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Characterization of Ganglionic Acetylcholine Receptor Autoantibodies

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Abstract

In myasthenia gravis (MG), autoantibodies bind to the $\alpha 1$ subunit and other subunits of the muscle nicotinic acetylcholine receptor (AChR). Autoimmune autonomic ganglionopathy (AAG) is an antibody-mediated neurological disorder caused by antibodies against neuronal AChRs in autonomic ganglia. Subunits of muscle and neuronal AChR are homologous. We examined the specificity of AChR antibodies in patients with MG and AAG. Ganglionic AChR autoantibodies found in AAG patients are specific for AChRs containing the $\alpha 3$ subunit. Muscle and ganglionic AChR antibody specificities are distinct. Antibody crossreactivity between AChRs with different α subunits is uncommon but can occur.

Keywords

autonomic; myasthenia; neuronal; acetylcholine receptor; immunoprecipitation; subunits; epibatidine; bungarotoxin

1. Introduction

Nicotinic acetylcholine receptors (AChRs) are a family of ligand-gated cation channels found throughout the central and peripheral nervous system. Every nicotinic AChR is formed by the association of five subunits of which at least two are α subunits. The α subunit contains important binding sites for acetylcholine.

The muscle-type AChR mediates neuromuscular transmission, and antibodies against the muscle AChR cause the characteristic defect in neuromuscular junction transmission and fatigable weakness in patients with myasthenia gravis (MG) (Drachman, 1994). Neuronal nicotinic AChRs are formed from a variety of subunits homologous to those in muscle AChRs. Many of the common monoclonal antibodies against muscle-type AChR recognize both muscle and neuronal nicotinic AChRs. Prior studies have defined a main immunogenic region (MIR) of the muscle AChR $\alpha 1$ subunit which is important for antibody binding (Tzartos et al.,

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1998; Tzartos and Lindstrom, 1980). Rat monoclonal antibodies to the MIR compete with MG patient autoantibodies for binding to muscle AChR but bind to distinct epitopes (Lindstrom et al., 2008). The MIR resides in the N-terminal extracellular domain of the AChR $\alpha 1$ subunit, and all AChR subunits have homologous amino acid sequences in this region. Although antibodies directed against the $\alpha 1$ subunit appear to be most important, MG patients may also have autoantibodies that bind to the $\beta 1$, γ , δ , and ϵ subunits of muscle AChRs (Kostelidou et al., 2007; Ragheb et al., 2005; Sideris et al., 2007).

Neuronal AChR serve many functions in the nervous system. In the peripheral autonomic nervous system, the ganglionic nicotinic AChR mediates fast synaptic transmission in all peripheral autonomic ganglia (sympathetic, parasympathetic and enteric ganglia). AChRs on autonomic neurons are typically composed of two $\alpha 3$ subunits in combination with three other AChR subunits. Although autonomic ganglia neurons can express numerous neuronal AChR subunits, including $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 7$, $\beta 2$, and $\beta 4$, the properties of the AChR at mammalian ganglionic synapses are most similar to AChRs formed by $\alpha 3$ and $\beta 4$ subunits (Skok et al., 1999). Transgenic mice lacking the $\alpha 3$ subunit have profound autonomic failure with prominent bladder distention, gastrointestinal dysmotility and lack of pupillary light reflexes indicating that the $\alpha 3$ subunit is required for ganglionic neurotransmission (Xu et al., 1999a).

Autoimmune autonomic ganglionopathy (AAG) is an acquired neurological disorder characterized by diffuse autonomic failure. Up to 50% of patients with the acute or subacute form of this disorder have high levels of autoantibodies that bind to neuronal ganglionic AChR (Vernino et al., 2000). The clinical features of AAG include orthostatic hypotension, inability to sweat, reduced lacrimation and salivation, bowel disturbances (ileus, abdominal colic, diarrhea, and constipation), atonic bladder, impotence, and a fixed heart rate. The constellation of tonic pupils and gastrointestinal dysmotility in the setting of severe orthostatic hypotension is suggestive of AAG (Klein et al., 2003). Serum ganglionic AChR antibody levels in AAG correlate with the severity of autonomic neuropathy clinically and with the severity on laboratory testing of autonomic function (Klein et al., 2003; Vernino et al., 2000). A decrease in antibody levels is associated with improvement in autonomic function (Vernino et al., 2000). Plasmapheresis to remove autoantibodies can produce a dramatic improvement in autonomic function in some cases (Gibbons et al., 2008; Schroeder et al., 2005).

Experimental AAG can be induced in animals either by active immunization with peptides derived from the ganglionic AChR $\alpha 3$ sequence or by passive transfer of IgG from patients with AAG (Vernino et al., 2004; Vernino et al., 2003). Additionally, in vitro studies show that IgG from AAG patients will reduce AChR current in cultured IMR-32 neuroblastoma cells (Wang et al., 2007). Together, these clinical and experimental findings indicate that AAG is an antibody-mediated disease caused by antibodies against ganglionic AChR.

Although muscle and ganglionic AChRs are structurally very similar, patients with AAG typically do not have weakness or other clinical features of MG. Patients with MG do not have prominent autonomic dysfunction. The exceptions are rare patients with an overlap syndrome of myasthenia with subacute autonomic failure often associated with thymoma (Vernino et al., 2001). Previous serological studies have shown little cross-reactivity between muscle and ganglionic AChR antibodies in patients with MG or AAG (Vernino et al., 1998). However, many of the MG patients examined in previous studies had been treated with immunosuppressive medications which may have modified their antibody profile.

The N-terminal extracellular domain of the $\alpha 1$ subunit is the putative location of autoantibody binding for many AChR antibodies in MG. The analogous region of the $\alpha 3$ subunit is quite similar, yet ganglionic and muscle AChR antibodies rarely co-exist. Ganglionic AChR antibodies could achieve specificity by recognizing unique sequences of the $\alpha 3$ subunit or by

binding to other components of the ganglionic AChR (such as the $\alpha 5$, $\beta 2$ or $\beta 4$ subunits, or even the $\alpha 7$ AChR present in ganglionic neurons and also in IMR-32 cells). Some patients with subacute autonomic failure do not have antibodies against the ganglionic AChR in IMR-32 cells but might harbor antibodies against one of the other AChR subunits expressed in autonomic ganglia. Differences in antibody specificity could potentially underlie some of the clinical differences between patients. The aim of this study was to confirm the clinical specificity of ganglionic AChR for AAG in a patient population different from previous studies and to characterize the subunit specificities of autoantibodies to ganglionic AChRs.

2. Materials and Methods

2.1 Clinical material

The collection and testing of serum samples was approved by the Institutional Review Board at UT Southwestern Medical Center (protocol 092004-041). With informed consent, serum was collected from patients with autoimmune autonomic ganglionopathy or related neurological disorders. The serum samples used in this study were collected between 2004 and 2007 and represent a different patient group than previous serological studies (Vernino et al., 1998; Vernino et al., 2000). Some individual patients have been described in published case reports (Gibbons et al., 2008; Schroeder et al., 2005). For purposes of this study, AAG was defined by the subacute onset of diffuse autonomic failure (sympathetic, parasympathetic and enteric function) and the lack of motor, sensory or central nervous system signs or symptoms.

Deidentified serum samples from patients with myasthenia gravis were collected as part of a recently completed treatment trial of mycophenolate mofetil (Muscle Study Group, 2008). All patients met strict clinical criteria for MG, all were seropositive for muscle AChR antibodies, none had thymoma, and none had been treated with immunosuppressive medications or other immunomodulatory therapies. The use of these specimens was reviewed and approved by the steering committee of the mycophenolate study.

Patients with other related neurological diseases (OND) included peripheral nerve hyperexcitability (11), idiopathic painful neuropathy (7), amyloidosis with neuropathy (3), multiple system atrophy (4), Parkinson's disease with dysautonomia (3), paraneoplastic sensory and autonomic neuropathy (6), limbic encephalitis (6), and Lambert-Eaton syndrome (2). Serum from these patients was collected at UT Southwestern Medical Center.

2.2 Cell culture

Transfected human embryonic kidney (HEK) tsA201 cells were maintained in culture medium consisting Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. This study utilized cell lines stably transfected with human $\alpha 3\beta 2$, $\alpha 3\beta 4$, $\alpha 4\beta 2$, or $\alpha 3\alpha 5\beta 4$ neuronal AChR subunits. These cell lines express functional membrane AChRs with physiological properties consistent with their subunit composition (Kuryatov et al., 2005; Nelson et al., 2001; Wang et al., 1998). Cytotoxic selection antibiotics were added to the media to ensure selection of transfected cells with AChR subunit expression (Nelson et al., 2001). TE671 cells were used as a source of muscle-type AChR, and IMR-32 cells were used as a source of heteromeric ganglionic AChRs and homomeric $\alpha 7$ AChRs (Vernino et al., 1998). These two cell lines were obtained from American Type Culture Collection (Manassas, VA).

2.3 Preparation of solubilized AChR from xenograft tumors

Athymic nude mice were obtained from Harlan (Indianapolis, IN). Each mouse was injected subcutaneously over the flank with approximately 10^7 cultured cells suspended in 1 ml of sterile media. When tumors grew to be 1.5 to 2 gram in size, the mice were euthanized and

tumors were removed, cut up, frozen in liquid nitrogen and stored at -80 degrees. Several tumors from each cell line were collected. Frozen tumors were homogenized and centrifuged to remove soluble components. The membrane fractions were subsequently solubilized in 2% Triton X-100 as previously described (Vernino et al., 1998; Vernino et al., 2002). Solubilized tumor membranes were snap frozen in liquid nitrogen and stored at -80°C . The presence of AChR antigen in the membrane preparations was confirmed by binding of radioactive epibatidine or α -bungarotoxin. The concentration of specific agonist binding sites was determined by a glass fiber filtration assay as previously described (Vernino et al., 2002). The yield of epibatidine binding sites (AChR) in the tumors derived from transfected cells ranged from 500 to 2,500 fmol per gram of tumor, similar to the yield from IMR-32 xenograft tumors. The concentration of bungarotoxin binding sites in IMR-32 or TE671 tumors were lower (150 to 200 fmol/gm). For each AChR type, preparations with the highest concentration of AChR were used in the immunoprecipitation assay. The volume of solubilized tumor extract used in each assay was determined according to the antigen concentration in order to produce a final concentration of 50pM of radioligand-AChR complexes in the final 300 μl immunoprecipitation reaction.

2.4 Immunoprecipitation Assays

Antibody assays were performed as described previously (Lennon et al., 2003; Vernino et al., 2002; Vernino et al., 2000). Solubilized AChR antigens were radiolabeled with 2 nM ^{125}I -epibatidine (for the heteromeric neuronal AChR cell lines) or 25 nM ^{125}I - α -bungarotoxin (for muscle AChR and homomeric $\alpha 7$ AChR) (Lennon et al., 2003; Vernino et al., 1998). The radiolabeled AChR preparation (approximately 15 fmol of AChR-radioligand complexes) was incubated with 5 μl of serum at 4°C overnight. The following morning, goat immune serum (against human IgG for patient sera, against rabbit IgG for rabbit sera, or against rat or mouse IgG for monoclonal antibodies, Equitech-Bio Inc., Kerrville, Texas) was added as secondary antibody. After 30 minutes at room temperature, 20 μl of 20% polyethylene glycol was added to each tube. After one hour further incubation at 4°C , immune complexes were precipitated by centrifugation. Pellets were resuspended in wash buffer (containing 0.1% Tween-20 and 0.7% PEG) and centrifuged twice more. Radioactivity in the washed pellet was measured in a gamma counter, and the antigen binding capacity of the serum sample was calculated in terms of moles of radioligand-AChR complexes bound per liter of serum.

Samples were always tested in duplicate, and positive and negative control samples of human and/or rabbit sera were included in each assay. The threshold for positive results was set at 0.05 nmol/L (based both on prior control data and on results from 20 additional healthy control serum used in this study)(Vernino et al., 2000). Positive results were confirmed and titrated so that less than 50% of the total AChR was bound under the conditions used to calculate the final binding capacity of the serum.

2.5 Electrophysiology

For electrophysiological recording, cells were plated on glass coverslips as described previously (Wang et al., 2007). Cells expressing $\alpha 3\beta 2$ AChR were exposed to 100 μM nicotine overnight prior to testing to increase the expression of surface AChR (Wang et al., 1998). AChR currents were obtained using conventional whole-cell patch-clamp techniques. Patch electrodes with a resistance of 5 to 8 M Ω were formed from borosilicate glass and filled with a solution containing (in mM): 140 cesium-methanesulfonate, 10 CsCl, 10 EGTA, 4 Na_2ATP , and 10 HEPES, adjusted to pH 7.2 with CsOH. Extracellular recording solution containing of (in mM) 150 NaCl_2 , 5 KCl, 1 MgCl_2 , 2 CaCl_2 , 5 HEPES, adjusted to pH 7.3 with NaOH. Solutions were applied to the cells by gravity-fed glass tubes connected to multiple reservoirs mounted above the recording chamber and moved by a computer controlled micromanipulator (Fast-Step, Warner Instrument Co Hamden, CT). Nicotinic AChR currents were recorded at a

holding potential of -70 mV and evoked by rapid application of the nicotinic against 1,1-dimethyl-4-phenylpiperazinium (DMPP; 50 μ M) for 4 seconds. Experiments were performed at room temperature (22 $^{\circ}$ C). Whole-cell membrane currents were recorded with an Axopatch 700B (Axon Instruments Inc., Foster City, CA, USA), and analyzed using PCLAMP software (version 9.2, Axon Instruments Inc.).

Currents in transfected HEK cells or IMR-32 cells were not affected by α -bungarotoxin and were inhibited by chlorisondamine and hexamethonium consistent with heteromeric neuronal AChR (data not shown). After recording stable baseline AChR currents, IgG was added to the solution perfusing the bath at a concentration of 1 mg/ml. The whole-cell AChR subunits currents were recorded after 2 minutes, 5 minutes, and subsequently every 5 minutes throughout the experiment. IgG was purified from patient plasma by protein A adsorption.

3. Results

3.1 Clinical specificity of AChR antibodies

The frequencies of autoantibodies to ganglionic and muscle AChRs in several clinical groups are shown in Table 1. Antibodies to ganglionic AChR were found in 23 of 32 patients with typical clinical features of subacute AAG. Most of these patients (18/23) had relatively high levels of binding activity (>1.0 nmol/L). The aim of this study, however, was to examine specificity and not the diagnostic sensitivity of ganglionic AChR antibodies in AAG. Autoantibodies to ganglionic AChR were not found in healthy control subjects or in patients with other autonomic or autoimmune neurological disorders (OND). Among 74 patients with MG who had autoantibodies to muscle AChR, autoantibodies to ganglionic AChR were detected in two (3%). None of these MG patients had been exposed to immunomodulatory therapy, and none had thymoma. The findings in this cohort of well-characterized MG patients is similar to results from our previous study (Vernino et al., 1998) indicating that immunosuppression did not significantly influence antibody specificity. Since the MG samples were deidentified, additional clinical information on the two patients with ganglionic AChR antibodies was not available.

Autoantibodies to muscle AChR were found in all MG patients in this study (by definition) but were found in only one patient with AAG. That AAG patient was carefully evaluated and had no clinical or electrophysiological evidence of MG and no radiological evidence of thymoma. Low levels of autoantibodies to muscle AChR were also found in two OND patients with clinical features of cramp-fasciculation syndrome, a disorder of peripheral nerve hyperexcitability (Table 1) (Vernino and Lennon, 2002).

In the three patients with autoantibodies to both muscle and ganglionic AChR antibodies, the antibody levels did not correlate. The AAG patient had a high level of ganglionic AChR antibody (11.9 nmol/L) and a much lower level of muscle AChR antibody (0.72 nmol/L). In the two MG patients, the levels of muscle AChR antibody (25.3 and 0.77 nmol/L) were much higher than the levels of ganglionic AChR antibody (0.24 and 0.12 nmol/L).

3.2 Subunit specificity of ganglionic AChR antibodies

To characterize the specificities of ganglionic AChR antibodies, AAG patient sera were tested for binding to neuronal AChRs with various subunit compositions. Each immunoprecipitation assay was performed in the same way except for the identity of the specific AChR antigen. Characterized specific rabbit polyclonal antibodies (AbCam, Cambridge, MA) and monoclonal mouse or rat antibodies (mAb) against various AChR subunits were tested to confirm the selectivity of the immunoprecipitation assay. The results of these control experiments (Table 2) indicate that these subunit-specific immunoprecipitation assays can accurately identify

subunit specificity. Antibodies specific for $\beta 2$ subunit bound to all AChR from transfected cell lines expressing $\alpha 3\beta 2$ or $\alpha 4\beta 2$ but to only 30% of AChR from IMR-32 cells. Although the predominant ganglionic AChRs in IMR-32 cells are $\alpha 3\beta 4$, some contain $\beta 2$ subunits (Nelson et al., 2001; Xu et al., 1999b).

The specificity of serum antibodies from AAG patients and EAAG rabbits are shown in Figure 1 and Table 3. All sera with autoantibodies to ganglionic AChR antibodies in IMR-32 cells (23 AAG patients and 8 EAAG rabbits) also showed binding to $\alpha 3\beta 4$, $\alpha 3\beta 2$ and $\alpha 3\beta 5\beta 4$ AChR. A minority of AAG patients (7 out of 23) also had antibodies against either $\alpha 4\beta 2$ or $\alpha 7$ AChR (Table 3). Immunized EAAG rabbits were more likely than AAG patients to have antibodies against $\alpha 4\beta 2$ AChR even though these rabbits were initially immunized with a peptide corresponding to the N-terminal region of the $\alpha 3$ subunit. Homologies between AChR subunits explains how antibodies raised against an $\alpha 3$ peptide could cross react with $\alpha 4$ or $\beta 2$ subunits.

Compared to native ganglionic AChR in IMR-32 cells, the human AAG patient sera generally bound less well to the three recombinant $\alpha 3$ AChR expressed in transfected HEK cells (Figure 1B). Only one of the AAG patients showed equivalent antibody levels in all four assays, and that patient also had low levels of antibodies to $\alpha 4\beta 2$ and $\alpha 7$ AChRs. All the EAAG rabbit sera bound equally well to all $\alpha 3$ -containing AChR.

The level of $\alpha 4\beta 2$ antibodies in the few seropositive patients and rabbits were very low (only 6–8% of the ganglionic AChR antibody level). Likewise, $\alpha 7$ AChR antibody levels (when present) were also low (< 0.5 nmol/L). The $\alpha 7$ AChR antibody levels, however, can not be directly compared because a different radioligand was used. Also the homomeric $\alpha 7$ AChR has more potential binding sites for radioligand which may lead to an overestimation of $\alpha 7$ AChR antibody binding.

None of the 9 patients lacking ganglionic AChR antibodies had antibodies against any other neuronal AChR type. Two control rabbits, which were immunized with $\alpha 3$ antigen but did not produce ganglionic AChR antibodies, were also negative in all other AChR antibody assays. None of the healthy control subjects had antibodies against any of the neuronal AChR subtypes. Among the group of patients with other neurological diseases (OND), none had antibodies against neuronal $\alpha 3$ or $\alpha 4$ -type AChR. Only one patient (with non-paraneoplastic limbic encephalitis) was positive for a low level of $\alpha 7$ antibodies (0.25 nmol/L).

3.3 Functional effects of ganglionic AChR antibodies

IgG from AAG patients and from rabbits with experimental AAG can produce a progressive decrease in AChR current in IMR-32 cells (Wang et al., 2007). To determine if the subunit specificity of these antibodies was reflected in specificity of their physiological effects, whole cell AChR currents in transfected HEK cells were recorded during exposure to IgG from three AAG patients. By immunoprecipitation, these three patients had antibodies against all $\alpha 3$ AChRs but not against $\alpha 4\beta 2$, $\alpha 7$ or muscle AChR. IgG from all three patients produced a reduction in $\alpha 3$ -type AChR current amplitude within 20 minutes of addition to the bath solution (Figure 2). At the same concentration, IgG from control patients had no effect on AChR current.

The effect on current amplitude was similar for $\alpha 3\alpha 5\beta 4$, $\alpha 3\beta 4$ and $\alpha 3\beta 2$ AChR currents and AChR currents in IMR-32 cells (20 – 35% reduction in current amplitude after 20 minutes). AAG patient IgG had no effect on the AChR currents in cells expressing $\alpha 4\beta 2$ AChR or on muscle-type AChR current in TE671 cells.

4. Discussion

Autoimmune autonomic ganglionopathy is an antibody-mediated neurological disorder. Many patients with AAG have antibodies that specifically recognize the $\alpha 3$ subunit of the ganglionic AChR. This specific binding leads to effects on $\alpha 3$ -type AChRs, perhaps including a reduction in surface AChR number as has been shown in MG (Sideris et al., 2007). Ganglionic AChR autoantibodies specifically reduce membrane current through AChR containing $\alpha 3$ subunits. Among patients with convincing clinical features of AAG who lacked antibodies to ganglionic AChRs, none had detectable antibodies against any other AChR subtype tested. Thus, if those patients have an antibody-mediated disorder of ganglionic transmission, the antibodies must be directed at a component of the ganglionic synapse other than the AChR.

Human autoantibodies to muscle AChR and those to ganglionic AChR are distinct with minimal cross reactivity between these AChRs. Only 3% of patients with MG or AAG had antibodies to both AChR types. On the other hand, rabbits immunized using a denatured N-terminal extracellular domain of the $\alpha 3$ subunit as antigen often develop antibodies to both ganglionic AChR and muscle AChR, as well as other neuronal nicotinic AChR types (Lennon et al., 2003). In some cases, the rabbits with both muscle and ganglionic AChR antibodies had clinical features of both MG and autonomic failure (Vernino, 2006). Rats with experimental autoimmune MG make some monoclonal antibodies to the MIR which also react with human $\alpha 3$ AChR (Wang et al., 1998) but not rat $\alpha 3$ AChR (Feng et al., 1998). Similarly, tolerance to $\alpha 1$ and $\alpha 3$ AChR appears to be quite discreet in humans. MG patients make autoantibodies to $\alpha 1$ AChR subunits but not to $\alpha 3$ subunits, and AAG patients make autoantibodies to $\alpha 3$ subunits but not to $\alpha 1$.

The neuronal AChRs in IMR-32 cells, as in autonomic neurons, are a mixture of $\alpha 3\beta 2$ and $\alpha 3\beta 4$ AChRs (some of which contain $\alpha 5$). Ganglionic AChR antibodies predominantly recognize the $\alpha 3$ subunit. The presence of $\alpha 5$ and the identity of the associated β subunit in the AChR did not have an important effect on antibody binding or on the antibody-mediated reduction in AChR current. However, serum antibodies from AAG patients had greater binding activity for the ganglionic AChR (as expressed in IMR-32 cells) compared to the recombinant AChR expressed in HEK cells. If most AAG patients produce a mixture of autoantibodies, some of which recognize $\alpha 5$, $\beta 2$ or $\beta 4$ subunits in addition to $\alpha 3$, the antibody binding would be higher using the mixture of AChR subtypes in IMR-32 extracts compared to binding to pure subtypes from transfected cell extracts. Alternatively, post-translational modifications of the AChR in different cell types could influence the binding of the human antibodies. Since the rabbit EAAG antisera were raised against a bacterially expressed denatured $\alpha 3$ extracellular domain, these antisera inevitably reacted equally well with all AChRs containing $\alpha 3$ subunits.

Although a few AAG patients had antibodies against other neuronal AChR ($\alpha 4\beta 2$ or $\alpha 7$), the binding levels of these antibodies were very low. This finding indicates that ganglionic AChR antibodies are quite specific for $\alpha 3$ -type AChR. Crossreactivity with other AChR α subunits, when present at all, is very weak. It is unlikely that circulating antibodies at low levels would reach the central nervous system or have significant pathophysiological effects. Unfortunately, there was insufficient material to directly test serum samples containing $\alpha 4\beta 2$ antibodies for functional effects on $\alpha 4\beta 2$ AChR current.

The data presented here regarding $\alpha 7$ AChR antibodies demonstrates the possibility of ganglionic AChR antibody cross-reactivity with the structurally-unique homomeric nicotinic AChR. However, detection of $\alpha 7$ AChR antibodies by immunoprecipitation can be problematic and will require further validation. While there are no clinical syndromes clearly associated with antibodies against central nicotinic AChR, $\alpha 7$ AChR antibodies were recently reported in two patients with Rasmussen encephalitis (Watson et al., 2005).

In both MG and AAG, major antigenic determinants for autoantibody binding reside within the N-terminal extracellular domain of α subunits. Even though various nicotinic AChR subunits are structurally similar, AChR antibodies are very specific for their respective AChR. Crossreactivity is uncommon and generally weak.

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Figure 1.

A) Neuronal AChR antibody levels for AAG patients (filled circles) and experimental rabbits with EAAG (triangles). B) Relative antibody levels were calculated by normalizing each antibody level to the value for ganglionic AChR antibody (in the same patient). The mean relative antibody levels ($\pm$ S.E.M.) are shown for each of the AChR subtypes. For $\alpha 3$ -type AChR, the data are from 23 patients with AAG and 8 seropositive experimental AAG rabbits. In the case of $\alpha 4\beta 2$, $\alpha 7$ and muscle AChR, the number above the data bar indicates the number of seropositive subjects. * $p < 0.005$ compared to IMR-32 assay (paired t-test).

Figure 2.

Antibody effects on AChR current. The whole cell AChR currents elicited by a 4 second application of 50 μ M DMPP are shown at baseline and 20 minutes after addition of IgG to the bath solution. IgG from an AAG patient with ganglionic AChR antibodies inhibits the current through all AChR containing the $\alpha 3$ subunit (A, B) but not current through $\alpha 4\beta 2$ (C) or muscle AChR (D, TE671 cells). IgG from a healthy control subject had no effect on any AChR currents (E, F).

Table 1
Clinical Specificity of Muscle and Ganglionic AChR antibodies

Diagnostic Category	No. of Patients	ganglionic AChR antibody			muscle AChR antibody, # (% positive)
		# (% positive)	Median (nmol/L)	Range (nmol/L)	
AAG	32	23 (72%)	5.36	0.29 – 40.83	1 (3%)
MG	74	2 (3%)	0.18	0.12, 0.24	74 (100%)
OND	42	0	0	0	2 (5%)*
Control	20	0	0	0	0%

* two positive OND were patients with cramp-fasciculation syndrome

Table 2

Validation of Neuronal AChR Antibody Assays *									
Antibody **	IMR-32	$\alpha 3 \beta 4$	$\alpha 3 \beta 2$	$\alpha 3 \alpha 5 \beta 4$	$\alpha 4 \beta 2$	$\alpha 7$	$\alpha 1$		
mAb 210	+++	+++	+++	+++	+	0	+++		
mAb 295 ($\beta 2$)	++	0	+++	0	+++	0	0		
mAb 299 ($\alpha 4$)	0	0	0	0	+++	0	0		
mAb 306 ($\alpha 7$)	0	0	0	0	0	+++	0		
rabbit $\beta 2$	++	0	+++	0	+++	0	0		
rabbit $\alpha 3$	+++	+++	+++	+++	0	0	0		
rabbit $\alpha 4$	0	0	0	0	+++	0	0		

* 0, no binding; +, <10% of total AChR; ++, 30–50% of total AChR; +++, 90–100% of total AChR bound

** mAb 210 binds to $\alpha 1$, $\alpha 3$ and $\alpha 5$ AChR subunits

Table 3

Subunit Specificity of AChR Antibodies in AAG

Group*	No. of patients	$\alpha 3\beta 4$	$\alpha 3\beta 2$	$\alpha 3\alpha 5\beta 4$	$\alpha 4\beta 2$	$\alpha 7$
AAG +	23	23 (100%)	23 (100%)	23 (100%)	3 (13%)	5 (22%)
AAG -	9	0	0	0	0	0
rabbit AAG +	8	8 (100%)	8 (100%)	8 (100%)	3 (38%)	4 (50%)
rabbit AAG -	2	0	0	0	0	0

* AAG + refers to subjects with serum autoantibodies to ganglionic AChR in IMR-32 cells