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HISTO-ULTRASTRUCTURAL ORGANIZATION OF NEUROMUSCULAR SYNAPSES OF THE MASSETER IN EXPERIMENTAL DIABETES MELLITUS

Abstract. *The paper deals with a study of the evolution of histological and ultrastructural changes in neuromuscular synapses (NMS) in streptozotocin-induced diabetes (SDM). We used histological and electron microscopic methods. It was established that on the 14th -28th days of SDM reactive changes dominated in the NMS, and they are characterized by a reduced length of the synaptic contact, perimeter of the axon terminal, the number and distance between the folds of the postsynaptic membrane. In the long term of SDM (42 days) the above changes become more intense, increasing dystrophic destructive processes in axon terminals and neurolemmocytes, which is confirmed by morphometry findings.*
Key words: *SDM, neuromuscular synapsis, muscle fiber.*

Introduction. The study of structural changes in organs and tissues in diabetes mellitus (DM) is one of the urgent problems of modern medicine [2]. Ukraine has officially registered more than 1 million diabetic patients, or 2% of the adult population [9]. The sharp rise in the prevalence of diabetes requires a deep study of the problem. The universal diabetic microangiopathy is one of the most frequent prognostically unfavorable manifestations of DM in which the vessels lesions of various organs are dominant [9]. In numerous scientific publications there are thorough data about the morphological manifestations of vascular disorders [8] in skeletal muscle in case of DM. However, almost no attention is paid to morphological changes in neuromuscular synapses (NMS) in the masticatory muscles (MM) in this disease [16]. There are no publications on the dynamics of morphometric changes of components of the NMS of MM in laboratories and in particular with streptozotocin-induced diabetes (SDM).

Objective: to study the dynamics of histo-ultrastructural changes in the NMS of MM during the development of streptozotocin-induced DM.

Materials and methods. The material for the study included the MM of twenty 12 month-old male Wistar rats, which were divided into 2 groups: the experimental and the intact ones. SDM in the experimental animals was simulated with a single intraperitoneal administration of streptozotocin (dissolved in 0.1 M citrate buffer with pH 4.5) at a dose of 6 mg per 100 g of weight [6]. The level of glucose in the experimental group of animals was measured daily in a drop of blood from the caudal vein using test strips for blood glucose meters by "Assu-Shec" (Germany). We selected the animals with the glucose rate higher than 13 mg / L for our study and sampled the material on the 14th, 28th, and the 42nd day of the experiment. We used histological and electron microscopic techniques. NMS was histologically found on tangential cuts of MM by impregnation with silver nitrate after Bilshovsky Gross. For the electron microscopic examination pieces of the material were fixed in 2% osmium tetroxide solution, and contrasted by a generally accepted method. Morphometry was performed on the electron-diffraction pattern using NIH USA "Image J" manually, taking into account the zooms. The computer data processing was performed using Statistical Package Stat.Soft.Inc; Tulsa, OK, USA; Statistica 6.

Results and discussion. As our study showed, the response of the NMS in SDM manifests itself on all structural components and has a strong

dynamic. After 14 days of SDM along the myelinated nerve fibers (MNF) on the histological specimens irregular narrowings and expansions appear. At the ultrastructural level in NMS, axon terminal perimeter is significantly reduced, so is the length of synaptic contacts, as well as the width and length of active zones of the presynaptic membrane (Table. 1). In the axoplasm the matrix of mitochondria is seen and their cristas get fragmented.

Table

Morphometric characteristics of axo-muscular synapses of the masseter at different times of SDM (M ± m, n = 20)

Structural elements and their parameters	intacts	duration		
		14th day	28th day	42nd day
Terminal perimeter ,microns	7,4± 0,02	7,1± 0,01#	6,7± 0,01#*	5,3± 0,01#*
Length of synaptic contact, microns	2,7± 0,01	2,4± 0,01#	2,1± 0,01#*	1,7± 0,02#*
Number of postsynaptic membrane folds	10,5± 1,23	9,9± 1,12#	8,8± 1,12#*	7,1± 1,01#*
The distance between the folds, microns	0,22± 0,004	0,26± 0,001#	0,31± 0,001#*	0,42± 0,002#*
The length of a fold, microns	2,7± 0,11	2,5± 0,08#	2,2± 0,08#*	1,9± 0,05#**
width of the active zone, microns	0,23± 0,001	0,21± 0,001	0,19± 0,001#*	0,14± 0,001#*
length of the active zone, microns	0,81± 0,003	0,78± 0,004	0,72± 0,004#*	0,54± 0,004#*
Number of vesicles throughout the active zone	165,3± 11,7	145,6± 6,52#	129,9± 6,35#*	102,1± 5,03#*
Number of vesicles in the active zone area	10,7± 0,33	9,8± 0,29#	9,1± 0,29#*	6,2± 0,22#*

Note: 1) #P <0,05 – reliability of the difference compared to the indices in the intact animals;
*2) *P <0,05; **P <0,01 – reliability of the values compared to the previous time of the experiment.*

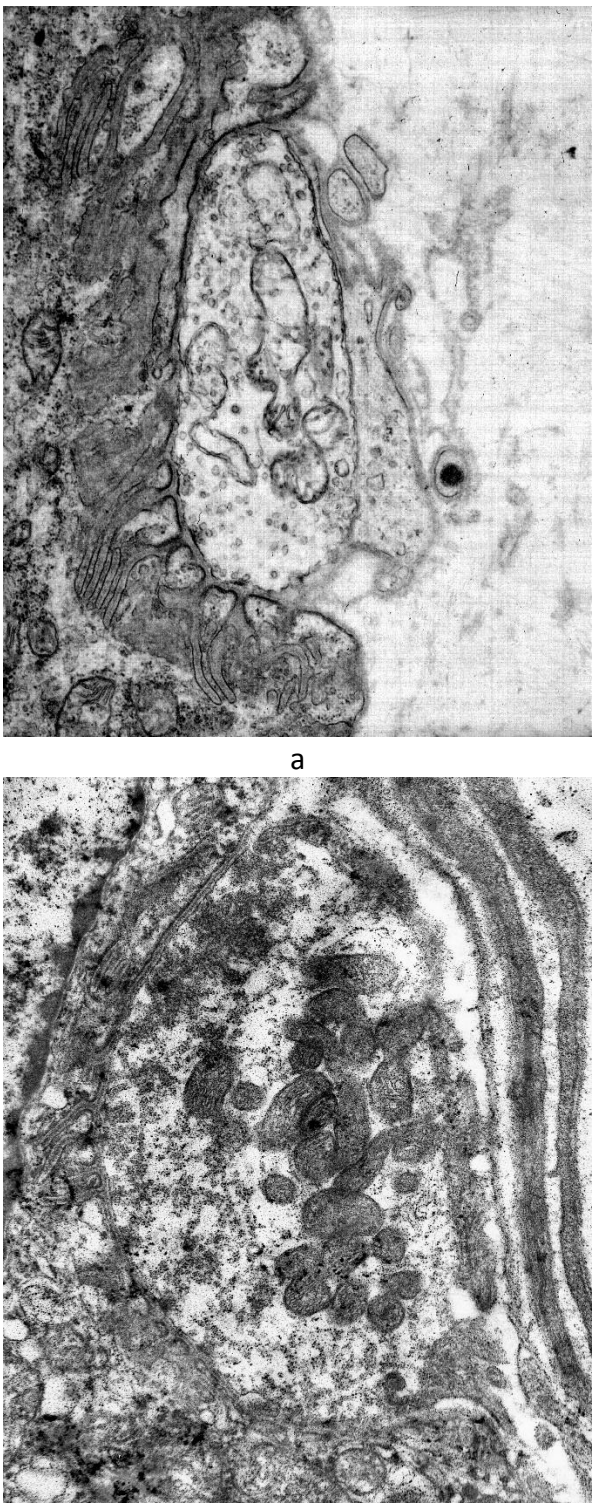
We found a swelling and separation of the myelin sheath (MS), which, according to many authors [3, 5], is a cause of its varicose. The chromatin in the neurolemmocyte nuclei condenses, a partial vacuolization of cytoplasm and the infiltration of mitochondrial matrix are observed. In the postsynaptic membrane the distance between synaptic folds increases due to their partial destruction. Changing the ultrastructural structure of the end neurolemmocytes in the experimental animals shows the development of stress reactions into a change in carbohydrate metabolism in SDM. An analysis of studies of a number of authors [1, 5] specifies that compensatory-adaptive response of neurolemmocytes results in hypertrophy of the morphological structures that are designed to ensure a sufficient level of synthetic processes in SDM.

On the 28th day from the start of simulating SDM the frequency and magnitude of MNF varicose on the histological specimens increases, particularly their preterminal departments, whereas the axon sprouting reduces. In electron microscopic examination the periaxonal space in the MNF is unevenly expanded. The degree of aggregation of filamentary-tubular structures in the axoplasm increases. This process is considered by some authors [1, 14] to be an outcome of axon transport disorders. O.S. Sotnikov and co-authors [10] indicate that neurofilaments aggregation and destructuration of microtubules occur under high acidity of the axoplasm, which, in turn, is the result of a release of oxidized products of protein

metabolism in the intercellular space, as a reflection of the distorted features of neurolemmocytes. Due to this stress reaction the cytoplasm of neurolemmocytes becomes oveloaded with lots of vesicles of different sizes [3], and the MS gets stratified [3, 14]. Degradation of MS is a nonspecific sign and a manifestation of a severe disturbance of phospholipid metabolism in the nervous system [4].

On the 28th day from the start of simulating SDM the total number of synaptic vesicles in the axoplasm of presynaptic membrane reduces. They usually have a different shape and small size. The synaptic fissure gets unevenly widened, the processes of the terminal neurolemmocytes grow into it, most of the postsynaptic membrane folds disintegrate. The number of mitochondria near the postsynaptic membrane decreases, they have small size and fragmented cristas (Fig. 1a). On the one hand it can serve as a morphological substrate of the disturbances in oxidative metabolism in SDM, on the other hand it explains the MM hypotrophy, resulting from impaired energy supply, axon transport delays, reduction of neurotransmitter and destructive changes in mitochondria [5, 11]. According to morphometry the terminal perimeter in this period decreases by 30.2%, and the length of synaptic contacts by 33.3%, resulting from a decrease in the number of subcellular components (see. Table. 1). According W.P. Hurlbrat [12] and H. Takekura et al. [15] the number of neurotransmitter vesicles and the number of mitochondria in the presynaptic axon terminal depends on synaptic neuronal activity and the axon transport speed [13].

Continuing the duration of SDM to 6 weeks leads to the destruction of certain MNF and their terminal branches, causing denervation of muscle fibers [1, 11]. Under these conditions the axoplasm is overloaded with synaptic vesicles of different diameters, and the length and number of active zones in the presynaptic membrane reduces. The length and width of synaptic contacts and of postsynaptic folds also decrease. The average area of NMS reduces compared with the control one by 25.6%, and compared to



a

b

Fig. 1. Ultrastructural changes in NMS on the 28th (a) and 42nd (b) days of SDM development: magn.: a, b) x 12000.

the last term of the experiment by 34.2% (see. Table. 1). It is noted that neurolemmocyte nuclei argyrophily increases in the area of NMS, and so does the number of these cells (satelitosis).

Conclusions. As a result of the study, histo-ultrastructural and morphometric changes in

the early stages of streptozotocin-induced diabetes were identified, and their pattern points to a close interaction between the neuromuscular endings and muscle fibers. It is shown that structural changes in neuromuscular endings depend on the duration of diabetes and include two stages: the first stage (14-28 days) reactive changes are observed, the second one (42 days) is dominated by degenerative processes.

Prospects for further research A comprehensive study of the patterns of changes in NMS and other constituents of MM in SDM.

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