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Inhibition of myosin ATPase activity by human myasthenia gravis antibodies reactive with the acetylcholine receptor

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Antibodies, obtained from myasthenia gravis patients, with reactivity for an immunodominant region of the nicotinic acetylcholine receptor were found to also react with muscle myosin. Since amino acid sequence analyses have previously suggested possible serological relationships between AChR and a head region sequence of myosin heavy chain, cross-reactive antibodies were examined for their ability to interfere with ATPase activities associated with this region of myosin. Results indicated that AChR-specific antibodies purified from MG patient serum by binding to and elution from antigen columns were found to inhibit Ca^{2+}-dependent, myosin-associated ATPase activity; interestingly, this inhibition appeared to be relatively selective in that neither K^+(EDTA)-dependent nor Mg^{2+}-dependent ATPase activities were sensitive to antibody-mediated interference.

Acetylcholine receptor antibody; Acetylcholine receptor/myosin cross-reactivity; ATPase inhibition; Myosin ATPase; Main immunogenic region

1. INTRODUCTION

Serum from myasthenia gravis (MG) patients has long been known to contain antibodies that react with the junctional muscle receptor for the neurotransmitter, acetylcholine [1–3]; it has also been previously observed that the same patient serum often also contains reactivity to other protein antigens associated with striated muscle [4–9]. A recent report from this laboratory described antibody cross-reactivities that revealed an unexpected serological relationship between the acetylcholine receptor (AChR) and myosin [10]. Thus, it was shown that whether affinity purification of MG plasma was performed using Sepharose coupled with myosin or the α61–76 AChR α subunit peptide, the binding activities recovered from the plasma of MG patients included highly overlapping antibody populations. That is, the majority of anti-myosin antibodies and the majority of anti-AChR peptide antibodies were one and the same. Although the specificity of these antibodies for AChR was clearly directed toward a region previously characterized [11,12] as being highly immunogenic and frequently involved in antibody responses in MG patients (i.e. against the so-called Main Immunogenic Region [MIR] of the alpha subunit), except for inferences derived from short stretches of identity observed by the examination of amino acid sequence data, the region of the myosin molecule that provided the cross-reactive antigenic determinant was not clearly defined; however, amino acid sequence comparisons suggested that homologies associated with residues 101–107 of the myosin heavy chain might be involved. Since this region lies relatively near to both the actin binding site, residues 142–148 [13], as well as the ATP binding site, residues 178–185 [14,15], it became of interest to examine the ability of AChR-reactive antibodies to interfere with myosin enzymatic function.

Reported below are observations indicating that AChR-reactive (MIR-specific) antibodies obtained from the plasma of myasthenia gravis patients are capable of inhibiting myosin-associated ATPase activity. Four myosin-associated ATPase activities were examined that have been described [16–18] based on the ions and other co-factors required for the activation of enzymatic function. First, myosin-mediated cleavage of phosphate from ATP associated with F-actin-activated, Mg^{2+}-dependent ATPase appears to be the most physiologically relevant activity. However, under controlled conditions (such as carefully paying attention to effects on the oxidation state of two important sulfhydryl groups), myosin ATPase activities can also be stimulated by (1) Mg^{2+} (in the absence of actin), (2) Ca^{2+}, and (3) NH_4 or K^+(EDTA). Even though activation of the intrinsic ATPases under these latter circumstances is of unsure physiological significance, the inhibition study presented below exploited the multiple means for activating myosin ATPase when bound by particular antibodies in order to gain some insight into the fine-specificity of the binding activity present.

2. MATERIALS AND METHODS

2.1 *ATPase assays*

Microassays for inhibition of ATPase activity were performed with

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some modification of published methods [13,19] in triplicate in 96-well microtiter plates following the incubation of 1 μg samples of myosin with a range of dilutions of affinity-purified anti-AChR peptide or anti-myosin antibodies (final volume of 30 μl); incubations were performed at 4°C for 2 h. Subsequently, ATP as the disodium salt, was added to each sample to a final concentration of 0.5 mM; when required, 10 μg of actin was added. The ATPase reactions were initiated by the addition of 10 μl (50 μl) of one of the following reaction buffers: (A) For Ca^{2+}-dependent ATPase, Tris-HCl (100 mM, pH 7.6), $CaCl_2$ (5 mM), KCl (660 mM); (B) for K^+(EDTA)-dependent ATPase, Tris-HCl (100 mM, pH 7.6), KCl (600 mM), EDTA (5 mM); (C) for Mg^{2+}-dependent ATPase, imidazole (20 mM, pH 7.0), 2 mM $MgCl_2$. Incubation times were chosen so that no more than 15% of the ATP was hydrolyzed and reaction rates were linear. Reactions were terminated by the addition of 200 μl of Malachite green reagent (see below) and the presence of liberated phosphate determined by measuring the absorbance of each sample at 630 nm using a Dynatech MR5000 ELISA reader; concentrations of phosphate were determined by comparison to phosphate standards prepared as 50 μl samples, representing concentrations of phosphate from 50–150 nM. The Malachite green reagent used in this assay was prepared by mixing the following stock solutions, respectively, in a 1:1:2:2 ratio: Ammonium molybdate, 5.72% (w/v) in 6 N HCl, polyvinyl alcohol, 2.32% (w/v) in water, Malachite green, 0.0812% (w/v) in water, glass-distilled, deionized water. Specific activities of myosin-associated ATPase under the three sets of conditions described above were determined as nmol phosphate released/mg myosin/min; data were sometimes converted to percent of uninhibited control. Negative control values (background), determined by the absorbance of samples lacking myosin, were subtracted from experimental values; this background of spontaneously hydrolyzed ATP routinely ranged from 0.5–1.0 nmol.

3. RESULTS AND DISCUSSION

Antibody binding was evaluated with regard to its effects on the enzymatic (ATPase) activity known to be localized in the head region of myosin [20,21]. Fig. 1 shows the consequences, on the Ca^{2+}-dependent ATPase associated with bovine myosin, of adding increasing amounts of two different preparations of myosin-reactive antibodies obtained from an MG patient characterized as a Class V myasthenic (severe generalized neuromuscular dysfunction) [10,22]. Antibodies present in plasma were obtained from this patient either by purification on myosin columns (Fig. 1, upper panel) or on α61–76 peptide columns (Fig. 1, lower panel). The rate of catalytic ATP hydrolysis was maximally inhibited by approximately 40–50%; moreover, the dose–responses of these inhibitions were virtually identical with regard to the two antibody preparations examined. In contrast to Ca^{2+}-activated ATPase activity, intrinsic Mg^{2+}-activated and K^+(EDTA)-activated ATPase activities appeared completely resistant to the inhibitory potential of both preparations of antibodies. This was also true when Mg^{2+} activation was more effectively performed in the presence of F-actin (not shown; see Table I). These percent inhibitions were derived from the specific activities summarized in Table I.

In searching for an explanation for the serological, and possible pathophysiological, relationships between myosin and AChR, we are left with the observation that only the ability of each of these proteins to interact with

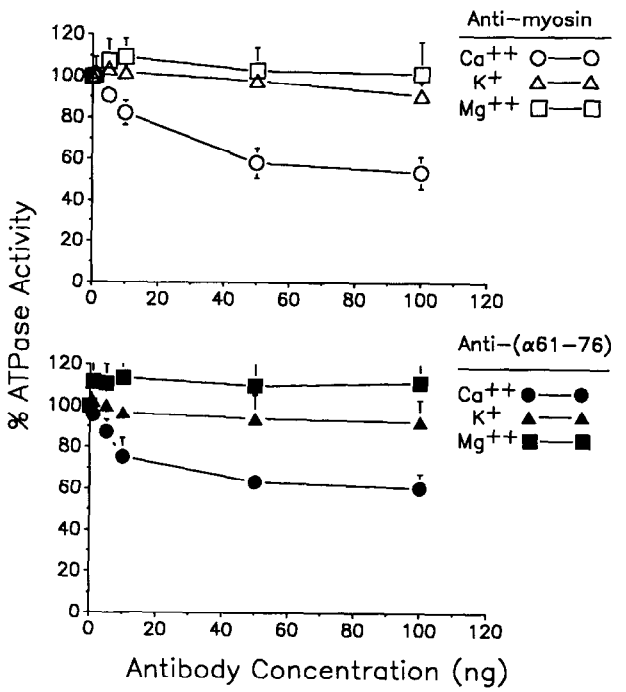

Fig. 1. Inhibition of intrinsic ATPase activities of bovine myosin by anti-myosin antibodies (upper panel) and anti-MIR peptide antibodies (lower panel). Each of these antibody preparations was obtained by affinity-purification of myasthenia gravis patient plasma on the appropriate antigen column; previous studies have demonstrated substantial cross-reactivity by these two sets of purified antibodies and their likely common B lymphocyte origins [10]. Results obtained from 3 individual assessments (each performed on triplicate samples) are given as the mean percents of ATPase activities (± S.E.M.) remaining after exposure to each antibody as a function of antibody concentration. Ca^{2+}-activated ATPase, (○,●); Mg^{2+}-activated ATPase, (□,■); K^+(EDTA)-activated ATPase, (△,▲). Maximum change in specific activities of ATPase in the presence of 2 μg/ml normal human Ig never exceeded 10%.

Ca^{2+} appears to weakly link the activities under study here. A similar structural region required for the binding of Ca^{2+} may therefore serve as the origin of serological cross-reactivity between AChR and myosin; that is, the purpose of the AChR, upon binding of acetylcholine, is to initiate muscle contraction by the translocation of Ca^{2+} across the muscle membrane. Interference of this AChR function (perhaps associated with or influenced by antibody binding to the MIR) could clearly impair neuromuscular transmission. Therefore, it may be no coincidence that the ATPase function of myosin that appears to be most directly, and apparently selectively, perturbed by antibody binding in this study is that which is dependent on interaction with Ca^{2+}. Although the contribution of the Ca^{2+} ATPase with regard to muscle contraction is unclear at this time, it is intriguing to consider that it might play an essential role in the energy dependent transportation of Ca^{2+} ions across the sarcolemma, analogous to the role played by the Ca^{2+}-dependent ATPase associated with ion influxes and effluxes across the sarcoplasmic reticulum [23].

Table I

Effects of antibody binding on the specific activities of myosin-associated ATPases

Antibody specificity[b]	ATPase activity[a]			
	Ca^{2+}	K^+(EDTA)	Mg^{2+}(actin$^-$)	Mg^{2+}(actin$^+$)
None	373 ± 6	443 ± 3	90 ± 6	317 ± 25
Anti-myosin	198 ± 36	411 ± 59	99 ± 27	346 ± 83
Anti-AChR (MIR)	193 ± 46	420 ± 67	137 ± 17	303 ± 110
Normal human Ig	349 ± 51	417 ± 20	N.D.[c]	N.D.

[a] ATPase activity is shown as specific activity (nmol inorganic phosphate released/mg myosin/min) activated under the conditions indicated. When indicated, actin was present at 200 μg/ml. Each individual 50 μl microassay of ATP hydrolysis was performed triplicate on each sample; values shown are mean specific activities (± S.E.M.) obtained from 3 independent assessments. Details are given in Fig. 1.

[b] Antibodies tested for their ability to inhibit ATP hydrolysis were either obtained, as indicated, from myasthenia gravis serum by affinity purification on columns coupled with myosin or columns coupled with the MIR-containing AChR peptide (α61–76). Resulting effects on ATPase activity are shown in comparison to enzyme activities in the absence of any antibody (None), or in the presence of the immunoglobulin fraction of human serum obtained from a healthy (non-myasthenic) individual. In all cases, antibody concentrations were 2 μg/ml.

[c] Not determined.

On the other hand, AChR and myosin play very different functional roles in muscle contraction (i.e. receptor and ion channel vs. enzyme and structural element of the contractile machinery, respectively), have different cellular locations (i.e. extracellular vs. intracellular), and do not have any apparent biochemical/structural similarity. Furthermore, the most clearly physiologically relevant form of the enzyme, F-actin-activated Mg^{2+} ATPase, appears to be resistant to the inhibitory effects of the antibodies tested. Therefore, even if mechanisms existed for getting these myasthenogenic antibodies to their cross-reactive cytoplasmic myosin target, no effects on Mg^{2+}/F-actin ATPase would be predicted. Taking these structural considerations together with the ATPase inhibition results makes the explanation for the potential importance of anti-AChR antibodies in MG patients that can also bind myosin totally unclear at this time. However, one must take this observed cross-reactivity into account when attempting to determine the etiology of the production of anti-AChR antibodies in MG patients, and particularly, the explanation for the immunodominance displayed by the MIR.

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