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ARTICLES

Analysis of Exo- and Endocytosis in the Mouse Nerve Ending in Experimental Diabetes Mellitus

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Abstract—Diabetes mellitus (DM) is a systemic disease characterized by changes in many organs and tissues, including the motor system. The processes of exo- and endocytosis in the motor nerve ending of the mouse diaphragm muscle during high-frequency activity in experimental alloxan model of DM were studied. End-plate potentials (EPPs) were recorded using intracellular microelectrodes during single and high-frequency (50 Hz, 1 min) stimulation. In mice with the experimental DM, the amplitude-time parameters of EPPs did not differ from those of the control; however, an increase in EPPs depression and a slower recovery were observed during high-frequency stimulation. Using an endocytosis marker FM 1-43, it was shown that in animals with experimental DM fluorescence intensity of the nerve terminals loaded with the dye by high-frequency stimulation increased that was prevented by 1-azakenpaullone (2 μM), an inhibitor of slow dynamin-1-mediated endocytosis. In addition, in the model animals, the destaining of the pre-loaded nerve terminals during high-frequency (50 Hz) stimulation slowed down. The obtained data indicate that in the experimental first type DM recycling of synaptic vesicles via long path becomes more pronounced and the mechanisms of the vesicular transport are impaired, which was confirmed by methods of mathematical modeling.

Keywords: diabetes mellitus, neuromuscular junction, synaptic vesicles, exocytosis, endocytosis, mathematical modeling

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INTRODUCTION

Diabetes mellitus (DM) is a chronic disease of the endocrine system of humans, characterized by a prolonged increase in the glucose concentration in the blood and concomitant changes in metabolism [1]. One of the serious complications of diabetes is peripheral neuropathy, characterized by muscle weakness, muscle atrophy [2], functional disorders of motor nerve fibers, as well as neuromuscular transmission changes. Several studies have shown morphological and functional changes of the neuromuscular synapse at pre- and postsynaptic levels [3–10].

Synaptic transmission in the neuromuscular junction is carried out by exocytosis of synaptic vesicles containing the neurotransmitter quanta. Synaptic vesicles in the nerve endings of mammals are divided into three functional pools. The first, readily releasable pool (docked vesicles), and the second (mobilization) pool are sometimes considered as a unified recycling pool [11]. The recycling pool provides maintenance of the synaptic transmission at moderate stimulation due to reuse of vesicles, which are recovering by rapid endocytosis [12, 13]. The third population consists of the reserve pool vesicles that slowly move into the recycling pool and are engaged in the response to prolonged high-frequency stimulation. The restoration of the reserve pool vesicles is due to the formation of invaginations and endosome-like structures, from the surface of which the vesicles are budding out [11, 12]. For realization of the processes of both fast and slow endocytosis, dynamin-1 GTPase activation is required [14], whereas, the slow endocytosis is exclusively controlled by the dephosphorylation and rephosphorylation cycle of dynamin 1. One of the key protein kinases, carrying out dynamin-1 rephosphorylation and, consequently, controlling the slow pathway of the endocytosis, is glycogen synthase kinase 3 (GSK3) [15]. Recycling of synaptic vesicles plays a key role in the secretion of neurotransmitters, ensuring the processes of synaptic plasticity both in the CNS and in the periphery [16–18].

The aim of our work was to study the processes of the transmitter release, as well as of exo- and endocytosis of the synaptic vesicles in the mouse motor nerve ending in the conditions of high-frequency activity in experimental DM.