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AlF_4^--induced inhibition of the ATPase activity, the Ca^{2+}-transport activity and the phosphoprotein-intermediate formation of plasma-membrane and endo(sarco)plasmic-reticulum Ca^{2+}-transport ATPases in different tissues

Evidence for a tissue-dependent functional difference

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AlF_4^- inhibits the $(Ca^{2+}+Mg^{2+})$-ATPase activity of the plasma-membrane and the sarcoplasmic-reticulum Ca^{2+}-transport ATPase [Missiaen, Wuytack, De Smedt, Vrolix & Casteels (1988) Biochem. J. **253**, 827–833]. The aim of the present work was to investigate this inhibition further. We now report that AlF_4^- inhibits not only the $(Ca^{2+}+Mg^{2+})$-ATPase activity, but also the ATP-dependent $^{45}Ca^{2+}$ transport, and the formation of the phosphoprotein intermediate by these pumps. Mg^{2+} potentiated the effect of AlF_4^-, whereas K^+ had no such effect. The plasma-membrane Ca^{2+}-transport ATPase from erythrocytes was 20 times less sensitive to inhibition by AlF_4^- as compared with the Ca^{2+}-transport ATPase from smooth muscle. The endoplasmic-reticulum Ca^{2+}-transport ATPase from smooth muscle was inhibited to a greater extent than the sarcoplasmic-reticulum Ca^{2+}-transport ATPase of slow and fast skeletal muscle.

INTRODUCTION

We have previously reported that AlF_4^- inhibited the ATPase activities of 'P'-type cation-transport ATPases, including the $(Ca^{2+}+Mg^{2+})$-ATPase activity of the plasma-membrane and sarcoplasmic-reticulum Ca^{2+}-transport ATPase (Missiaen *et al.*, 1988*a*). The aim of the present work was to provide evidence that, besides the ATPase activity, the $^{45}Ca^{2+}$ transport and the formation of the phosphoprotein intermediate by these Ca^{2+}-transport ATPases were also affected by AlF_4^-. We have at the same time addressed the question whether the Ca^{2+}-transport ATPases in different tissues are equally sensitive to inhibition by AlF_4^-.

Because AlF_4^- interacts with the phosphate-binding site of the ATPase (Missiaen *et al.*, 1988*a*), its action resembles that of vanadate. Mg^{2+} and K^+ facilitate the inhibition of the plasma-membrane Ca^{2+}-transport ATPase by vanadate (Bond & Hudgins, 1980; Barrabin *et al.*, 1980; Rossi *et al.*, 1981). Vanadate binding to the sarcoplasmic-reticulum Ca^{2+}-transport ATPase also requires the presence of Mg^{2+} (Pick, 1982; Dupont & Bennett, 1982). It was therefore decided to investigate whether Mg^{2+} is also an essential component for the inhibition of these Ca^{2+}-transport ATPases by AlF_4^-, and whether these inhibitions are enhanced by K^+ ions.

EXPERIMENTAL

Membrane preparation and purification of the plasma-membrane Ca^{2+}-transport ATPase

KCl-extracted microsomes (microsomal fractions) from pig stomach smooth muscle were prepared as described by Wuytack *et al.* (1981). An endoplasmic-reticulum fraction from stomach smooth muscle was prepared as described by Raeymaekers *et al.* (1985). Pig erythrocyte vesicles were prepared as described by Steck & Kant (1974). Fragmented sarcoplasmic reticulum from fast (psoas) and slow (soleus) pig skeletal muscle was prepared as described by Heilmann *et al.* (1977). The plasma-membrane Ca^{2+}-transport ATPase was purified from stomach smooth muscle and erythrocytes by calmodulin affinity chromatography (Vrolix *et al.*, 1988), and re-activated by addition of phosphatidylcholine (1 mg/mg of ATPase).

Preincubation with AlF_4^-

The membranes or Ca^{2+}-transport ATPases were preincubated for 10 min at 37 °C in a medium containing (unless otherwise indicated): 30 mM-imidazole/HCl (pH 6.8), 100 mM-KCl, 1 mM-$MgCl_2$, 1 mM-NaF and the indicated concentration of $AlCl_3$. When no $AlCl_3$ was added, 0.5 mM-deferoxamine was included in order to complex contaminating Al^{3+} in the solutions and membranes. The protein concentration in this preincubation medium was 100 μg/ml for the two purified plasma-membrane Ca^{2+}-transport ATPases and the fragmented sarcoplasmic-reticulum fraction, 500 μg/ml for the pig erythrocyte vesicles, and 1 mg/ml for the KCl-extracted microsomes from pig stomach smooth muscle. After this preincubation, 50 μl of the preincubation medium was added to 950 μl of assay medium (for ATPase and $^{45}Ca^{2+}$-uptake measurements) or 150 μl of assay medium (for phosphorylation experiments).

ATPase measurements

ATPase activities were measured at 37 °C with an NADH-coupled enzyme assay (Wuytack & Casteels,

Abbreviation used: $(Ca^{2+}+Mg^{2+})$-ATPase, Ca^{2+}-stimulated and Mg^{2+}-dependent ATPase.

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1980) in a medium of the following composition: 100 mM-KCl, 30 mM-imidazole/HCl (pH 6.8), 5.7 mM-$MgCl_2$, 5 mM-ATP, 1 mM-EGTA, 1.5 mM-phosphoenolpyruvate, 0.26 mM-NADH, 5 mM-NaN_3, 0.1 mM-ouabain, pyruvate kinase (40 units/ml) and lactate dehydrogenase (36 units/ml). The $(Ca^{2+}+Mg^{2+})$-ATPase activity was measured by comparing the rate of ATP hydrolysis in this assay medium and that obtained at a free Ca^{2+} concentration of 10 μM, which was obtained by addition of 0.87 mM-$CaCl_2$. For measuring the maximum activity of the plasma-membrane $(Ca^{2+}+Mg^{2+})$-ATPase, calmodulin was added at a saturating concentration of 0.6 μM. The calmodulin-stimulated $(Ca^{2+}+Mg^{2+})$-ATPase activity was measured by comparing the rate of ATP hydrolysis in the assay medium containing 10 μM-Ca^{2+} and that obtained after addition of 0.6 μM-calmodulin.

$^{45}Ca^{2+}$ uptake

ATP-dependent $^{45}Ca^{2+}$ uptake was measured at 37 °C in a solution similar to that used for determining the $(Ca^{2+}+Mg^{2+})$-ATPase activity, except that the coupled enzyme system and ouabain were omitted, and that 40 mM-phosphate was added for measuring the $^{45}Ca^{2+}$ uptake in plasma membranes, and 5 mM-oxalate for measuring the $^{45}Ca^{2+}$ uptake in the endo-(sarco-)plasmic reticulum. The reaction was initiated by adding the vesicles to the medium. The membranes were separated from the medium after 10 min by Millipore filtration (the reaction was linear up to that time). The values were corrected for the passive binding of $^{45}Ca^{2+}$ to the vesicles, which was determined in a medium without ATP. The calmodulin-stimulated $^{45}Ca^{2+}$ uptake is the increase in the ATP-dependent $^{45}Ca^{2+}$ uptake by 0.6 μM-calmodulin, in a medium containing 40 mM-phosphate. The oxalate-stimulated $^{45}Ca^{2+}$ uptake is the increase in the ATP-dependent $^{45}Ca^{2+}$ uptake by 5 mM-oxalate.

Phosphorylation experiments and electrophoresis

Phosphorylation was conducted at 0 °C as described by Wuytack *et al.* (1982). The reaction medium contained 30 mM-imidazole/HCl (pH 6.8), 1 mM-$MgCl_2$, 100 mM-KCl and 50 μM-$CaCl_2$. The reaction was started by addition of 6 μM-ATP containing [γ-^{32}P]ATP (Amersham International) at 3000 Ci/mmol. The reaction was stopped after 10 s, and electrophoresis was performed as described by De Smedt *et al.* (1984).

RESULTS

Effect of AlF_4^- on the plasma-membrane Ca^{2+}-transport ATPase in different tissues

AlF_4^- inhibited the calmodulin-stimulated ATP-dependent $^{45}Ca^{2+}$ uptake in inside-out vesicles from pig erythrocytes and in the KCl-extracted microsomes from stomach smooth muscle. The latter microsomes contained both plasma-membrane and endoplasmic-reticulum elements, but only the plasma-membrane Ca^{2+}-transport ATPase was stimulated by calmodulin (Wuytack *et al.*, 1984). As a consequence, the calmodulin-stimulated ATP-dependent $^{45}Ca^{2+}$ uptake in the KCl-extracted microsomes was taken to represent the activity of the plasma-membrane Ca^{2+}-transport ATPase. The concentration of $AlCl_3$ required for the inhibition of the ATP-dependent $^{45}Ca^{2+}$ uptake at a constant NaF concentration of 1 mM is illustrated in Fig. 1(*a*). It can be

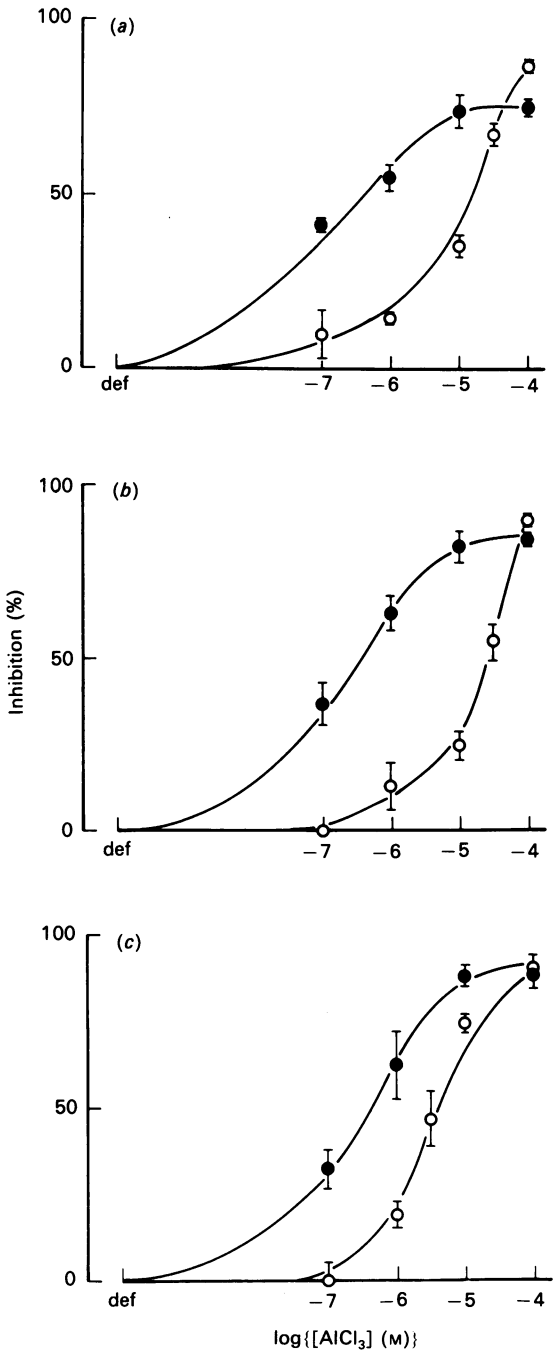

Fig. 1. Inhibition by AlF_4^- of the plasmalemmal Ca^{2+} uptake and Ca^{2+}-transport ATPase in pig stomach smooth muscle and in pig erythrocytes

In (*a*) and (*b*), the KCl-extracted microsomal fraction from pig antral smooth muscle (●) and pig erythrocyte inside-out vesicles (○), and in (*c*) the purified plasma-membrane Ca^{2+}-transport ATPase from stomach smooth muscle (●) and from pig erythrocytes (○), were preincubated for 10 min at 37 °C in a medium containing 1 mM-NaF, 30 mM-imidazole/HCl (pH 6.8), 100 mM-KCl, 1 mM-$MgCl_2$ and $AlCl_3$ (concentration indicated on the abscissa). 'def' indicates the inhibition observed in the presence of 0.5 mM-deferoxamine. (*a*) represents the inhibition of the calmodulin-stimulated $^{45}Ca^{2+}$ uptake, and (*b*) and (*c*) show the inhibition of the calmodulin-stimulated $(Ca^{2+}+Mg^{2+})$-ATPase activity after preincubation in this medium. The indicated points are mean values ± S.E.M. for four experiments.

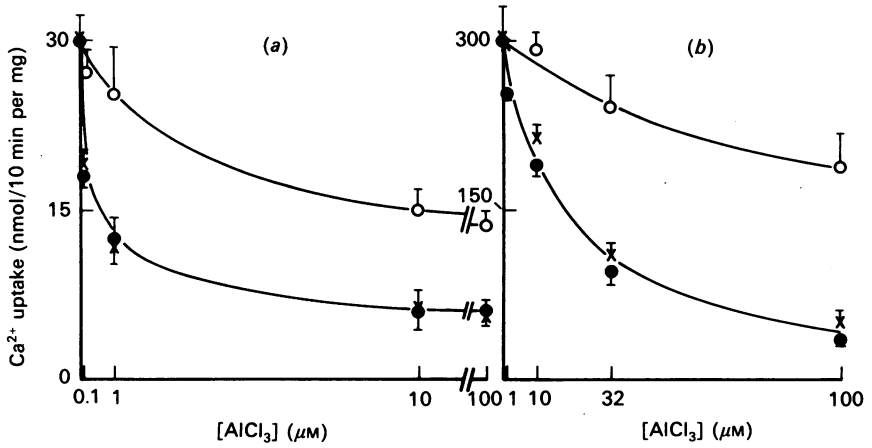

Fig. 2. Inhibition of the plasma-membrane Ca^{2+}-transport ATPase in pig stomach smooth muscle and in pig erythrocytes: dependence on Mg^{2+} and K^+

The KCl-extracted microsomal fraction from pig antrum (*a*) and erythrocyte inside-out vesicles (*b*) were preincubated for 10 min at 37 °C in a medium containing 1 mM-NaF, the indicated concentration of $AlCl_3$, 30 mM-imidazole/HCl (pH 6.8) (○), or in the same medium supplemented with 1 mM-$MgCl_2$ (●), or with 1 mM-$MgCl_2$ plus 100 mM-KCl (×). The calmodulin-stimulated $^{45}Ca^{2+}$ uptake remaining after this preincubation was expressed in nmol/10 min per mg of protein. The results represent means ± S.E.M. for three different experiments.

inferred that the calmodulin-stimulated ATP-dependent $^{45}Ca^{2+}$ uptake by stomach smooth-muscle microsomes was more sensitive to inhibition by AlF_4^- than was the erythrocyte enzyme. That the observed effects on the calmodulin-stimulated ATP-dependent $^{45}Ca^{2+}$ uptake were indeed related to the Ca^{2+}-transport ATPase is illustrated in Fig. 1(*b*). AlF_4^- inhibited the calmodulin-stimulated ($Ca^{2+}+Mg^{2+}$)-ATPase in a concentration-dependent manner both in inside-out vesicles from pig erythrocytes and in KCl-extracted microsomes from stomach smooth muscle. The erythrocyte Ca^{2+}-transport ATPase was again less sensitive to AlF_4^- than the plasma-membrane Ca^{2+}-transport ATPase from stomach smooth muscle.

The difference in sensitivity to AlF_4^- of the Ca^{2+}-transport ATPases in these two tissues was also found in preparations of isolated Ca^{2+}-transport ATPases from pig erythrocytes and from the plasma membranes of pig stomach smooth muscle, purified by calmodulin affinity chromatography and re-activated by addition of phosphatidylcholine. Similar inhibition curves were obtained whether the ($Ca^{2+}+Mg^{2+}$)-ATPase was measured in the presence of 10 μM-Ca^{2+} or in the presence of 10 μM-Ca^{2+} plus 0.6 μM-calmodulin (0.6 μM-calmodulin stimulated the erythrocyte enzyme at 10 μM-Ca^{2+} 1.9-fold, and the smooth-muscle enzyme 1.3-fold). The inhibition of the calmodulin-stimulated ($Ca^{2+}+Mg^{2+}$)-ATPase activity is illustrated in Fig. 1(*c*): $AlCl_3$ inhibited both ATPases in a concentration-dependent manner, with a K_i for the erythrocyte ATPase of 4 μM and for the stomach smooth-muscle ATPase of 0.2 μM. This finding also indicates that the purified erythrocyte enzyme was about 20 times less sensitive to AlF_4^- than was the stomach smooth-muscle Ca^{2+}-transport ATPase. In the absence of NaF, up to 100 μM-$AlCl_3$ did not inhibit these ATPases (results not shown).

The potentiating effect of Mg^{2+} on the AlF_4^--induced inhibition of the plasma-membrane Ca^{2+}-transport ATPase is illustrated in Fig. 2. In the presence of 1 mM-NaF, 1 mM-Mg^{2+} enhanced the inhibition of the plasma-membrane Ca^{2+}-transport ATPase from stomach smooth muscle and erythrocytes by $AlCl_3$. In the absence of added Mg^{2+}, AlF_4^- still inhibited the Ca^{2+}-transport ATPase to some extent. Mg^{2+} potentiated the effect of AlF_4^-, with a K_m of 83 μM for the stomach smooth-muscle Ca^{2+}-transport ATPase and 186 μM for the erythrocyte Ca^{2+}-transport ATPase. Maximal potentiation was reached at 1 mM for both ATPase preparations. KCl (100 mM) did not potentiate the AlF_4^--induced inhibition of the two Ca^{2+}-transport ATPases in the presence of 1 mM-Mg^{2+}.

Fig. 3(*a*) shows the effect of AlF_4^- on the formation of the Ca^{2+}-dependent phosphoprotein of 140 kDa, which represents the catalytic intermediate of the plasma-membrane Ca^{2+}-transport ATPase (Wuytack *et al.*, 1983). AlF_4^- inhibited the phosphorylation at 140 kDa in inside-out erythrocyte vesicles (track 3, compared with track 4). A fluoroaluminate complex, and not F^- or Al^{3+} alone, was involved in this inhibition, as F^- alone (track 1) or Al^{3+} alone (track 2) did not abolish the phosphorylation, whereas the combination of F^- and Al^{3+} (track 3) did. The same inhibitory effect of AlF_4^- on the formation of the phosphoprotein intermediate was also observed for the purified plasma-membrane Ca^{2+}-transport ATPase from erythrocytes (track 5 compared with track 6) and from stomach smooth muscle (track 7 compared with track 8).

Effect of AlF_4^- on the endo-(sarco-)plasmic-reticulum Ca^{2+}-transport ATPase

Fig. 3(*b*) shows the effect of AlF_4^- on the Ca^{2+}-dependent phosphoprotein of 100 kDa, which represents the catalytic intermediate of the endo-(sarco-)plasmic-reticulum Ca^{2+}-transport ATPase. AlF_4^- inhibited the phosphorylation at 100 kDa in KCl-extracted microsomes from stomach smooth muscle (track 3, compared with track 4). Note also the decrease of phosphorylation at 140 kDa, which represents the plasma-membrane Ca^{2+}-transport ATPase in these microsomes. A fluoroaluminate complex, and not F^- or Al^{3+} alone, was

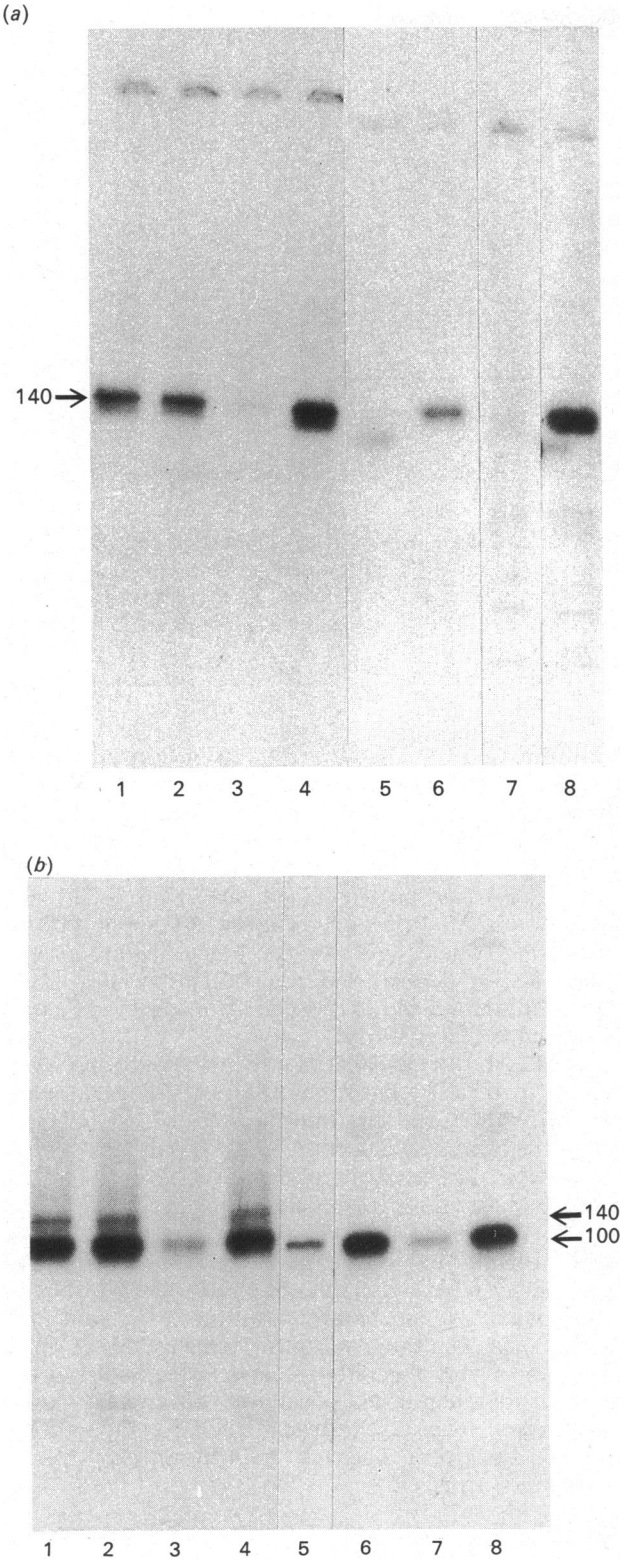

Fig. 3. Autoradiogram of a polyacrylamide slab gel showing the effect of AlF_4^- on the phosphoprotein intermediates of the plasma-membrane and the endo-(sarco-)plasmic-reticulum Ca^{2+}-transport ATPases of different tissues

Tracks 1–4 of panels (*a*) and (*b*) represent the phosphoprotein intermediates after preincubating erythrocyte inside-out vesicles (*a*) or KCl-extracted microsomes from stomach smooth muscle (*b*) in a medium containing 30 mM-imidazole/HCl (pH 6.8), 1 mM-$MgCl_2$, 100 mM-KCl, 50 μM-$CaCl_2$ and supplemented with 1 mM-NaF plus 0.5 mM-deferoxamine (track 1), or with 100 μM-$AlCl_3$ (track 2), or with 1 mM-NaF plus 100 μM-$AlCl_3$ (track 3), or with 0.5 mM-deferoxamine (track 4). Tracks 5–6 and 7–8 in panel (*a*) show the phosphoprotein intermediates of the purified plasmalemmal Ca^{2+}-transport ATPase from erythrocytes and stomach smooth muscle respectively, and tracks 5–6 and 7–8 in panel (*b*) show the phosphoprotein intermediates of fragmented sarcoplasmic reticulum from slow and fast skeletal muscle respectively after preincubation in a medium containing 30 mM-imidazole/HCl (pH 6.8), 1 mM-$MgCl_2$, 100 mM-KCl, 50 μM-$CaCl_2$ and supplemented with 1 mM-NaF plus 100 μM-$AlCl_3$ (tracks 5, 7), or with 0.5 mM-deferoxamine (tracks 6, 8). The autoradiograms illustrated are typical for three experiments. Molecular masses (kDa) are indicated.

Table 1. Inhibition by AlF_4^- of the endo-(sarco-)plasmic-reticulum Ca^{2+}-transport ATPase in pig stomach smooth muscle and in pig slow and fast skeletal muscle

The subcellular fractions were preincubated for 10 min at 37 °C in a medium containing 1 mM-NaF, 100 μM-$AlCl_3$, 30 mM-imidazole/HCl (pH 6.8), 100 mM-KCl and 1 mM-$MgCl_2$, and in a medium containing no $AlCl_3$ and supplemented with 0.5 mM-deferoxamine. ($Ca^{2+}+Mg^{2+}$)-ATPase activity and oxalate-stimulated $^{45}Ca^{2+}$ uptake were then measured. The results are expressed as percentage inhibition by AlF_4^- (means ± S.E.M.) for the numbers of observations given in parentheses.

	Inhibition (%)	
Muscle	($Ca^{2+}+Mg^{2+}$)-ATPase	Oxalate-stimulated $^{45}Ca^{2+}$ uptake
Smooth	76 ± 4 (5)	77 ± 5 (5)
Fast skeletal	40 ± 2 (6)	23 ± 4 (5)
Slow skeletal	43 ± 6 (7)	14 ± 3 (6)

involved in these inhibitions, as F^- alone (track 1) or Al^{3+} alone (track 2) did not decrease the phosphorylation, whereas the combination of F^- and Al^{3+} (track 3) did. The same inhibitory effect of AlF_4^- on the formation of the phosphoprotein intermediate of the Ca^{2+}-transport ATPase was also observed for the sarcoplasmic-reticulum fraction from slow skeletal muscle (track 5, compared with track 6) and from fast skeletal muscle (track 7, compared with track 8).

AlF_4^- also inhibited the ($Ca^{2+}+Mg^{2+}$)-ATPase activity, as well as the oxalate-stimulated ATP-dependent $^{45}Ca^{2+}$ transport by the endoplasmic-reticulum Ca^{2+}-transport ATPase in stomach smooth muscle (Table 1). The inhibition of the ($Ca^{2+}+Mg^{2+}$)-ATPase was measured in an endoplasmic-reticulum-enriched fraction. The contamination of this fraction with plasma-membrane fragments was 10–20% (Raeymaekers *et al.*, 1985). The oxalate-stimulated ATP-dependent $^{45}Ca^{2+}$ uptake was measured in a KCl-extracted microsomal fraction and was only due to $^{45}Ca^{2+}$ uptake in the endoplasmic reticulum, as plasma membranes are impermeable to oxalate (Raeymaekers *et al.*, 1985). We did not measure $^{45}Ca^{2+}$ transport in the endoplasmic-reticulum fraction, but instead used the microsomal fraction for that

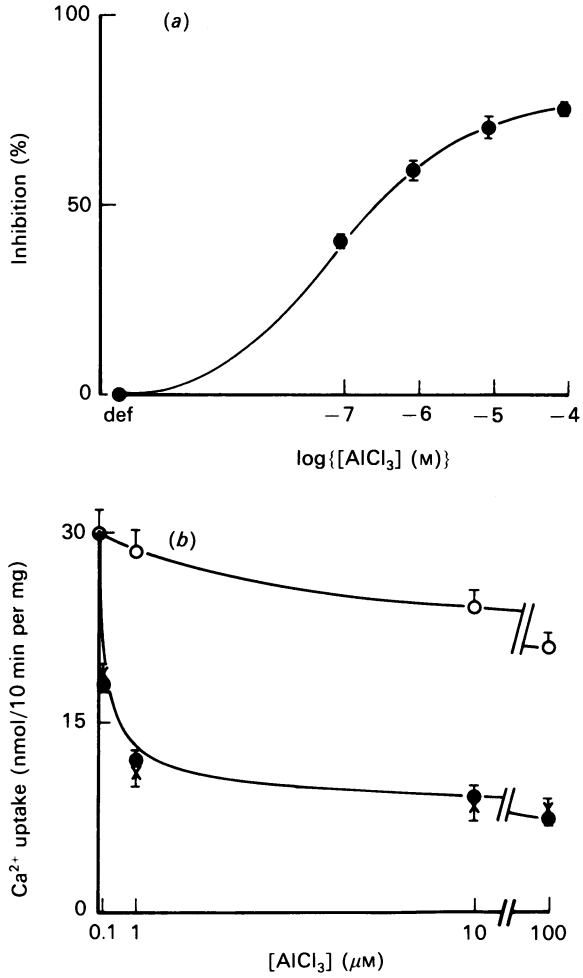

Fig. 4. Inhibition by AlF_4^- of the endoplasmic-reticulum Ca^{2+} transport in pig stomach smooth muscle: dependence on Mg^{2+} and K^+

In (*a*), the KCl-extracted microsomal fraction was pre-incubated for 10 min at 37 °C in a medium containing 1 mM-NaF, the indicated concentration of $AlCl_3$, 30 mM-imidazole/HCl (pH 6.8), 100 mM-KCl and 1 mM-$MgCl_2$. $^{45}Ca^{2+}$ uptake was then measured. 'def' indicates the inhibition observed in the presence of 0.5 mM-deferoxamine. The percentage inhibitions of the oxalate-stimulated ATP-dependent $^{45}Ca^{2+}$ uptake are expressed as means ± S.E.M. ($n = 3$). In (*b*), the KCl-extracted microsomal fraction was preincubated for 10 min at 37 °C in a medium containing 1 mM-NaF, the indicated concentration of $AlCl_3$, 30 mM-imidazole/HCl (pH 6.8) (○), or in the same medium supplemented with 1 mM-$MgCl_2$ (●), or with 1 mM-$MgCl_2$ plus 100 mM-KCl (×). The oxalate-stimulated $^{45}Ca^{2+}$ uptake remaining after this pre-incubation is expressed in nmol/10 min per mg of protein (mean ± S.E.M. for three observations).

purpose, because the technique used for preparing the endoplasmic-reticulum fraction did not yield enough membranes to perform the $^{45}Ca^{2+}$-uptake studies. The concentration of $AlCl_3$ required to inhibit the $^{45}Ca^{2+}$ uptake at a constant NaF concentration of 1 mM is illustrated in Fig. 4(*a*). $AlCl_3$ inhibited the Ca^{2+}-transport ATPase in a concentration-dependent manner, with a K_i of 0.2 μM. Fig. 4(*b*) indicates that 1 mM-Mg^{2+} enhanced the inhibition at different concentrations of $AlCl_3$, and also that, in the absence of added Mg^{2+}, AlF_4^- still inhibited the Ca^{2+}-transport ATPase to some extent. Mg^{2+} potentiated the effect of AlF_4^- with a K_m of 50 μM and a maximum at 1 mM-$MgCl_2$. KCl (100 mM) did not potentiate the AlF_4^--induced inhibition of the endoplasmic-reticulum Ca^{2+}-transport ATPase of stomach smooth muscle in the presence of 1 mM-Mg^{2+} (Fig. 4*b*).

AlF_4^- also inhibited the ($Ca^{2+} + Mg^{2+}$)-ATPase activity and the oxalate-stimulated ATP-dependent $^{45}Ca^{2+}$ uptake by a sarcoplasmic-reticulum fraction from slow and fast skeletal muscle (Table 1). The inhibition of the ($Ca^{2+} + Mg^{2+}$)-ATPase was more pronounced than the inhibition of the $^{45}Ca^{2+}$-uptake. Table 1 furthermore illustrates that the endoplasmic-reticulum Ca^{2+}-transport ATPase in stomach smooth muscle is inhibited to a greater extent than the sarcoplasmic-reticulum Ca^{2+}-transport ATPases in slow and fast skeletal muscle.

DISCUSSION

AlF_4^- decreased in parallel the ($Ca^{2+} + Mg^{2+}$)-ATPase activity, the active $^{45}Ca^{2+}$ transport, and the level of phosphoenzyme of the plasma-membrane and endo-(sarco-)plasmic-reticulum Ca^{2+}-transport ATPases. In the absence of deferoxamine, it was not possible to lower the concentration of $AlCl_3$ below 0.1 μM in the dose–response curves of Figs. 1 and 4, because the water, the tissues and the reagents are contaminated with trace amounts of $AlCl_3$ (Sternweis & Gilman, 1982). The efficiency of AlF_4^- as an inhibitor of the plasma-membrane Ca^{2+}-transport ATPase resembles that described for vanadate (Barrabin *et al.*, 1980; Bond & Hudgins, 1980; Rossi *et al.*, 1981) by its strong dependency on the presence of Mg^{2+}, but differs from it by its lack of potentiating effect by K^+ ions. We previously reported that AlF_4^- inhibited the purified plasma-membrane Ca^{2+}-transport ATPase in the absence of added Mg^{2+} (Missiaen *et al.*, 1988*a*). In that paper, we erroneously did not take into account that the ATPase preparation contained 1 mM-$MgCl_2$. The present finding that in the absence of added Mg^{2+}, AlF_4^- still inhibited the Ca^{2+}-transport ATPase to some extent, might be explained by the fact that the membranes contain endogenous Mg^{2+}.

The finding that AlF_4^- inhibited the plasma-membrane Ca^{2+}-transport ATPase from erythrocytes with lower affinity than that of smooth muscle suggests that the two plasma-membrane Ca^{2+}-transport ATPases may differ by their phosphate-binding site or by the accessibility of this site to AlF_4^-. It has become apparent that indeed at least two plasma-membrane Ca^{2+}-transport ATPase isoforms exist (Shull & Greeb, 1988), as deduced from their nucleotide sequence. Michaelis *et al.* (1987) reported two distinct vanadate sensitivities of the Ca^{2+}-transport ATPases in synaptic plasma membranes, in which one Ca^{2+}-transport ATPase was 33 times more sensitive than the other (K_i 0.2 μM and 6.0 μM).

The tissue-dependent differences in sensitivity of the endo-(sarco-)plasmic-reticulum Ca^{2+}-transport ATPases to AlF_4^- is consistent with the observation that different isoforms of this ATPase are expressed in smooth and fast skeletal muscle as deduced from isoform typing by means of the phosphoprotein intermediate and antibodies (Wuytack *et al.*, 1989), and from Northern-blot analysis of smooth-muscle RNA (de la Bastie *et al.*, 1988). The discrepancy between the degree of inhibition of the ($Ca^{2+} + Mg^{2+}$)-ATPase activity and the formation of the

Ca^{2+}-induced phosphoprotein intermediate on the one hand and that of the inhibition of the ATP-dependent $^{45}Ca^{2+}$ uptake can be explained by the fact that these assays measure different aspects of the same parameter. Whereas the $^{45}Ca^{2+}$-transport studies only determine the $^{45}Ca^{2+}$ transport in closed inside-out vesicles, the $(Ca^{2+}+Mg^{2+})$-ATPase assay will measure the enzyme activity in closed inside-out vesicles and in open vesicles. A similar discrepancy has been described for the effect of agonists (Missiaen *et al.*, 1988*b*), and of inositol trisphosphate and guanosine 5′-[γ-thio]triphosphate (Kuo, 1988) on the plasma-membrane Ca^{2+}-transport ATPase.

In conclusion, AlF_4^- could be a useful tool for discriminating isoform diversity of both the plasma-membrane Ca^{2+}-transport ATPase, and the Ca^{2+}-transport ATPases of the endo-(sarco-)plasmic reticulum.

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