Synaptic Compensatory Mechanism and its Impairment in Autoimmune Myasthenic Diseases

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ABSTRACT

The neuromuscular junction (NMJ) is organized by a complex architecture and various signals orchestrated by sophisticated interactions. They include the presynaptic Ca\textsuperscript{2+} homeostasis for acetylcholine (ACh) release in the active zone organization, the post-synaptic ACh receptor (AChr) clustering at endplate membranes, the trans-synaptic communication from muscle to nerve, and the synaptic stabilization. The present data and discussions are concerned in an adaptive change of ACh release from the nerve terminal and its immunological impairment in the post-synaptic disease (myasthenia gravis, MG) and the presynaptic disease (Lambert-Eaton myasthenic syndrome, LEMS). Discussions mainly focus the antibody-induced failure of the synaptic compensatory mechanisms that are brought about by the presynaptic autoreceptors (the M1-type muscarinic AChR [mAChR] cooperated with adenosine receptors), and the non-voltage-gated Ca\textsuperscript{2+}-dominant influx channel (transient receptor potential canonical [TRPCs], particularly its phenotype TRPC3). Besides the synaptic transmission fatigue, the TRPC3 antibodies are discussed in terms of their implication in the muscle contraction fatigue that often occurs in the thymoma-associated MG and reflects a defect in the physiological association of TRPC3 with the ryanodine receptor-1 in the excitation-contraction coupling in which the sarcoplasmic Ca\textsuperscript{2+} release takes place. In addition to the modulating role in the NMJ functions, the mAChRs participate in the innate and adaptive immunity by MG thymus and in the lung cancer (often associated with LEMS) growth.

Post-synaptic organization and its contribution to trans-synaptic communication

In the neuromuscular junction, the presynaptic calcium homeostasis for acetylcholine (ACh) release and the postsynaptic nicotinic ACh receptor (nAChR) clusters anchored to the membrane by rapsyn (immobilized by heat-shock proteins) are modulated by the molecular mechanisms underlying synaptic plasticity.\textsuperscript{1-4} The former depends on functional organizations including the active zone architecture (piccolo, bassoon, RIM, RIM-BP, ELKS, Munc 13, α-liprin, Munc 1B, complexin, Neurexins, etc.), calcium channels, SNARE complex, synaptotagmins, synaptic vesicles and presynaptic autoreceptors\textsuperscript{5-9}; the latter depends on agrin, Wnts (wingless integration site family of glycoproteins), Lrp4 (low-density lipoprotein receptor-related protein 4), MuSK (muscle-specific tyrosine kinase) and Dok 7 (downstream kinase 7).\textsuperscript{10-18} The postsynaptic structure to be precisely opposed to the nerve terminal is stabilized by the extracellular matrix (including synaptic collagens such as Q, IV and XIII, perlecain, biglycan, laminins and α-dytroglycan) and dystrophin-associated glycoprotein complex (including β-dystroglycan [linking to rapsyn and utrophin], cortactin...
(acting as a synaptic kinase substrate and a regulator of
actin polymerization), caveolin 3, coronin 6, α-dystrobrevin
(conducted by neuregulin 1-Erb B receptor interaction),
and Sorbs 1/2 (conducted by Dok7-Crk-L).19-34

In the trans-synaptic communication (Figure 1), the
signals are mediated via (1) the Wnt canonical signaling by
way of Wnt-MuSK Ig 4 (cytotoxic-rich domain)/Dishevelled
(scaffold protein)/ inhibition of glycogen synthase kinase
3/β-catenin/Slit 2 in the muscle35-37; (2) muscle-derived
Lrp 4 (eight low-density lipoprotein a [LDLa] repeats),38
interacting with an Lrp 4-binding protein in motor
neuron38,39; (3) muscle laminin β2 tethering the P/Q-type
and N-type voltage-gated calcium channels (VGCCs) to the
presynaptic active zone via α3 integrin10,41; (4) muscle-
derived laminins α4, α5 and β2 in the laminin-network
including muscle-agrin.42,43 Neuregulin-1,44 endostatin,45
and bone morphogenic protein pathway members46
have been suggested as candidate retrograde signaling
molecules.47

Modes of compensatory vesicle endocytosis in the
nerve terminal

The fast-mode of endocytosis (clathrin-
independent synaptic vesicle recycling) operates by

![Figure 1: Schematic presentation of acetylcholine (ACh) release which is upregulated to compensate presynaptic dysfunctions by the
homeostatic mechanisms operating in the active zone of the nerve terminal.](Image)

The fast-mode of endocytosis (clathrin-independent synaptic vesicle recycling) requires a large influx of external Ca$^{2+}$ to compensate postsynaptic dysfunction. This homeostatic Ca$^{2+}$ is promoted by phospholipase C (PLC) activation via the activation of G-protein-coupled receptor, such as the presynaptic M1-type muscarinic acetylcholine receptor (mAChR), and also via the interaction of brain-derived
neurotrophic factor (BDNF) with receptor tyrosine kinase (TrkB). These biological mechanisms share a common pathway mediated by
PLC/phosphatidylinositol-4,5-bisphosphate (PtdIns (4,5)P2)/diacylglycerol (DAG)/protein kinase C (PKC); these in turn potentiate Ca$^{2+}$ influx
voltage-dependently via DAG/PKC-activated P/Q-type voltage-gated Ca$^{2+}$ channel [VGCC] and voltage-independently via DAG-activated
transient receptor potential canonical (TRPCs). The interaction of inositol-1,4,5-triphosphate (IP3) with IP3 receptor allows the
TRPC-mediated Ca$^{2+}$ influx to be free from the inhibitory control by scaffolding protein, Homer1. DAG also regulates the vesicle priming
protein (Munc13-1) to recruit ACh-containing vesicles for immediately releasable pool in the active zone of the nerve terminal. In
the physiologic condition, the mechanisms to control Ca$^{2+}$ influx is based on the balance between the promotive effect by M1-type mAChR
and the inhibitory effect by M2-type mAChR (modulated by adenosine and TrkB). The presynaptic architecture including SANREs (synaptobrevin,
syntaxin and SNAP-25) and Company are detailed in references 5-9. Retrograde signals (from muscle to nerve) organize the active zone
architecture contributing to synaptic vesicle recycling for ACh release. Y1: P/Q-type VGCC antibodies; Y2: antibodies to the extracellularly
exposed segment of synaptotagmin 1 (N-terminus) during exocytosis; Y3: antibodies to M1-type mAChR; Y4: antibodies to TRPC3.
promoting recruitment of dynamin 1/synaptophysin/synaptobrevin or by calcium/calmodulin/calcinulin/dynamin signaling (endocytosis overshoot); these mechanisms require a large influx of external calcium. This homeostatic calcium influx is promoted by the activation of G protein-coupled receptors (GPCRs), such as the presynaptic M1-type muscarinic acetylcholine receptor (mAChR) and adenosine receptor (A2A), and the interaction of brain-derived neurotrophic factor (BDNF) with receptor tyrosine kinase (TrkB) in the nerve terminal. The positive effect of calcium influx for the compensatory presynaptic M2-type mAChR/protein kinase A signaling; the balance is modulated by adenosine receptors: A2A for activation; A1 for inhibition) and TrkB.

The mechanisms for the positive effect of GPCR (M1-type mAChR) and BDNF share a common pathological pathway mediated by phospholipase C (PLC)/phosphatidylinositol-4,5-bisphosphate (PIP2)/diacylglycerol (DAG)/protein kinase C (PKC); these biological mechanisms stimulate the P/Q-type voltage-gated calcium channel (VGCC) and also regulates the vesicle priming protein (Munc13-1) to recruit ACh containing vesicles for the immediately releasable pool. The PLC-generated inositol triphosphate (IP3) interacts with the IP3 receptor; this allows the TRPC-mediated calcium influx to be free from inhibitory control by scaffolding protein, Homer 1. Among the seven members in TRPC family, TRPC3, TRPC6 and TRPC7 are the close homologues; it is structurally confirmed that the TRPC3 is abundantly expressed in the nervous system and has a unique feature with the remarkable long S1-S4 domain stretching into the extracellular side and supporting the formation of the extracellular domain; thereby, the TRPC3 could be accessible to antibodies in the manner similar to the M1-type mAChR containing the extracellularly exposed domain. The M1-type mAChR is physiologically associated with the non-voltage-gated channel (TRPC)-mediated calcium influx (as shown by brown and blue lines with arrow-head in Figure 1). This association may thus be cooperative to act on the mechanism and function compensating synaptic disorders. As mentioned above, however, we should take notice that the large Ca\(^{2+}\) influx for the compensatory presynaptic mechanisms via M1-type mAChR, TRPCs/IP3R and adenosine A2A receptor are controlled (in order to prevent injurious Ca\(^{2+}\)-excess in the nerve terminal) by the balance with the inhibitory mechanisms based on M2-type mAChR, Homer 1, adenosine A1 receptor and TrkB.

Immunology in myasthenia gravis and Lambert-Eaton myasthenic syndrome focusing presynaptic autoreceptors and channels for synaptic compensatory mechanisms

In view of the above-mentioned synaptic physiology, serum samples from myasthenia gravis (MG) and Lambert-Eaton myasthenic syndrome (LEMS) were assayed by immunoblotting using the human M1-type mAChR fusion protein, and by ELISA using the peptide synthesized based on the sequences of the human TRPC3 (extracellularly exposed segment: Phe611-His640). Of 25 MG patients with postsynaptic nAChR antibodies, seven (28 %) were positive for presynaptic M1-type mAChR antibodies, and 9 (36 %) were positive for TRPC3 antibodies (Table 1). The clinical severity of these patients (graded by Myasthenia Gravis Foundation of America, MGFA, Clinical Classification) tends toward higher, more severe grades, suggesting that their synaptic functions may not be adequate to upregulate ACh quantal release to compensate postsynaptic defect.

Reportedly, the electrophysiological study in MG with AChR antibodies showed that the compensatory ACh release upregulation cannot be sustained at the high frequency of nerve stimulation, possibly because the reduction in homeostatic small reserve pool of vesicles that depends on presynaptic adenosine A2A receptor operating Ca\(^{2+}\) influx via RyR1. In a long-tetanic load in muscle, the complex compensatory mechanisms including the presynaptic autoreceptor and channel in this signaling pathway may underlie to compensate postsynaptic dysfunction.

As an additional consideration to the physiological role of TRPC3, it is worthy to note that the TRPC3 takes part in muscle contraction in addition to above-mentioned synaptic transmission. Many of the severe-graded MG patients studied herein have thymoma and are positive for antibodies to the ryanodine receptor 1 (RyR1) (Table 1). RyR1 activates the sarcoplasmic Ca\(^{2+}\) release in the excitation-contraction coupling via membrane-depolarization; this cooperates with TRPC3 which is activated by PLC-PIP2-DAG signaling pathway and conducts the activating signal to RyR1 via triadic bridging proteins, including TRPC1 and possibly coregulatory proteins (junctophyins 1 and 2, homer, mitsugumin 29, calreticulin and calmodulin). In our experiment (Table 1), three synthetic RyR1 peptides were used as antigens to determine antibodies: (1) the residues 1191-1216 (a region toward the N-terminus that faces the loop between domains II and III of dihydropyridine receptor [voltage sensor]), (2) the residues 4997-5017 (C-terminal transmembrane segment, which is expressed in thyomma epithelial cells and is suspected of inducing breakdown in tolerance to RyR1), and (3) the residues 5019-5038 (C-terminal tail which protrudes into cytoplasm outside the...
Table 1: Clinical and immunological profiles in 25 patients with myasthenia gravis.

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<tr>
<th>No. of Patients</th>
<th>Gender (M/male, F/female)/ age of onset (years)</th>
<th>MGFA grades</th>
<th>AChR (nM) (control: &lt;0.1)</th>
<th>M1 mAChR (immunoblots) (control: &lt;0.17 at OD450)</th>
<th>Antibodies to TRPC3 (ELISA) (control: &lt;0.10 at OD450)</th>
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<td>IIIa</td>
<td>73</td>
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Referenced to references 48, 69 and 89. MGFA, Myasthenia Gravis Foundation of America; AChR, acetylcholine receptor; M1 mAChR, M1-type muscarinic acetylcholine receptor; TRPC3, Transient receptor potential classical isoform-3; RyR1, ryanodine receptor-1; N: N-terminus (residues 1191-1216); C-tr:C-terminal transmembrane (residues 4997-5017); C: C-terminal tail (residues 5019-5038). RyR1 antibodies determined by ELISA at OD450: expressed by + (positive, above 0.10) and – (negative, below 0.10). None of 25 patients showed antibodies to TRPC3 (data not shown).

Table 2: Clinical, electrophysiological and immunological profiles in 25 patients with Lambert-Eaton myasthenic syndrome.

<table>
<thead>
<tr>
<th>No. of patient</th>
<th>Gender (M/male, F/female)/ age of onset (years)</th>
<th>Amplitude of single CMAP (mV)</th>
<th>Potentiation at 50Hz RNS (%)</th>
<th>SCIC antibodies to Synpt I (ELISA) (control: &lt;0.1)</th>
<th>Antibodies to RyR1 (ELISA) (control: &lt;0.10 at OD450)</th>
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Referenced to references 85-89. CMAP, compound muscle action potential; RNS, repetitive nerve stimulation; SCIC, small-cell lung carcinoma; VGCC, voltage-gated calcium channel.

sarcoplasmic reticulum (SR) and can form a functional Ca²⁺ release channel[63], they were highly positive for antibody-detection particularly by (2) and (3). The experiment using TRPC3-knockdown skeletal myotubes showed that the SR Ca²⁺ release after direct RyR1 activation by caffeine was decreased regardless of SR Ca²⁺ content.[63,64]

In LEMS which is often associated with small-cell lung carcinoma (SCLC) expressing antigens immunologically cross-reactive with the nerve terminal proteins, we showed that M1-type mAChR antibodies are implicated in this presynaptic disorder, the pathogenicity of which has mainly referred to the antibodies directing to presynaptic components, P/Q-type VGCC and synaptotagmin 1.[65-69]. In 25 LEMS patients whose diagnosis was electrophysiologically confirmed (low amplitude of muscle action potential evoked with single nerve stimulation and its marked potentiation after tetanic nerve stimulation), 19 patients (76 %), 4 of whom were negative for antibodies to both P/Q-type VGCC and synaptotagmin 1, were positive for presynaptic M1-type mAChR antibodies.[66,68,69] (Table 2). Passive transfer
of serum IgG from a patient solely positive for presynaptic M1-type mACHR antibodies to mouse produced the electrophysiological features of LEMS (reduced ACh quantal contents confirmed by the microelectrode study). The compensatory mechanism that activates presynaptic M1-type mAChR and modulates the fast-mode of synaptic vesicle recycling may be restricted by coincident M1-type mAChR antibodies in LEMS. Pharmacologically, ACh quantal release is reduced by specific M1-type mAChR blockade in mice at standard ion concentrations without quantal release. None of 25 patients with LEMS showed antibodies to M1-type mAChR-dependent compensatory mechanism CD25 mAChR antibodies in LEMS.

Thymus in myasthenia gravis and cancer in Lambert-Eaton myasthenic syndrome

The MG thymus play a pivotal role in the pathogenesis of the disease. The hyperplastic MG thymus contains the components necessary for an anti-nACH immune response including activated autoreactive B and T cells, the antigen-presenting cells, the autoantigen and the activated complement receptors in the epithelial cells. The thymoma-associated MG brings about the abnormal T cell selection caused by the defective expression of tolerogenic autoimmune regulator (AIRE)-positive thymic medulla, the epithelial cells defective in the expression of HLA class II molecules, the lack or reduction of immune-tolerated AChR-positive myoid cells and the failure to generate CD4+, CD25+ and Fox P3-positive regulatory T cells. The mTOR (mammalian target of rapamycin)/Akt (serine/threonine kinase) pathway participates to the cell proliferation associated with thymoma growth. In the innate and adaptive immunity in MG thymus, the crosstalk between the complement (C5a and C3a) and the Toll-like receptors in thymic epithelial cells causes (1) promoting abnormal B cell activation, leading to ectopic germinal center development; (2) Th1/Th17-related pro-inflammatory cytokine production based on T cells disproportion (Th effector>Th regulator); (3) antigen-presentation by dendritic cells recruited by chemokine (CCL17 and CCL22). The inflammatory cytokine elevation are brought about by the activation of mitogen-activated protein kinases (MAPKs), transcriptional nuclear factor-κB (NF-κB) and activated protein-1 (AP-1). The mAChRs (M1 and M5) expressed in thymocytes and lymphocytes contribute to the regulation of pro-inflammatory cytokine production related to adaptive immunity. In view of the MG immune pathogenicity, the target-specific immunotherapies are developing; the specific therapies are adapted or in expectation of trial to the MG patients refractory to thymectomy and/or usual immunosuppressants by use of (1) IgG-κ chain targeting CD20 B-cell antibody, (2) Complement C5 inhibitors, (3) antibody to cytotoxic T lymphocyte antigen, (4) IL-6 receptor blocker, (5) IL-10 modulated human dendritic cells to generate regulatory T cells (to be superior to effector T cells), (5) recombinant AChR fragment for antigen-specific removal of AChR antibodies, (6) human neonatal Fc receptor blocker, (7) selective inhibitor of immunoproteasome, and (8) inhibitor of inflammatory factors via TRL2/4-P13K-NFκB signaling.

The SCLC, which tends to associate with LEMS, expresses mACHRs as well as VGCC and synaptotagmins, suggesting that the antibodies to neuronal antigens expressed in SCLC cross-react with those on nerve terminals leading to the neurological defects. Besides the functional role as the presynaptic autoreceptor which modulates the synaptic transmission, the mAChRs are expressed in lung cancer and their activation (via the cholinergic autocrine loop expressed in cancer and stimulated by ACh secreted by cancer) provides an effect for cancer growth via the activation in mitogen-activated protein kinase and serine/threonine kinase Akt (involved in the phosphatidylinositol-3 kinase signaling pathway).

References


58. Amaral MD, Pozzo-Miller L. Intrinsic cellular Ca2+ stores and Ca2+ influx are both required for BDNF to rapidly increase quantal vesicular transmitter release. Neural Plast. 2012; 2012: 203536.


