MYASTHENIA GRAVIS ASSOCIATED WITH NEUROPSYCHOSIS : SERUM AUTOANTIBODIES RECOGNIZE NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS

SATOSHI UENO
Department of Medical Genetics,, Nara Medical University
Received February 7, 1997

Abstract: The α_4 subunits of neuronal nicotinic acetylcholine receptor (nAChR) are widely expressed throughout the brain, and the α_1 subunits of muscle nAChR are localized at the muscle endplates. Most patients with myasthenia gravis (MG) are seropositive for anti-muscle nAChR antibody, and the peptide comprising residues 125-147 of the human α_1 subunit is myasthenogenic. We synthesized a peptide of the α_4 subunit counterpart of the α_1 125-147 to investigate cross-reactivity of neuronal nAChR with autoantibodies in MG patients. The synthetic peptide α_4 160-182 was injected into rabbits, and produced antibodies caused a loss of net muscle nAChR. Three myasthenic patients with neuropsychiatric symptoms had autoantibodies of which bindings to muscle nAChR were inhibited with anti-α_4 160-182 antibodies. The serum autoantibodies from these patients recognized the α_4 160-182.

Index Terms

myasthenia gravis, acetylcholine receptor, neuropsychosis, autoantibody

INTRODUCTION

Myashenia gravis (MG) is the best characterized, autoimmune disease in which autoantibodies to the peripheral nicotinic acetylcholine receptors (nAChR) impair the neuromuscular transmission[1-7]. While peripheral effects of MG have been well known, there is an increasing amount of data suggesting central cholinergic effects of MG[8]. MG patients have a higher incidence of seizure and psychiatric problems than do normal populations[9,10]. Lefvert and Priskanen reported that MG patients had anti-nAChR antibodies in both serum and cerebrospinal fluid[11]. The central effect of MG was more directly supported by animal experiments in which rabbits immunized with nAChR showed the finding of EEG abnormalities. EEG abnormalities were also induced by microinjection of human myasthenic serum into the caudata nucleus of the rabbits[12]. Whitehouse et al. demonstrated a 50 % reduction of nAChR in the brain from patients with Alzheimer's disease[13]. These facts suggest that the antibody-mediated loss of nAChR causes the transmission failure of central cholinergic systems in MG patients. Lewis et al., however, failed to demonstrate central cholinergic deficits in MG patients[14].

Among the members of a gene family of nAChR α subunit, the α_4 subunits are widely expressed throughout the thalamus, hypothalamus and cortex[15]. In contrast, the expression of the α_1 subunit is localized at the muscle endplate of neuromuscular junction. Most MG patients are seropositive for anti-α_1 subunit antibodies, and the synthetic peptide 125-147 of the α_1

subunit is shown to be myasthenogenic in humoral and cellural immune processes[16,17].

In this study, we synthesized the α_4 subunit counterpart of the α_1 125-147, and investigated the possibility that the α_4 subunit is a target for autoantibodies in MG patients.

PATIENTS AND METHODS

Measurement of anti-muscle nAChR sntibody titer

Test sera were obtained from 219 patients with MG. In all cases, diagnosis was made on a positive Tensilon test or decremental responses of compound muscle action potentials to repetitive motor nerve simulations. Clinical classification was according to the modified Osserman criteria[18]. Antibody titers were measured by radioimmunoassay and expressed as moles of ^{125}I α-bungarotoxin (αBGT)-muscle nAChR complexes per liter of serum according to the method of Lindstrom et al[19].

Antibody production against synthetic peptide α_4 160-182

The α_4 subunit cDNA fragment encoding the counterpart of the human muscle α_1 125-147 was amplified by reverse-transcriptase initiated PCR of human brain mRNA[15,20]. The primer sequences encoding the conserved regions between rat and chick α_4 subunits are shown in the

```
Chick α4   HVETRAHAEERLLKKLFSGYNKWSRP VANISDVVLVRFGLSIAQLIDVDEKNQMMTTNVWHDYKLR
Rat  α4    ·I···········R···········  ·G··································

WDPQEYENVTSIRIPSELIWRPDIVLYNNADGDFAVTHLT KAHLFYDGRIKWMPPAIYKSSCSIDVTFFPFDQQNCKMKF
···GD·····································  ·········VQ·T·····················T···
                          Primer S
GSWTYDKAKIDLVSMHSHVDQLDYWESGEWVIINAVGNYN SKKYECCTEIYPDITYSFIIRRLPLFYTINLIIPCLLISC
·············I··R·····F·········VD···T·· TR·····A·········A·····················
                                                        Primer a-S
LTVLVFYLPSECGEKITLCISVLLSLTVFLLLITEIIPST SLVIPLIGEYLLFTMIFVTLSIIITVFVLNVHHRSPRTHT
·············V·····························  ·····················V···············

MPDWVRRVFLDIVPRLLFMKRPSTVKDNCKKLIESMHKLT NSPRLWSETDMEPNFTTSSSPSPQSNEPSPTSSFCAHLEE
··A·····················V·····RR·······MA ·A··F·P·PVG··GIL//·DICN·GLS·A··FCNPTDTAV

PAKPMCKSPSGQYSMLHPEPPQVTCSSPKP/SCHPLSDTQ T/TSISKGRSLSVQQMYSPNKTEEGSIRCRSRSIQYCYLQ
ETQ·T·R··PLEVPD·KTSEVEKASPC·S·G··P·PKSSS GAPMLI·A·····HVP·SQEAA·DG··········VS·

EDSSQTNGHSSASPASQRCHLNEEQPQHKPHQCKCKCRKG EAAGTPTQGSKSHSNKGEHLVLMSPALKLAVEGVHYIADH
DGAALADSKPTS··T·LKARPSQLPVSDQASP···T·KEP SPVSPV·VLKAGGTKAPPQHLPL····TR·····Q·····

LRAEDADFSVKEDWKYVAMVIDRIFLWMFIIVCLLGTVGL FLPPWLAGMI
·K···T····································  ··········
```

Fig. 1. Alignment of amino acid sequence between chick and rat α_4 subunits. The primer sequences were based on the conserved amino acid sequence. C-C is predicted as an acetylcholine binding site. Primer S: 5'-GACATCGTCCT-CTACAACAATGCG-3', Primer a-S: 5'-GTTGATGGTGTAGAATAGCGG-3'

legend to Fig. 1. PCR run consisted of 25 cycles of denaturation at 94℃, annealing at 55℃ and extension at 74℃ for one min. for each step, and the nucleic acid sequence of the product was determined (Fig. 2). On the basis of the deduced residue sequence, nondisulfide-looped peptide of the human α_4 160-182 was synthesized. The numbering of residues is based on the published sequence of rat α_4 subunit[15]. 100 μg of the α_4 160-182 emulsified in complete Freund's adjuvant was injected into rabbits at three times at 30-60 day intervals. The antibody concentration of rabbit serum was measured by radioimmunoassay, using the α_4 160-182 labeled with ^{125}I. The rabbit IgG was purified by chromatography on DE-52 DEAE cellulose.

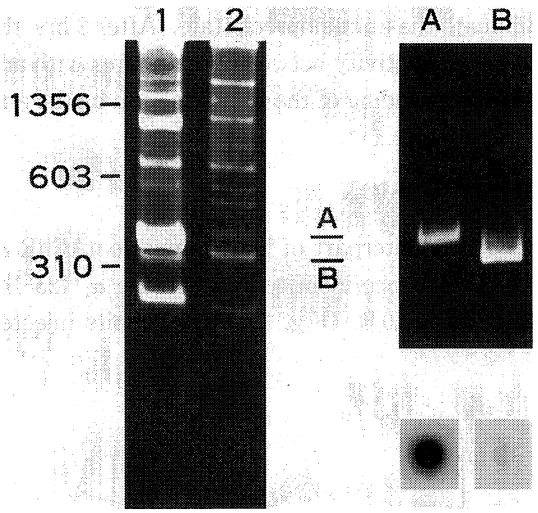

Fig. 2. PCR amplified fragment of rat brain cDNA (lane 1) and human brain cDNA (lane 2) in the left gel. Human brain amplified cDNA (indicated with A and B) were re-amplified and electrophoresed (right gel). As dot hybridization of re-amplified cDNA with rat α4 cDNA was positive for the lane A, the cDNA in this lane was cloned and sequenced.

Human α1 DLVLYNNADG DFAIVKFTKV LLQYTGHITW
Human α4 [ ]•• •••VTHL••A H•F•D•RVQ•

TPPAIFKSYC EIIVTHFPFD EQNCSMKLGT WTYDGSVVAI
••••Y•••S• S•D••F•••• Q•••T••F•S ••••KAKIDL

NPESDQPDLS NFMESGEWVI KESRGWKHSV TYSCCPDTPY
VSMHSRVDQL D•W••••••• VDAV•TYNTR K•E••AE/I•

LDITYHFVMQ RLPLYFYVN
P••••A•IIR ••[ ]

Fig. 3. Alignment of amino acid sequence of human α1 and α4 subunits

Degradation of muscle cell nAChR

Patient IgG and rabbit anti-α_4 160-182 IgG were incubated (concentrations of≤200 mg per ml) with cultured muscle cells of rat fetus limbs for 16 hrs at 37℃. The amount of nAChR remaining on muscle cell surfaces was measured by ^{125}I-αBGT binding, and percentage loss of ^{125}I-αBGT binding site was quantitated according to method of McCormick et al.[17].

Specificities of MG autoantibodies

Human muscle nAChRs (100 fmoles) labeled with ^{125}I-αBGT were mixed with a 20-fold excess of anti-α_4 160-182 IgG overnight. After 4 hr incubation of the mixture with serum (0.2 -2 μl) from MG patient, goat anti-human IgG was added to the reactions. Goat anti-human IgG was depleted of antibodies cross-reactive with rabbit IgG by preincubation with normal rabbit serum overnight and centrifugation to eliminate the immunoprecipitate. After 2 hrs, the reaction tubes were centrifuged. The difference in radioactivity between precipitates with and without the protecting IgG represented the inhibition of binding of the sample serum by the anti -α_4 160-182 IgG[21].

RESULTS AND DISCUSSION

We have determined the residues of the α_4 subunit counterpart of human muscle nAChR α_1 125-147 (KSYCEIIVTHFPDEQNCSMIKLG). The sequence identity between the α_1 125-147 and α_4 160-182 (KSSCSIDVTFFPFDQQNCTMKFG) is 70 % (Fig. 3). The rabbits injected

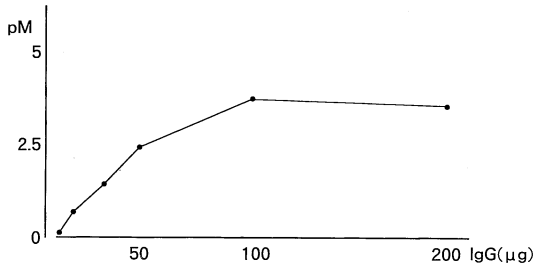

Fig. 4. Binding of anti-α4 160-182 IgG to muscle nAChR

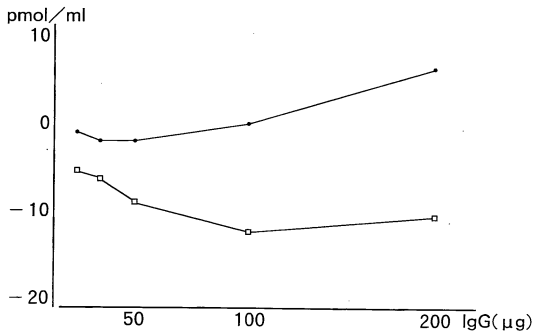

Fig. 5. Inhibition of myasthemic IgG-muscle nAChR bindings by preincubation of the receptors with anti α4 160-182 IgG (open square) and normal rabbit IgG (closed circle).

Table 1. Binding and antigenic modulation of antibodies to muscle nAChR and neuronal α_4 160-182

Immunogen	Antibody [nM] against nAChR (human/rat)	α_4 160-182	% loss[2]
Human nAChR[1]	1350/27.8	5.82	40.2±3.01
α_4 160-182	2.85/0.21	375	16.3±2.05
Adjuvants	n. d./n. d.	n. d.	1.23±1.12

1. pooled serum from MG patients ; 2. mean value±2SD (n=5) ; n. d. not detected.

Table 2. MG patients with neuropsychiatric disorders

Patient, age/sex	33/F	55/M	43/M	other MG
Myasthenia	IIb	IIb	IIb	I-IIb
Anti-muscle nAChR [nM]	1470	675	540	23.5±12.1
Anti-α_4 160-182 [nM]	5.45	3.21	3.97	0.03±0.02
% inhibition	4.81	2.53	2.87	<0.01
Consciousness	dreamy	dreamy	dreamy	—
Psychosensory	change of smell	change of taste	change of smell	—
Cognitive	recent memory loss	—	—	—
Emotional	fear	fear and anger	fear	fear and anger[1]
Psychotic	delusion	hallucination	—	—

1. Emotional disturbances with fear and anger were seen in 7 of 216 MG patients. However their autoantibodies showed no significant binding to thce α_4 160-182.

with the α_4 160-182 produced antibodies cross-reacting with muscle nAChR from rat and human (Fig. 4). Further, we demonstrated that the anti-α_4 160-182 antibodies caused a 16.3 % loss of nAChR content on the rat muscle cell surfaces, whereas control sera had no effects (Table 1). These facts indicated that similar antigenicity is shared by the α_1 125-147 and α_4 160-182, although its extent is very limited. Next, we examined the reactivity of serum autoantibodies in MG patients with the α_4 160-182. By the ELISA, we falied to detect bindings of MG antibodies to the immobilized α_4 160-182 (not shown). By radioimmunoassay, we demonstrated that the patients' antibodies bound to ^{125}I labeled α_4 160-182. Antibody-antigen reactions are summarized in Table 1.

We have screened sera from 219 MG patients for the antibodies to the same antigenic determinants on the muscle nAChR as the antigenic determinants for anti-α_4 160-182 antibodies. With higher concentration of the protecting antibodies and shorter incubations with MG serum, the binding of patients antiboidies to the muscle nAChR was inhibited in the 3 cases (Fig. 5). Table 2 shows the clinical profiles of these patients. They had slightly abnormal orientation and occasionally failed to distinguish their dream from the real world. In case of these rabbits, their consciousness was in neither a cloudy nor a delirious state. This is a provocative report to describe MG patients with neuropsychiatric disorders, whose autoantibodies bound to the neuronal nAChR at the molecular level. Our findings support that the central disturbanes observed in MG result from an antibody-mediated loss of nAChR.

Further investigations are required to address several questions; these are whether the cross-reaction could alter transmission in the central nervous systems, provided the extremely low concentration of these antibodies are present, and whether autoantibodies bind to other members of the α subunit gene family. The possibility that other factors than autoantibodies are involved in the central cholinergic transmission block should be investigated.

REFERENCES

1) **Ueno, S., Wada, K., Takahashi, M.** and **Tarui, S.**: Acetylcholine receptor in rabbit thymus: antigenic similarity between acetylcholine receptors of muscle and thymus. Clin. Exp. Immunol. **42**: 463-469, 1980.

2) **Ueno, S., Kang, J., Takeuchi, H., Takahashi, M.** and **Tarui, S.**: Specificities of antibody to acetylcholine receptor in rabbits with experimental myasthenia gravis. Clin. Exp. Immunol. **41**: 13-18, 1980.

3) **Ueno, S., Kang, J., Takeuchi, H., Takahashi, M.** and **Tarui, S.**: Experimental myasthenia gravis:

isolation and quantitative assay of anti-acetylcholine receptor antibody protein concentration in sera of rabbits immunized with narke acetylcholine receptor. Exp. Neurol. **68**: 512-520, 1980.

4) **Ueno, S., Kang, J., Takeuchi, H., Takahashi, M., Tarui, S.** and **Kasai, M.**: An experimental model of myasthenia in rabbits. Univ. Tokyo Press 93-104, 1981.

5) **Ueno, S.** and **Takahashi, M.**: Myasthenia gravis and nicotinic acetylcholine receptors: recent research on neurotransmitter receptors. Excerpta Medica, Amsterdam. 1986.

6) **Ueno, S., Takahashi, M., Hara, Y., Kajiyama, K., Okahisa, N., Hazama, T., Yorifuji, S.** and **Tarui, S.**: Parkinson's disease and myasthenia gravis. Neurology **37**: 832-833, 1987.

7) **Patrick, J., Ballivetet, M., Boas, L., Claudio, T., Forrest, J., Ingraham, H., Mason, P., Stengelin, S., Ueno, S.** and **Heinemann, S.**: Molecular cloning of the acetylcholine receptor. Cold Spring Harbor Symposia on Quantitative Biology XL Ⅷ: 71-78, 1983.

8) **Tucker, D. M., Roeltgen, D. P., Wann, P. D.** and **Wertheimer, R. I.**: Memory dysfunction in myasthenia gravis: Evidence for central cholinergic effects. Neurology **38**: 1173-1177, 1988.

9) **Hoeffer, P. F. A., Aranow, H.** and **Rowland, L. P.**: Myasthenia gravis and epilepsy. Arc. Neurol. Psychiat. **80**: 10-17, 1958.

10) **Snead, O. C. Ⅲ, Benton, J. W., Dwyer, D., Morley, B. J., Kemp, G. E., Bradley, R. J.** and **Oh, S. J.**: Juvenile myasthenia gravis. Neurology **30**: 732-739, 1980.

11) **Lefvert, A. K.** and **Priskanen, R.**: Acetylcholine-receptor antibodies in cerebrospinal fluid of patients with myasthenia gravis. Lancet **2**: 351-352, 1977.

12) **Fulpius, B. W., Fontana, A.** and **Cuénoud, S.**: Central-nervous-system involvement in experimental autoimmune myasthenia gravis. Lancet **2**: 350-351, 1977.

13) **Whitehouse, P. J., Martino, A. M., Price, D. L.** and **Kellar, K. J.**: Reductions in nicotinic but not muscarinic cholinergic receptors in Alzheimer's disease measured using[^{3}H] acetylcholine. Ann. Neurol. **18**: 145, 1985.

14) **Lewis, S. W., Ron, M. A.** and **Newson-Davis, J.**: Absence of central functional cholinergic deficits in myasthenia gravis. J. Neurol. Neurosurg. Psychiat. **52**: 258-261, 1989.

15) **Goldman, D., Deneris, E., Luyten, W., Kohchar, A., Patrick, J.** and **Heinemann, S.**: Members of a nicotinic acetylcholine receptor gene family are expressed in different regions of the mammalian central nervous system. Cell **48**: 965-973, 1987.

16) **Lennon, V. A., McCormick, D. J., Lambert, E. H., Griesmann, G. E.** and **Atassi, M. Z.**: Region of peptide 125-147 of acetylcholine receptor α subunit is exposed at neuromuscular junction and induces experimental autoimmune myasthenia gravis, T-cell immunity, and modulating autoantibodies. Proc. Natl. Acad. Sci. USA **82**: 8805-8809, 1985.

17) **McCormick, D. J., Griesmann, G. E., Huang, Z. X., Lambert, E. H.** and **Lennon, V. A.**: Myasthenogenicity of human acetylcholine receptor synthetic α-subunit peptide 125-147 dose not require intramolecular disulfide cyclization. J. Immunol. **139**: 2615-2619, 1987.

18) **Osserman, K. E.** and **Genkins, G.**: Studies in myasthenia gravis: review of a twenty year experniece in over 1200 patients. Mt. Sinai. J. Med. **38**: 497-537, 1971.

19) **Lindstrom, J. B., Einarson, B.** and **Tzartos, J.**: Production and assay of antibodies to acetylcholine receptors. Methods Enzymol. **74**: 432-460, 1987.

20) **Nef, P., Oneyser, C., Alliod, C., Couturier, S.** and **Ballivet M.**: Gene expressed in the brain define three distinct neuronal nicotinic acetylcholine receptors. EMBO J. **7**: 595-601, 1986.

21) **Tzartos, S. J., Seybold, M. E.** and **Lindstrom, J. M.**: Specificities of antibodies to acetylcholine receptors in sera from myasthenia gravis patients measured by monoclonal antibodies. Proc. Natl. Acad. Sci. USA **79**: 188-192, 1982.