Wuytack *et al.*, 1984; Raeymaekers *et al.*, 1985).

These results furthermore confirm the conclusion of Raeymaekers *et al.* (1985) that the low-density membrane fraction, obtained by a combination of digitonin treatment of the postmitochondrial supernatant and of centrifugation in a sucrose gradient containing 0.6 M-KCl, consists largely of endoplasmic-reticulum vesicles. This low-density fraction contains only small amounts of plasma-membrane markers such as a wheat-germ-agglutinin-binding activity, 5'-nucleotidase, $(\text{Na}^{+} + \text{K}^{+})$ -dependent ATPase and Mg^{2+} -dependent ATPase.

In addition, we have observed that antibodies against the calmodulin-binding $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase inhibit the $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase activity of the endoplasmic-reticulum-enriched fraction to a much smaller extent (25%) than in a plasma-membrane-enriched fraction (80–85%). The 25% inhibition by these antibodies in the endoplasmic-reticulum fraction can be explained by its contamination with plasma-membrane vesicles. A minor binding of these antibodies to a protein band of approx. M_r 140000 present in the endoplasmic-reticulum fractions was also observed (Fig. 3).

The 80–85% inhibition by the antibodies of the ATPase activity in the KCl-extracted microsomes from pig stomach smooth muscle suggests that these microsomes consist mainly of plasma membranes containing the calmodulin-binding Ca^{2+} -transporting ATPase. The binding of the antibodies did not modify the stimulation by calmodulin of the $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase activity either in the purified enzyme solubilized in the presence of detergent, or in the different smooth-muscle membrane preparations. A similar observation was made by Verma *et al.* (1982), who could not detect any inhibitory effect of their antibodies on the binding of calmodulin to erythrocyte membranes.

Our findings indicate that the $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase of endoplasmic reticulum and the plasmalemmal $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase display highly distinctive antigenic determinants. Therefore it may be possible to use these antibodies for immunocytochemical localization of the plasma-membrane $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase in smooth-muscle cells and for immunochemical detection of this Ca^{2+} -transport ATPase in other tissues.

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REFERENCES

- Carafoli, E. & Zurini, M. (1982) *Biochim. Biophys. Acta* **683**, 279–301
- Caroni, P. & Carafoli, E. (1981) *J. Biol. Chem.* **256**, 3263–3270
- Carsten, M. E. & Miller, J. D. (1984) *Arch. Biochem. Biophys.* **232**, 616–623
- Clark, A. & Carafoli, E. (1983) *Cell Calcium* **4**, 83–88
- Chiesi, M., Gasser, J. & Carafoli, E. (1984) *Biochem. Biophys. Res. Commun.* **124**, 797–806
- De Schutter, G., Wuytack, F., Verbist, J. & Casteels, R. (1984) *Biochim. Biophys. Acta* **773**, 1–10
- Gietzen, K., Tejcka, M. & Wolf, H. V. (1980) *Biochem. J.* **189**, 81–89
- Gopalakrishna, R. & Anderson, W.B. (1982) *Biochem. Biophys. Res. Commun.* **104**, 830–836
- Laemmli, U. K. (1970) *Nature (London)* **227**, 670–685
- Marionosi, A. & Halpin, R. A. (1971) *Arch. Biochem. Biophys.* **144**, 66–77
- Niggli, V., Penniston, J. T. & Carafoli, E. (1979) *J. Biol. Chem.* **254**, 9955–9958
- Raeymaekers, L., Wuytack, F. & Casteels, R. (1985) *Biochim. Biophys. Acta*, **875**, 447–454
- Steck, T. L. & Kant, J. A. (1974) *Methods Enzymol.* **31**, 172–180
- Steinbuch, M. & Audran, R. (1969) *Rev. Fr. Etud. Clin. Biol.* **14**, 1054–1058
- Suko, J. & Hasselbach, W. (1976) *Eur. J. Biochem.* **64**, 123–130
- Verbist, J., Wuytack, F., De Schutter, G., Raeymaekers, L. & Casteels, R. (1984) *Cell Calcium* **5**, 253–263
- Verma, A. K., Gorski, J. P. & Penniston, J. T. (1982) *Arch. Biochem. Biophys.* **215**, 345–354
- Wuytack, F. & Casteels, R. (1980) *Biochim. Biophys. Acta* **595**, 857–863
- Wuytack, F., De Schutter, G. & Casteels, R. (1981) *FEBS Lett.* **129**, 297–300
- Wuytack, F., Raeymaekers, L., De Schutter, G. & Casteels, R. (1982) *Biochem. J.* **193**, 45–52
- Wuytack, F., De Schutter, G., Verbist, J. & Casteels, R. (1983) *FEBS Lett.* **154**, 191–195
- Wuytack, F., Raeymaekers, L., Verbist, J., De Smedt, H. & Casteels, R. (1984) *Biochem. J.* **224**, 445–451

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