Role Sympathetic Autonomic Nervous System in the Regulation of Immune Response during Myasthenia

V. B. Lantsova, E. K. Sepp, and A. S. Kozlovsky

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In 12 patients with myasthenia, the content of β_2-adrenoreceptors on the cell surface and activity of intracellular lymphocytic enzymes were determined by EIA and biochemical methods, respectively. In comparison with the normal, these patients demonstrated pronounced elevation in the content of β_2-adrenoreceptors and significant changes in activity of lymphocytic enzymes. In 10 of 12 patients, administration of the agonists to β_2-adrenoreceptors resulted in health improvement accompanied by normalization of EMG and immunobiochemical indices. Our findings suggest that intra- and intercellular signaling pathways and their modification can serve as potential targets for the therapy.

Key Words: *myasthenia; β_2-adrenoreceptor; enzyme immune assay; salbutamol*

Myasthenia is a neurocrine autoimmune neuromuscular disease with unknown etiology. The basic pathogenic mechanism of myasthenia is the formation of autoreactive clones of lymphocytes and high-affinity complement-fixing antibodies, which block or lyze acetylcholine receptors (AChR) in the postsynaptic membranes of neuromuscular junction.

Interaction of the nervous, immune, and endocrine systems determines their interdependence in such a way that the primary damage to any component of this triad pronouncedly increases the risk of system dysregulation [2]. The hypothalamus is key element of the central apparatus of nervous regulation of the immune system. Its structures react to antigens with minimum latency and are directly coupled to the organs of the immune system via the autonomic nervous system (ANS) [2]. However, little is known on the role of ANS during autoimmune diseases. It is noteworthy that the immune system can affect both sympathetic and parasympathetic compartments of ANS in various organs and tissues via antibodies [4].

The key structures of ANS are presented on peripheral blood lymphocytes. All lymphoid cells express various receptors of the adreno- and cholinergic systems. Specifically, β_2-adrenoreceptors (β_2-AR) are expressed on the surface of Th0, Th1, and B cells [6] and M3 and M5 subtypes of muscarinic AChR are present on the some of T and B cells [5]. β_2-AR and muscarinic AChR belong to metabotropic receptors that are coupled to G-proteins, which control the diverse cell effector functions. Activation of these receptors is related to enhancement of cAMP and cGMP levels. The blood serum of myasthenic patients contains cAMP and cGMP in elevated concentrations [10] together with antibodies against β_1-AR and β_2-AR [8,9]. The studies of the mechanisms of catecholamine action on cell immunity showed that these neurotransmitters inhibit secretion of IL-12 and up-regulate secretion of IL-10 in a dose-dependent manner within the physiological range of concentrations [3]. These effects of catecholamines were completely blocked by propranolol, an antagonist of β-AR. These data

State Research Institute of General Pathology and Pathological Physiology, Russian Academy of Medical Sciences; Children's Clinical Hospital, Ministry of Health Care and Social Development of the Russian Federation, Moscow, Russia. ***Address for correspondence:*** sepp.e@mail.ru. V. B. Lantsova

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showed that ANS could regulate Th1/Th2 balance. Under conditions of sympathicotonia, this balance is shifted towards the Th2 response. The effect of sympathetic neurotransmitters on the immunocompetent cells impairs their immune function, which can culminate in apoptosis [6]. Thus, ANS plays an important role in the regulation of local immunity.

There are various tests for evaluation of ANS function in clinical practice [1]. However, they score only systemic effects exerted by ANS activity in the whole organism. By the mode of action, the effects of ANS on immune cells principally differ from the direct adrenergic or cholinergic stimulation of the major effector organs and tissues. Moreover, both kinds of processes can develop simultaneously and independently on each other.

There are indications that lymphocytic β_2-AR are involved in the pathogenesis of multiple sclerosis [12]. Increase number of AR on lymphocytes is detected during exacerbation of this disease, which is explained by the cell immune reaction manifested in activation of Th1-cells. β_2-AR agonists down-regulated the expression of β_2-AR on lymphocytes, which correlated with improvement of the state of the patients and animals with experimental allergic encephalomyelitis [7].

However, the autoimmune and allergic diseases in most cases develop in patients with prevailing parasympathetic influences. Under conditions of vagotonia, plastic processes characterized by intensified protein synthesis (cytokines included) predominate in lymphoid cells, which leads to proliferation of autoaggressive clones of lymphocytes.

Myasthenia occupies a special place among autoimmune disorders. Acetylcholine esterase (AChE) inhibitors increasing the concentration of acetylcholine in the synaptic cleft not only in the neuromuscular junction, but also in synapses of CNS and autonomic ganglia are still used as drugs of choice for the correction of neuromuscular disorders. This treatment induces effects mediated by nicotinic and muscarinic cholinergic receptors. Chronic administration of AChE inhibitors provokes drug-induced vagotonia in myasthenic patients. In some of them, the quantity of kalymin, proserine, and other inhibitors amounted to 16-20 and more doses per day.

Therefore, the study of ANS regulation of the immune system in myasthenia is extremely important for understanding of individual elements in pathogenesis of this disease affecting clinical manifestation, severity, and the development of the pathological process. For evaluation of the role of the sympathetic division of ANS in the pathogenesis of myasthenia, we studied the number of surface β_2-AR and activity of intracellular enzymes in lymphocytes before and during administration of β_2-AR agonists.

MATERIALS AND METHODS

Seventeen individuals were examined: 12 patients with generalized myasthenia (6-23 years) including 2 patients receiving salbutamol and healthy volunteers (n=5). According to international classification, the severity of the disease was III-IVB in 10 patients and IIA in 2 patients.

The content of lymphocytic β-AR was determined by ELISA in Immunochemistry Department of National Narcologic Scientific Center of the Ministry of Health of the Russian Federation. Salbutamol was conjugated with the carrier proteins according to Jamamoto-Jwata method [11] in original modification. Metabolism of lymphocytes was assessed by photometric methods by activity of transaminases, γ-glutamyl transpeptidase, lactate dehydrogenase, and alkaline phosphatase on a FP 901M Semi-Automatic Biochemical Analyzer (Labsystem) with a Biocon kits.

RESULTS

In myasthenic patients, a significant elevation of the content of β_2-AR on the surface of lymphocytes to 0.95±0.05 optical density units was observed compared to the corresponding parameter in the control group of the healthy donors (0.35±0.50 optical density units).

In lymphocyte cytoplasm, activities of γ-glutamyl transpeptidase and alkaline phosphatase increased to 10 and 51 U/ml (*vs*. 4.4 and 19.4 U/ml in the control), respectively, while activities of AST and ALT decreased to 2.5 and 1.1 U/ml (*vs*. 30.4 and 3.7 U/ml in the control), respectively. In addition, activity of lactate dehydrogenase decreased from 762.8 U/ml to 450.8 U/ml. Elevated γ-glutamyl transpeptidase indicates the autoimmune reaction during myasthenia. Probably, this enzyme can be a factor needed for maintaining proliferation of the autoreactive lymphocytic clone.

In 10 of 12 patients treated with β_2-AR agonists (salbutamol in various drug formulations, 2-14.5 mg/day *per os* for 3-4 weeks), improvement of the health status and normalization of EMG and immunobiochemical indices were observed. In one case (female patient C, 24 years), the course of salbutamol (Saltos) increased the contraction force of the deltoid muscle from 1 to 4 points, potentiated M-response from 3.3 to 11 mV, and reduced the decrement of M-response by 10%. The drug normalized both cell-mediated and humoral immune depression manifested by decreased levels of IgM and IgA, respectively; the severity of IFN-α insufficiency decreased from III to II degree (the titer elevated from 40 to 160), although the titer of IFN-γ remained at a low level (16). The amount of

lymphocytic β_2-AR decreased from 0.95 to 0.3 optical density units; activity of γ-glutamyl transpeptidase disappeared (10 U/ml before treatment). Activities of alkaline phosphatase and lactate dehydrogenase returned to normal: alkaline phosphatase activity decreased from 51 to 24 U/ml and lactate dehydrogenase activity increased from 400 to 750 U/ml.

Thus, the study revealed efficiency of β_2-AR agonists in compensation of the motor disorders and selective immunocorrection in myasthenic patients.

The molecular mechanisms of the effects of ANS dysfunction on the pathological processes during myasthenia can be related to an increase in the number of β_2-AR, modification of these receptors, AChR desensitization, and activation of the synthesis of anti-inflammatory cytokines and complement-fixing antibodies.

It can be hypothesized that the direct effect of β_2-AR agonists on muscles in myasthenia consists probably in, first, cleavage of glycogen yielding ATP to supply energy for muscle contraction and, second, phosphorylation of AChR with elimination of receptor desensitization. This mode of action of β_2-AR agonists can partially compensate for muscle weakness and open vista to diminish the dosage of acetylcholine esterase inhibitors used in the therapy of myasthenic patients. It can also be hypothesized that disorders in signal transmission from the sympathoadrenal system via the lymphocytic receptor machinery aggravated by proliferation of the autoreactive (Th1) cell clone is a physiological mechanism underlying the development of myasthenia. This hypothesis is corroborated by changes in the density of β_2-AR, normalization of metabolism in lymphocytes, recovery of cell and humoral immunity indices resulting in improvement of the health status of myasthenic patients combined with positive EMG dynamics after course salbutamol treatment.

Therefore, clinical testing of β_2-AR agonists open new avenues to study the immune mechanisms and to use novel approaches to treat the Th1-related diseases. The pathways of intra- and intercellular signaling and their modification can be viewed as the potential targets for therapeutic tools.

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