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Antigenic Structure of the Human Muscle Nicotinic Acetylcholine Receptor Main Immunogenic Region

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Abstract

The main immunogenic region on the $\alpha 1$ subunits of muscle nicotinic acetylcholine receptors provokes half or more of the autoantibodies in myasthenia gravis and its animal model. Many of these autoantibodies depend on the native conformation of the receptor for their ability to bind with high affinity. We mapped this region and explained the conformation-dependence of its epitopes by making chimeras in which sequences of human muscle $\alpha 1$ subunits were replaced in human neuronal $\alpha 7$ subunits or *Aplysia* acetylcholine binding protein. These chimeras also revealed that the main immunogenic region can play a major role in promoting conformational maturation, and, consequently, assembly of receptor subunits.

Keywords

Nicotinic acetylcholine receptor; Myasthenia gravis; Experimental autoimmune myasthenia gravis; Main immunogenic region; Acetylcholine binding protein; Monoclonal antibodies

Introduction

Nicotine acetylcholine receptors (AChRs) are formed from five homologous subunits organized like barrel staves around a central cation channel (Albuquerque et al. 2009). The primordial AChR is thought to have been homomeric, with five identical subunits, which through gene duplication evolved into a variety of subunits with basically similar structures. Neuronal $\alpha 7$ AChRs are homomeric. There are five ACh binding sites found at interfaces between the $\alpha 7$ subunits. Muscle AChRs are heteromeric. Adult muscle AChRs have the subunit composition and arrangement $(\alpha 1\delta)(\alpha 1\epsilon)\beta 1$, with two ACh binding sites, one between $\alpha 1$ and δ and the other between $\alpha 1$ and ϵ . ACh binding proteins (AChBPs) are water soluble homomeric proteins resembling in structure the extracellular domain of $\alpha 7$ AChRs (Brejc et al. 2001; Hansen et al. 2005). These are secreted by mollusc glia to modulate cholinergic transmission, but have been extremely valuable for structural studies because their crystal structures are easily obtained at high resolution, thereby defining the basic structures of the extracellular domains of subunits of AChRs and other receptors in their superfamily.

Myasthenia gravis (MG) is caused by an antibody-mediated autoimmune response to muscle AChRs (Lindstrom 2000; Conti-Fine et al. 2006). Experimental autoimmune MG (EAMG) can be induced by immunizing animals with AChRs from fish electric organs (Lindstrom 2000). Half or more of the autoantibodies to AChRs in MG and EAMG bind to the main immunogenic region (MIR) on $\alpha 1$ subunits (Lindstrom 2000; Tzartos et al. 1998). The MIR is

defined by the ability of mAbs to compete for binding to this region (Tzartos and Lindstrom 1980; Tzartos et al. 1982). mAb 35 is a classic example of a mAb to the MIR (Tzartos et al. 1982). mAbs to the MIR can passively transfer EAMG and exhibit the two primary activities which make autoantibodies pathologically significant in MG and EAMG: 1) they fix complement, which causes focal lysis of the postsynaptic membrane resulting in loss of AChRs and disruption of the architecture of the synapse; and 2) they crosslink AChRs, which causes antigenic modulation, an increase in AChR turnover that reduces the number of AChRs (Lindstrom 2000). The MIR is located at the extracellular tip of $\alpha 1$ subunits (Beroukhim and Unwin 1995). Amino acids contributing to the MIR have been mapped to the region $\alpha 1$ 66–76 (Tzartos et al. 1998). All AChR subunits have a homologous MIR loop (Albuquerque et al. 2009). For example, some mAbs made to muscle AChRs, e.g. mAb 210, react not only with muscle $\alpha 1$ subunits but also with neuronal $\alpha 3$, $\alpha 5$, or $\beta 3$ subunits (Wang et al. 1998; Kuryatov et al. 2008). For high affinity binding, most mAbs to the MIR depend on the $\alpha 1$ subunit being in its native conformation (Tzartos et al. 1998; Lindstrom 2000).

We made chimeras consisting of the $\alpha 1$ 66–76 MIR loop alone or in combination with other $\alpha 1$ subunit extracellular domain sequences replacing the corresponding parts of $\alpha 7$ subunits or AChBPs (Lindstrom et al. 2008). These chimeras enabled our discovery that the antigenic structure of the MIR in the native conformation of $\alpha 1$ subunits depends on both the MIR loop and its interactions with the N-terminal α helix and adjacent regions. Further, we discovered that the $\alpha 1$ MIR greatly promotes expression of chimeric $\alpha 1/\alpha 7$ AChRs, overcoming the intrinsic inefficient expression of wild type $\alpha 7$ AChRs in *Xenopus* oocytes. And we found that the MIR influences AChR function.

Results and Discussion

Mapping the Antigenic Structure of the MIR

The crystal structures of AChBP and $\alpha 1$ subunits indicate that the MIR loop at the extracellular tip of the subunit is paralleled by the N-terminal α helix on one side and the turn between β sheets 5 and 6 on the other (Brejc et al. 2001; Hansen et al. 2005; Dellisanti et al. 2007). This forms a structure angled out from the central axis of the AChR, as would be expected from the orientation of mAb 35 located bound to the MIR by electron crystallography and from the ability of mAb 35 and other mAbs to efficiently crosslink AChRs (Conti-Tronconi et al. 1981; Beroukhim and Unwin 1995).

Chimeras were constructed and characterized as described in Lindstrom et al., 2008. Examples of the data are shown in Figure 1. The chimera $\alpha 1(66-76)/\alpha 7$ expressed in *Xenopus* oocytes produced AChRs containing only the MIR loop. These could be immune precipitated by mAbs to the MIR such as mAb 6 or 210, which can bind both native and denatured $\alpha 1$. But this chimera bound poorly to mAb 198, which binds less well to denatured $\alpha 1$, and virtually not at all to mAb 35, which does not bind at all to denatured $\alpha 1$ on western blots. Increasing the loop region in the $\alpha 1(60-81)/\alpha 7$ chimera did not greatly increase binding of mAbs 35 or 198. A chimera with only the N-terminal α helix of $\alpha 1$ subunits, $\alpha 1(2-14)/\alpha 7$ did not form functional AChRs. However, a chimera with both the N-terminal sequence and the MIR loop, $\alpha 1(2-14, 60-81)/\alpha 7$ bound both conformation-dependent and conformation-independent mAbs to the MIR. Only longer sequences, $\alpha 1(1-32, 60-81)/\alpha 7$, allowed binding of a MG patient-derived mAb to the MIR (Luo et al. unpublished).

These results imply that the native structure of the MIR depends on both the MIR loop and its interaction with the N-terminal α helix. The N-terminal α helix from $\alpha 1$ expressed alone in $\alpha 7$ presumably could not form mature AChRs because the $\alpha 1$ terminal region could not interact with the $\alpha 7$ MIR loop sufficiently to stabilize a conformation which would permit subunits to

conformationally mature, assemble, and form functional AChRs. These results explain the conformation-dependence of binding of mAbs and autoantibodies to the MIR.

A similar $\alpha 1(1-32, 60-81)/\text{AChBP}$ chimera also bound all rat mAbs to the MIR and both EAMG and MG sera, as shown in Fig. 2. We previously reported that it did not bind well to MG patient sera (Lindstrom et al. 2008). However, subsequent studies with this chimera revealed binding to MG patient sera, antisera from both cats and dogs with MG, and an MG patient-derived mAb to the MIR (Luo et al., unpublished).

These data reveal that rats with EAMG and human, dogs, or cats with MG recognize the MIR in slightly different ways. The epitopes to which antibodies bind can be determined by a small number of amino acids, and binding can be prevented by changing a single amino acid, even though the area on a protein obscured by the binding of an antibody can be very large (Amit et al. 1985; Konstantakaki et al. 2007). This explains why mAbs and serum autoantibodies can be mutually competitive for binding to the MIR while recognizing discrete epitopes and conformations within this region.

Influence of the MIR on Expression and Function

$\alpha 1/\alpha 7$ chimeras expressed much more efficiently in *Xenopus* oocytes than did native human $\alpha 7$ AChRs (Lindstrom et al. 2008). $\alpha 1(60-81)/\alpha 7$ expressed 10 fold better than $\alpha 7$, and $\alpha 1(2-14, 60-81)/\alpha 7$ expressed 26 fold more than wild type $\alpha 7$.

These data suggest that the $\alpha 1$ MIR in the chimeras increases expression by improving conformational maturation. Synthesis of $\alpha 1$ subunits requires only 1 minute, but conformational maturation prior to assembly requires 30 minutes (Merlie et al. 1983). This conformational maturation was detected by the simultaneous acquisition of the ability of $\alpha 1$ subunits to bind both α bungarotoxin and mAb 35. A further 60 minutes are required for assembly of subunits into mature AChRs in the endoplasmic reticulum. Yet another 60 minutes are required for transport of the mature AChRs to the cell surface. $\alpha 7$ AChRs require special chaperones for assembly (Millar 2008). The chaperone RIC-3 greatly increases expression of $\alpha 7$ AChRs in cells which would not otherwise express $\alpha 7$ AChRs, but even then expression is not altogether efficient. It seems likely that, in chimeras, interaction between the $\alpha 1$ N-terminal α helix and the $\alpha 1$ MIR loop nucleates much more efficient conformational maturation of $\alpha 7$ subunits than occurs with $\alpha 7$ subunits in oocytes, which permits assembly of these subunits into mature AChRs much more efficiently. This hypothesis is consistent with the observation that the N-terminal α helix is required for assembly of mature pentameric AChRs both for $\alpha 7$ and heteromeric AChRs (Castillo et al. 2009).

MIR chimeras also influence conformation changes in mature AChRs (Lindstrom et al. 2008). The EC_{50} for activation of $\alpha 1(1-32, 60-81)/\alpha 7$ AChRs by ACh, 17.8 μM , indicates that these are 10 fold more sensitive to activation than is wild type $\alpha 7$, $\text{EC}_{50} = 182 \mu\text{M}$. Conversely, the $\alpha 1(2-14, 60-81)/\alpha 7$ chimera, $\text{EC}_{50} = 2353 \mu\text{M}$, is 13 fold less sensitive.

These data suggest that the MIR takes part in global conformation changes associated with activation of AChRs. The resting conformation of the $\alpha 1(1-2, 60-81)/\alpha 7$ chimera may be less stable than wild type and more easily triggered into the open channel conformation by binding of ACh. Conversely, the resting conformation of the $\alpha 1(2-14, 60-81)/\alpha 7$ chimera may be stabilized and less easily triggered into the open channel conformation.

Summary

$\alpha 1/\alpha 7$ chimeras permit mapping of the antigenic structure of the MIR. These reveal interactions between the N-terminal α helix and the MIR loop which explain the conformation-dependence of the antigenic structure of the MIR.

Future crystallographic studies of mAbs bound to $\alpha 1$ /AChBP chimeras might permit mapping of the antigenic structure of the MIR at atomic resolution.

The profound influence of the MIR on both the expression and function of $\alpha 1/\alpha 7$ chimeras suggests that the MIR at the extracellular tip of AChR subunits may have much greater roles in AChR conformation changes than previously appreciated. Interaction between the N-terminal α helix and the MIR loop may nucleate conformational maturation of the subunit, and this maturation may permit assembly of mature AChRs. The MIR may also influence global conformation changes in mature AChRs associated with activation and desensitization.

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Figure 1. Immunoprecipitation of human $\alpha 1/\alpha 7$ chimeras and an $\alpha 1/AChBP$ chimera by mAbs to the MIR. Data were derived from figures 2 and 5 in Lindstrom et al. 2008. Results were normalized to the binding of mAb 210.

Figure 2.

The MIR expressed in the α 1(1–32, 60–81)/AChBP chimera was recognized by MG patient sera. Sera from 7 MG patients and a pool of sera from rats with EAMG were determined by immune precipitation of ^{125}I α 1(1–30, 60–81)/AChBP. Radioimmunoassay was conducted as described in Lindstrom et al. 2008.