THE EFFECT OF ANTI-ATPase ANTIBODIES UPON THE Ca^{++} TRANSPORT OF SARCOPLASMIC RETICULUM

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Summary: Sheep or guinea pig antisera against the purified Ca^{++} transport ATPase of sarcoplasmic reticulum inhibit Ca^{++} transport due to a complement-dependent damage of the membrane, which causes massive leakage of Ca^{++}. The Ca^{++}-activated ATPase activity is only slightly affected even at ten times higher antibody concentration than that required for inhibition of Ca^{++} transport. Antibodies prepared against the Ca^{++} binding protein (C_1 protein) have no influence upon either ATPase activity or Ca^{++} transport and ferritin-labeled anti-C_1 antibodies do not bind to microsomes.

INTRODUCTION

The precise localisation of the Ca^{++} transport ATPase and the Ca^{++} binding proteins within the sarcoplasmic reticulum membranes is the subject of much current debate (1,2) with significant implications upon the mechanism of Ca^{++} translocation and the binding of accumulated Ca^{++} to the membrane.

We approached the problem using antibodies prepared against the purified Ca^{++} transport ATPase of sarcoplasmic reticulum (3) and the C_1 protein (4,5) and correlating the binding of antibodies to the membrane with their effect upon the ATPase activity and Ca^{++} transport.

METHODS AND MATERIALS

The Ca^{++} transport ATPase was purified from rabbit skeletal muscle according to MacLennan (3). The calcium binding protein (C_1-protein) was isolated either according to Duggan and Martonosi (4) by extraction of sarcoplasmic reticulum at pH 8.0 with 1 mM EDTA followed by DEAE-cellulose chromatography or as described by MacLennan and Wong (5).

Guinea pigs were immunized against the purified Ca^{++} transport ATPase, the calcium-binding protein or whole rabbit skeletal muscle microsomes by monthly intramuscular injections of 1-2 mg antigen dispersed in 0.9% NaCl and 0.01 M Na-phosphate buffer pH 7.0 mixed with an equal volume of Freund's complete adjuvant. Sheep were immunized in a similar manner using 2-4 mg antigen. The anti-ATPase antibodies of sheep serum were obtained after Na_2SO_4 precipitation (6) and DEAE cellulose chromatography (7) in the IgG fraction. The antibodies were further purified by adsorption on particles of the purified ATPase at 5^{o} C in a medium of 0.9% NaCl and 5 mM imidazole. After washing with 0.9% NaCl and 5 mM imidazole the anti-ATPase antibodies were eluted with 0.1 M glycine at pH 3.0. The labeling of purified anti-ATPase with ^{125}I was performed essentially as described by McConahey and Dixon (8).

Before measurement of their effect upon Ca^{++}-uptake or ATPase activity all sera were dialyzed against 0.9% NaCl-5 mM imidazole pH 7.3 and 0.1% Na-azide in order to remove interfering serum calcium. Ca uptake and ATPase activity were measured as described earlier (4).

For the Ouchterlony test the antigens were dissolved in 20 mM Na-phosphate buffer, pH 7.0 containing 2% Triton x-100 and used immediately. The agar gel (1%) contained 20 mM Na-phosphate pH 7.1 and 1% Triton x-100, with merthiolate added as preservative.

Ferritin labeled antibodies were prepared using either glutaraldehyde (9) or 2,4-toluene diisocyanate (10) as crosslinking agents and their binding to microsomes was studied by electron microscopy after repeated washing with 0.9% NaCl, 5 mM imidazole and 0.10% sodium azide by centrifugation at 7,000 g for 20 minutes.

RESULTS

The effect of anti-ATPase serum on the Ca^{++} transport and ATPase activity of fragmented sarcoplasmic reticulum

Immune sera produced in guinea pigs (Fig. 1) or in sheep (not shown) against the purified Ca^{++} transport ATPase inhibited the oxalate-potentiated

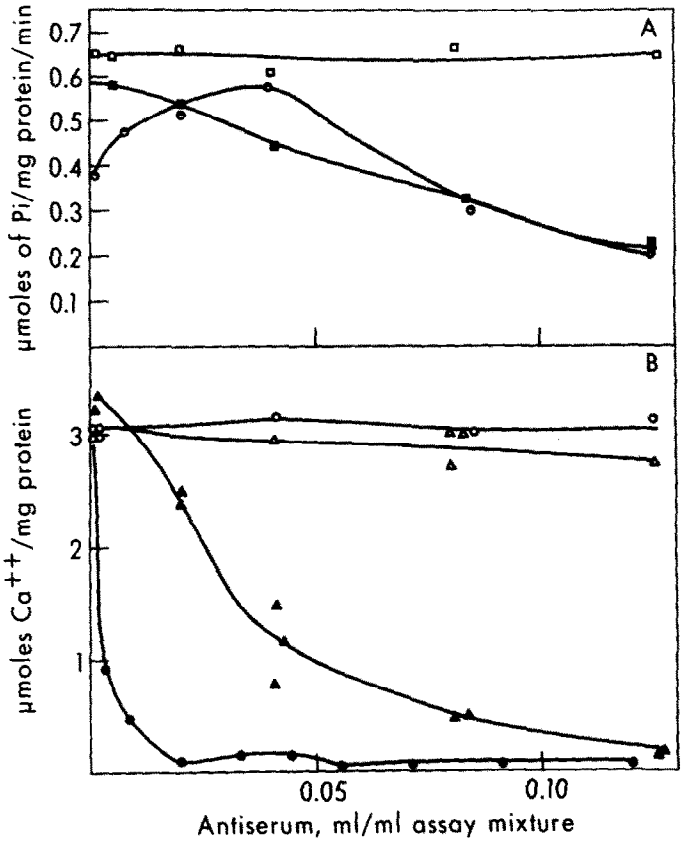

Fig. 1. Effect of guinea pig anti-ATPase sera upon the ATPase activity and Ca^{++} transport of rabbit sarcoplasmic reticulum

For details see Methods.
A. ATPase activity: □ -- □, control guinea pig serum; ■ -- ■ guinea pig anti-ATPase serum; ⊖--⊖ guinea pig anti-ATPase serum with 0.1 ml control guinea pig serum as the source of complement.
B. Ca uptake: O--O control guinea pig serum; Δ--Δ heated guinea pig serum (56° C for 30 min); ▲--▲, guinea pig antiserum; ●--● guinea pig antiserum with 0.1 ml control guinea pig serum per ml.

Ca^{++} transport of rabbit sarcoplasmic reticulum in the presence of complement. The inhibitory effect on the Ca^{++} transport was abolished by exposure of the serum to 56° C for 30 minutes, which inactivates complement, and could be restored by the addition of serum obtained from nonimmunized guinea pigs. Even at antiserum concentrations 10-50 times greater than those required for inhibition of Ca^{++} transport the Ca^{++} activated ATPase activity of sarcoplasmic reticulum was only slightly affected. Anti-ATPase sera obtained in the early phase of immunization usually had no effect upon the ATPase

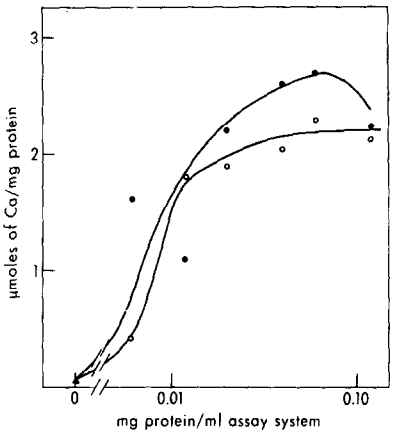

Fig. 2. Neutralization of guinea pig antimicrosome serum by preincubation with rabbit microsomes or with the purified ATPase

Guinea pig anti-microsome serum (0.04 ml/ml assay system) and guinea pig control serum (0.08 ml/ml assay system) were preincubated with heat inactivated (100° C for 10 min in water) rabbit sarcoplasmic reticulum O--O, or with purified ATPase (●--●) for 15 minutes at 25° C in the final concentrations indicated on the abscissa. Ca^{++} transport was measured as described under Methods.

activity. The inhibition of Ca^{++} transport in the presence of complement is noticeable already after 1 minute preincubation of microsomes with antisera. Addition of antisera and complement to microsomes which accumulated calcium in the absence of oxalate produced rapid Ca^{++} release.

The inhibitory effect of guinea pig anti-microsome or anti-ATPase serum upon the Ca^{++} transport is readily neutralized by preincubation of antiserum with purified Ca^{++} transport ATPase (Fig. 2) indicating that the principal antigen involved in the inhibition of Ca^{++} transport is the Ca^{++} transport ATPase; isolated microsomal phospholipids and the purified C_1 protein were ineffective. The complement dependence of the inhibitory effect on Ca^{++} transport indicates that the inhibition is not simply the result of the binding of anti-ATPase antibody to the transport ATPase but represents a form of complement-dependent membrane damage.

Similar inhibition of Ca^{++} transport was obtained with antisera whether the measurements were performed by Millipore filtration or by the ultra-

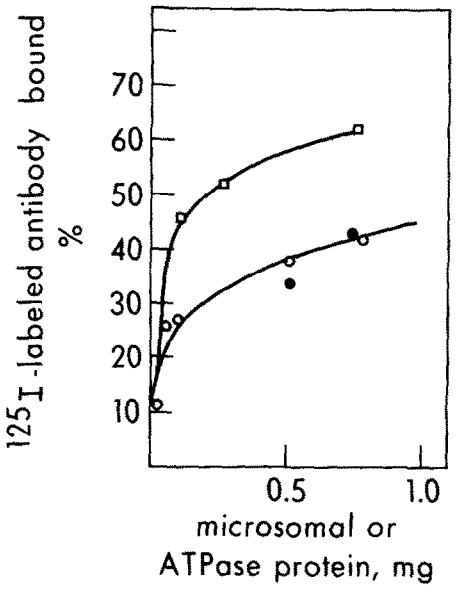

Fig. 3. The binding of ^{125}I-labeled purified sheep anti-ATPase IgG to microsomes and the purified Ca transport ATPase

Microsomes (O,●) or the purified ATPase (□) were incubated with ^{125}I-labeled purified anti-ATPase antibodies with (●) or without (O,□) guinea pig complement in a medium of 0.1 M KCl, 10 mM imidazole pH 7.3 for 10 minutes at 25° C in an assay system of 0.8 ml total volume. The amount of antibody was 0.1 mg in the microsome (O,●) and 0.05 mg in the ATPase experiment.

centrifuge technique indicating that the effect is not dependent upon the technique used for measurement of Ca^{++} transport.

Purification of sheep anti-ATPase antibody

The inhibitory effect on Ca^{++} transport was associated with the IgG fraction. Purified IgG immunoglobulins obtained from sheep anti-ATPase sera as described under Methods were labeled with ^{125}I and their binding to rabbit sarcoplasmic reticulum vesicles and to the purified ATPase was measured (Fig. 3). The inclusion of IgG immunoglobulins isolated from non-immunized animals had little or no effect on the antibody binding. The results indicate a reasonable access of the antigenic sites to anti-ATPase globulins in both purified ATPase and sarcoplasmic reticulum membranes. The maximum antibody:antigen weight ratio was 0.55 for microsomes both in the presence or absence of complement. The corresponding value for the purified

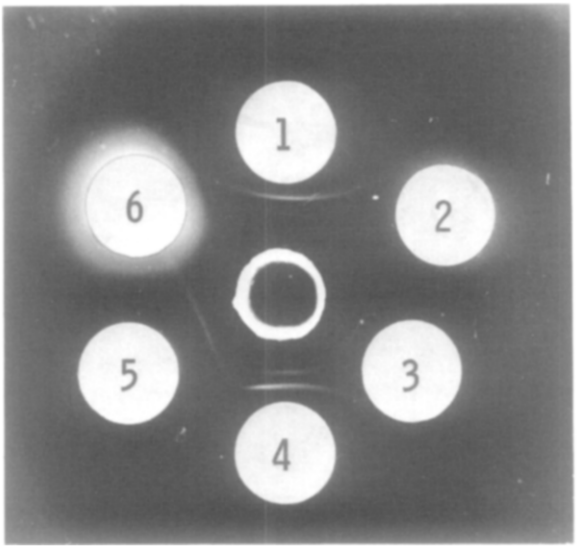

Fig. 4. Precipitin reactions of antisera with solubilized rabbit sarcoplasmic reticulum

Center well: solubilized rabbit sarcoplasmic reticulum; 1 and 4 guinea pig anti-microsome serum; 2. guinea pig anti-ATPase serum; 3. guinea pig anti-C_1 serum; 5. sheep anti-C_1 serum; 6. sheep anti-ATPase serum.

ATPase was 0.57 mg antibody per mg ATPase. Considering that about 70% of the protein content of sarcoplasmic reticulum is the Ca^{++} transport ATPase the similarity between these values is not surprising.

Antibodies against the Ca^{++} binding (C_1) protein

Guinea pig or sheep antisera against the C_1 protein of rabbit sarcoplasmic reticulum so far had no detectable influence upon either ATPase activity or Ca^{++} transport. On Ouchterlony-plates anti-C_1 sera gave a single sharp precipitin line with pure C_1 protein or with Triton solubilized whole microsomes (Fig. 4). Under the same conditions guinea pig antiserum against rabbit muscle microsomes gave multiple precipitin lines, only one of which resulted from reaction with the C_1 protein. With these sera a weak complement dependent lysis of rabbit erythrocytes was observed which may indicate the presence of antibodies directed against nonspecific membrane antigens, but the titer of these is very low. Anti-ATPase sera gave no precipitin lines with microsomes as antigens due to the limited diffusion of

the antigen. Ferritin labeled antibodies against the C_1 protein did not bind demonstrably to microsomes. Further work is necessary to decide whether this indicates inside localisation of the antigen within the vesicles or inaccessibility of the antigen located on the outside.

DISCUSSION

The increase in the Ca^{++} permeability of the membrane induced by anti-ATPase antibody in the presence of complement is similar in nature to the complement-dependent lesion of various artificial and natural membranes (11). It could serve as a model for studying the effect of local changes in membrane structure upon the rate of Ca^{++} release. Interaction between sarcoplasmic reticulum and other elements of the triad could play a role in the initiation of Ca^{++} release during excitation (12).

Binding of anti-ATPase antibodies to fragmented sarcoplasmic reticulum in the absence of complement did not effectively inhibit the Ca^{++} transport ATPase or the active Ca^{++} transport. This suggests that major changes in the orientation of the Ca^{++} transport ATPase, as envisaged in various carrier mechanisms, may not constitute an important feature of Ca^{++} translocation. The possibility of a gated-pore mechanism gains relevance in which the transport ATPase instead of serving as a mobile carrier regulates the permeability of a Ca^{++}-channel. Incorporation of the purified Ca^{++} transport ATPase into liposomes increases the passive Ca^{++} permeability of the membrane several thousand-fold (13).

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