Role of Antibodies to Neuronal α7-Acetylcholine Receptors in Myasthenia

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The role of antibodies to a fragment of neuronal acetylcholine receptor was studied by EIA in patients with myasthenia. Antibody levels were significantly higher in patients with generalized myasthenia. Enzyme immunoassay of antibodies by the reaction with acetylcholine receptor fragment can serve as an additional method for studies of autoimmune myasthenia pathogenesis.

Key Words: *myasthenia; antibodies to neuronal acetylcholine receptor; muscle acetylcholine receptor; enzyme immunoassay*

The role of antibodies and structure of antigen target in the pathogenesis of autoimmune and degenerative diseases of the nervous system are now intensely studied [1,3,5]. The role of neuronal acetylcholine receptors (nACR) expressed on immune system cells (macrophages, T and B cells) is less known. At the same time, cholinergic structures are involved in the regulation of target cell metabolism through biochemical processes modifying the status of membrane lipids, calcium-dependent signal transduction routes, and protein phosphorylation. Acetylcholine or nicotine stimulation of nACR leads to rapid entry of Ca^{2+} through α7-nACR [7], which are expressed by macrophages, monocytes, and lymphocytes [11,12,14]. These receptors are involved in the regulation of lymphocyte (particularly B cells) development and behavior in the primary lymphoid organs and spleen [10]. In addition, α7-nACR are involved in the regulation of Ca^{2+} local metabolism [11].

Through the vagus nerve the nervous system significantly and rapidly inhibits secretion of TNF-α by macrophages and suppresses systemic inflammatory responses. However, the molecular mechanisms of cholinergic regulation of inflammation had remained unknown until the role of α7-nACR in the realization of inflammation was established [12-14]. It was found that α7-nACR agonists, *e.g.* nicotine or acetylcholine, by interacting with the receptor inhibit production of Th1-proinflammatory cytokines (IL-12, IL-18, TNF-α) through modulation of the nuclear factors phosphorylation. This could be paralleled by a shift of the immune response towards Th2 type.

Reports about the contribution of disorders in nACR function to the pathogenesis of diseases of the central and peripheral nervous system are scanty [4-6].

We studied the role of antibodies to α7-nACR in myasthenia.

MATERIALS AND METHODS

Twenty-two patients with myasthenia (3 men and 19 women aged 13-73 years) were examined. All patients were treated at Russian Myasthenic Center, Institute of Pathology and Pathophysiology.

The disease was diagnosed by case history (dynamics of symptoms over 24 h, history of partial or complete remissions), clinical examination (distribution of muscle weakness characteristic of myasthenia, pathological muscle fatigue), electromyographic (EMG) signs of neuromuscular transmission disorders, and positive reaction (clinical and EMG) to injection of anticholinesterase drugs. By the International Clas-

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sification the following conditions were diagnosed: 1 patient aged 65 years with ocular myasthenia (observed over 14 years) – I, 4 women with IIA, 2 men and 13 women with IIB (of these 1 patient (female aged 60 years) with generalized myasthenia and thymoma), and 2 patients with IIIB. Disease duration varied from 1 month to 21 years. Dynamic studies were carried out in 2 patients. Control group consisted of 6 volunteers (3 men and 3 women) aged 20-52 years without signs of neuromuscular diseases.

Extracellular domain of nACR α7-subunit, a kind gift from Laboratory of Neuropeptide Reception, M. M. Shemyakin and Yu. V. Ovchinnikov Institute of Organic Biochemistry, served as the antigen in ELISA [2].

The domain in a concentration of 0.04 mg/ml was adsorbed (50 μl/well) in 96-well plates (Costar) and incubated for 60 min at 37°C. Rows 1, 4, 7, and 10 of the wells were left free for conjugate control. Nonspecific binding was blocked by 60-min incubation with 200 μl/well 2% BSA (Sigma) in PBS with Twin-20 (0.1%) and incubation at 37°C. After incubation, serial dilutions of polyclonal IgG, obtained by immunization of rabbits with nACR α7-subunit fragments [8] were added in row 2. The test sera diluted 1:80 were added in all other rows. Antibodies were visualized by staining using peroxidase conjugate with human anti-IgG (Sigma) and rabbit anti-IgG (Sigma), which were added in a dose of 100 μl/well at 1:1000 dilution and incubated for 60 min at 37°C. After incubation, the plates were washed (0.2% BSA in PBS with 0.1% Twin, 200 μl/well) for 3 min. *o*-Phenylene diamine (Sigma) served as the chromogen: 1 tablet was dissolved in 5 ml citrate buffer (pH 4.5) and 15 μl hydrogen peroxide and 100 μl/well was added. Staining developed during 10 min. The reaction was stopped by adding 3 N HCl (50 μl/well). Optical density was measured at 492 nm. Antibody level was evaluated by calibration curve reflecting the relationship between optical density and IgG concentration in the calibration reagent. Polyclonal rabbit IgG in concentrations of 10, 5, 2.5, and 1.25 μg/ml served as the reference samples.

The results were expressed in optical density units (arb. units) or in μg/ml. The mean content of antibodies to nACR fragment in the sera of 6 healthy adult individuals served as the reference (normal) level.

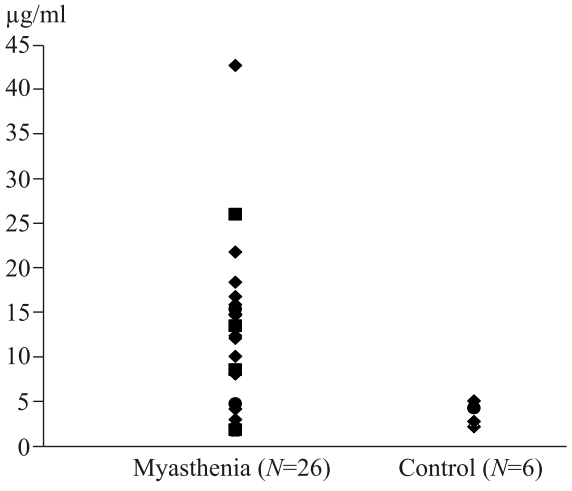

Fig. 1. Serum antibody level by interaction with α7-208-nACR fragment.

RESULTS

The results of evaluation of serum antibodies in patients with myasthenia and donors are presented in Figure 1. Antibody levels were increased in 15 (68.2%) patients. The level of antibodies to α7-nACR in generalized myasthenia was 12.87±9.73 μg/ml *vs.* 3.10±1.14 μg/ml in the control. Antibody level in patients receiving no immunosuppressive therapy (16.30±9.94 μg/ml, N=10) was higher than during glucocorticosteroid treatment (9.6±8.8 μg/ml, N=10; p=0.06). The level of antibodies to α7-nACR was higher (10.55) in grave patients than in those with less severe disease (5.91). No correlations between the levels of antibodies to α7-nACR and predominant location of the process, disease duration, and presence of concomitant diseases, including neuroendocrine disorders, were detected. No antibodies to α7-nACR were detected in the male patient with ocular myasthenia and female patient with myasthenia and thymoma (2.3 and 3.0 μg/ml, respectively).

It is known that 80% patients with myasthenia have antibodies to muscular ACR. These antibodies are characterized by a high pathogenetic potential, they fix the complement, which leads to lysis of ACR on the postsynaptic membrane. The homology of ACR family proteins is very high, reaching 70% for some subunits. High antibody levels were detected in almost all patients by the interaction with muscular ACR fragment (1-209) [1]. Appearance of anti-nACR in the serum in myasthenia was presumably caused by possible cross-reactions and by their presumable role in immune response regulation [6]. It is known that α7-nACR receptors are expressed on stimulated T and B lymphocytes. Chronic cholinergia (continuous treatment of myasthenia patients with acetylcholinesterase inhibitors) results in α7-nACR activation, which leads to massive entry of Ca^{2+} into the cell and inhibition of proinflammatory cytokine production. In addition, it is known that Ca^{2+} ions regulating adenylate cyclase and phosphodiesterase activities through calmodulin (Ca^{2+} binding protein) play an important role in the regulation of metabolic processes in cells via cAMP. The function of antibodies to α7-nACR probably consists in competition with the agonists for receptor binding modulation of receptor conformation, inhibition of

Ca^{2+} entry into the cell, stimulation of IL-12 synthesis and proliferation of autoreactive T-cell clone. Hence, immune response disorders in myasthenia are presumably caused by changes in cholinergic regulation of lymphocyte metabolism.

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