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MORPHOMETRY OF SYNAPTIC VESICLES OF THE NEUROMUSCULAR JUNCTION UNDER DIFFERENT CONDITIONS OF TRANSMITTER RELEASE

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The axon terminal of the neuromuscular junction is the classical object for study of the structural and functional aspects of neurotransmitter secretion and its disturbances [3]. The abundant experimental data on which the quantum vesicular theory is based [4] have been obtained mainly on this object. Meanwhile there is evidence that is incompatible with this hypothesis [5]. Investigations of the vesicular apparatus of the axon terminal of the intact neuromuscular synapse in different functional states have also yielded contradictory results.

The aim of this investigation was to study the ultrastructure of the neuromuscular junction when functioning under different conditions, by means of a morphometric method.

EXPERIMENTAL METHOD

Experiments were carried out on August rats weighing 100-120 g. During the experiments the rats were anesthetized with ether. The diaphragm muscle was fixed in situ by injecting the cold formol-sucrose fixative into the peritoneal and both pleural cavities. Fixation was carried out 20 min after unilateral division of the phrenic nerve, i.e., in the "resting" state, and also during supramaximal electrical stimulation (square pulses, frequency 50 Hz) of the nerve isolated in the neck. Stimulation began 20 min after division of the nerve. A method of fixation preceded by freezing in situ [2] also was used both "at rest," during supramaximal electrical stimulation of the phrenic nerve, and also during physiological contraction and relaxation of the diaphragm during breathing. Postfixation of the material was carried out in buffered OsO_4 solution, followed by dehydration in ethyl alcohol and acetone, and embedding in Araldite. Ultrathin sections were cut on the LKB-III Ultrotome and examined in the JEM-7A electron microscope. The electron micrographs thus obtained were analyzed by means of a modified Leitz ASM apparatus for semiautomatic image analysis (West Germany). The perimeter of the synaptic vesicles (SV) was measured; for statistical analysis this was converted into volume as being more appropriate for the physiological significance of the parameter. Since, as was shown previously, formaldehyde during fixation causes release of synaptic transmitter [1], the number of SV was counted in material fixed after preliminary freezing, in the zone 200 nm wide adjacent to the presynaptic membrane. Student's and the Kolmogorov–Smirnov tests were used for statistical comparisons.

EXPERIMENTAL RESULTS

Analysis of the electron micrographs showed that supramaximal electrical stimulation of the phrenic nerve causes changes in the ultrastructure of the neuromuscular junction (Fig. 1a, b). These changes were manifested in the axon terminals as widening of elements of the smooth endoplasmic reticulum, displacement of the mitochondria closer to the presynaptic membrane, an increase in the number of SV in contact with the presynaptic membrane (Fig. 1d), marked tortuosity of that membrane, and the formation of invaginations (Fig. 1b). The synaptic folds were shortened and thickened, the synaptic cleft was irregular in width, and the electron density of its material was reduced.

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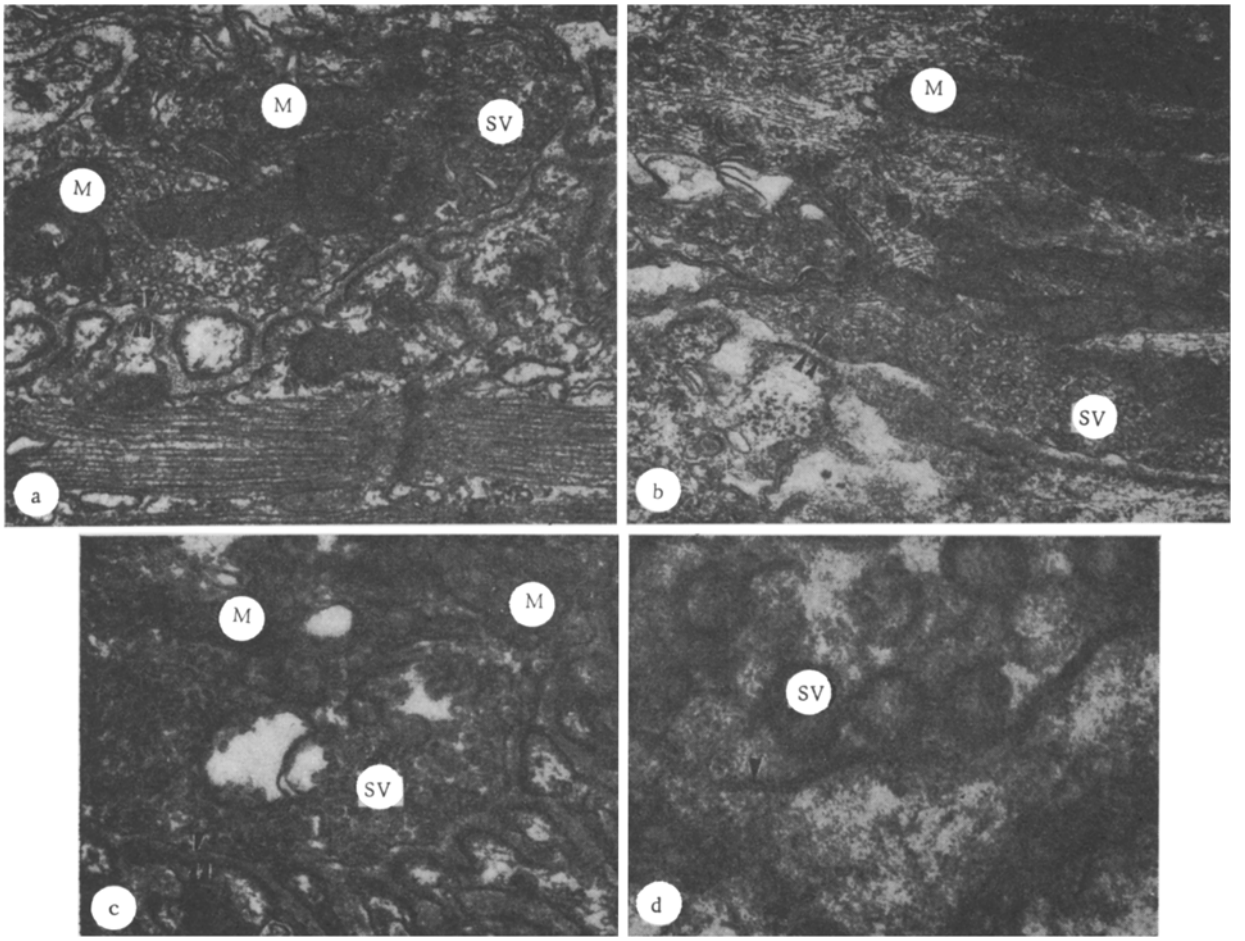

Fig. 1. Neuromuscular junction of rat diaphragm in different functional states: a) "resting" state. Fixation with formol-sucrose. 50,700×; B) supramaximal electrical stimulation of phrenic nerve with frequency of 50 Hz. Formal-sucrose fixation. 50,700×; c) "Resting" state. Fixation with previous freezing. 62,400×; d) Synaptic vesicles in contact with presynaptic membrane. Supramaximal electrical stimulation of phrenic nerve with frequency of 50 Hz. Fixation after preliminary freezing. 340,000×. M) Mitochondria. One arrow indicates presynaptic, two arrows postsynaptic membrane.

The structure of the neuromuscular junction in muscle fixed after preliminary freezing differed only a little from that observed after the use of other methods of fixation (Fig. 1c).

The distribution of SV by volume differed from the Gaussian distribution whatever the conditions of function of the neuromuscular junction (Fig. 2). In all cases considerable skewedness and scatter of the data were observed. Comparison by the Kolmogorov–Smirnov test showed that the distribution of SV by size in all the states studied differed significantly. The "resting" state was characterized by one distinct mode in the 25,000-30,000 nm^3 range. During supramaximal electrical stimulation this mode was less well marked and, in addition, a second mode appeared in the 50,000 nm^3 region. States of contraction and relaxation of the muscle during breathing were characterized by a more uniform distribution of SV by size; most lay within the 20,000-40,000 nm^3 range during inspiration (physiological stimulation of synaptic activity) and 20,000-35,000 nm^3 during expiration (physiological rest of synaptic activity).

Counting the number of SV (Fig. 3) showed that an increase in the intensity of synaptic activity leads to a statistically significant decrease in their number in the zone 200 nm wide near the presynaptic membrane.

It must be emphasized that the difference in the number or size of SV during supramaximal stimulation and during physiological stimulation was not statistically significant. This is evidence of the high structural and functional reliability of the neuromuscular

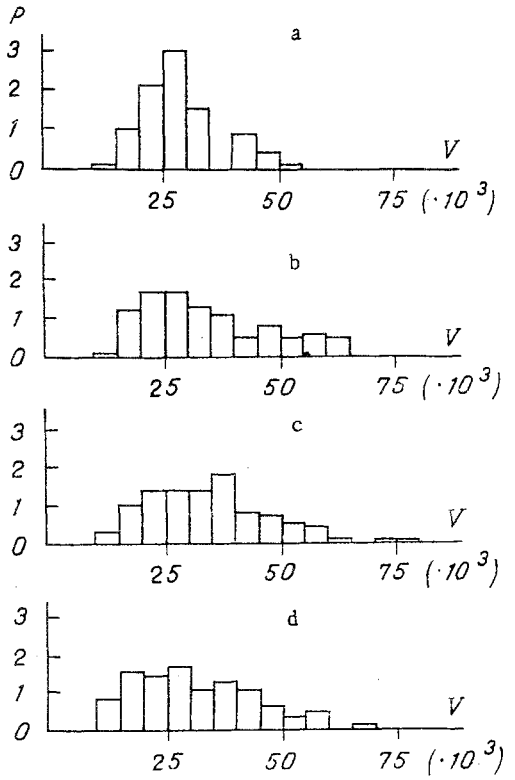

Fig. 2. Distribution of SV by volume in axon terminals of neuromuscular synapses in various functional states. Abscissa, volume of SV (in nm^3); ordinate, frequency. a) "Resting" state; b) stimulation; c, d) inspiration and expiration respectively.

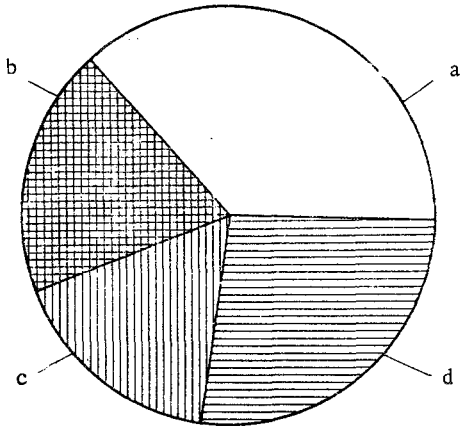

Fig. 3. Mean number of SV per 40,000 nm^2. a) "Resting" state (10.83 ± 0.34); b) stimulation (6.50 ± 0.34); c, d) inspiration (5.26 ± 0.27) and expiration (7.72 ± 0.29) respectively.

junction, due in part to the fact that SV, which are capable of exocytosis, can be quickly and adequately restored. This may perhaps be connected with the failure of previous attempts to exhaust SV pools in the intact neuromuscular synapse by prolonged stimulation. Moreover, in most investigations fixation was carried out not during stimulation but after its end, and the role of chemical fixatives as agents capable of inducing transmitter release was disregarded, not to mention the fact that chemical fixation over a long period of time compared with the rate of restoration of the pool of vesicles makes interpretation of the results very difficult.

Analysis of the data obtained by estimation of the size of SV leads to the conclusion that they constitute a heterogeneous population and that each different functional state of the neuromuscular synapse corresponds to its own type of distribution of SV by this parameter. This is evidence that heterogeneity of SV for size may reflect their different functions. In investigations conducted on the electric organ of the skate [6] heterogeneity of SV with respect to biochemical (functional) properties and correlation between the latter and the size of the vesicles also have been demonstrated.

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CARDIOMYOCYTE ULTRASTRUCTURE IN THE PERIFOCAL ZONE OF AN EXPERIMENTAL MYOCARDIAL INFARCT IN RATS TREATED WITH THE HEXAPEPTIDE DALARGIN

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Intensive research is in progress at the present time to find drugs capable of reducing the size of a zone of myocardial infarction [1, 2, 10, 14]. Much attention is being paid to the perifocal zone of the myocardial infarct as the target object for therapeutic activity aimed at restricting the spread of necrosis among cardiomyocytes [6, 9]. Enkephalins are known to have an antistressor action and to prevent release of various hormones that participate in the catabolic phase of stress, as well as preventing the peripheral action of catecholamines [4, 7]. The authors showed previously that administration of the hexapeptide dalargin to rats after coronary artery occlusion leads to a decrease in size of the infarct [8].

The aim of this investigation was to determine the most effective dose of dalargin in experimental myocardial infarction and also to assess its effect on cardiomyocytes of the perifocal zone surrounding the infarct at the ultrastructural level.

EXPERIMENTAL METHOD

Myocardial infarction was induced in 76 noninbred albino rats weighing 180-200 g by ligation of the descending branch of the left coronary artery in its upper third. The rats of the experimental groups received dalargin by intraperitoneal injection in doses of 10, 50, 100, 500, and 1000 μg/kg and rats of the control group received physiological saline 1 h after ligation. The rats were decapitated 24 h after coronary occlusion and the size of the zone of infarction in the left ventricle was determined by demonstration of phosphorylase activity followed by planimetry [15], and also by the macroscopic reaction with nitro-BT followed by gravimetry [3]. Specimens of myocardium for electron microscopy were excised from the region around the infarct, fixed, and embedded in Araldite in the usual way. Some

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