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CHANGES IN RELATIONS BETWEEN QUANTUM-VESICULAR PARAMETERS WHEN SECRETION OF SYNAPTIC TRANSMITTER IS DISTURBED IN MAMMALIAN NEUROMUSCULAR JUNCTIONS

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Data which evidently are opposed to the quantum-vesicular hypothesis of secretion have recently been obtained as a result of the development of new, notably biochemical approaches to the study of the mechanisms of secretion of synaptic transmitter [8, 10]. This has drawn added attention to its basic principles, especially in relation to exocytosis of transmitter from the vesicles, as the basis of the quantum character of secretion [7-10]. One of the most valid methods of testing these principles is assessment of the structural and functional correlation under the influence of factors causing oriented changes in the state of the secretion process. In the investigation described below this type of analysis was used to study the mammalian neuromuscular junction poisoned by tetanus toxin (TT), which inhibits the liberation of transmitter [1, 2, 4]. To test the hypothesis of formation of "quanta" of transmitter within the cytoplasm, in the synaptic vesicles (SV), some parameters of SV were compared in motor endings and in spontaneously arising "quantum" reactions — in miniature end-plate potentials (MEPPs).

EXPERIMENTAL METHOD

August rats weighing 100-120 g were used. The test objects were isolated preparations of the diaphragm and phrenic nerve, placed in a controlled-temperature (35°C) chamber through which was passed carbogenized (95% O_2 + 5% CO_2) Tyrode's solution of the following ionic composition (in mM): Na^+ 150; K^+ 2.7; Ca^{2+} 2.0; Mg^{2+} 1.0; Cl^- 145.7; HCO_3^- 12.0; $H_2PO_4^-$ 1.0; glucose 11.0. MEPPs were recorded intracellularly, using glass microelectrodes filled with KCl solution (2.5 M), with a resistance of 10-20 MΩ, selected for low noise level. Electrical activity was photographed from an oscilloscope screen. For electron-microscopic investigation the diaphragm was fixed successively in formaldehyde (4%) and osmium tetroxide and embedded in Araldite. TT in a dose of 2×10^5 MLD for mice was injected under ether anesthesia into the substance of the diaphragm 3-3.5 h before isolation of the preparation.

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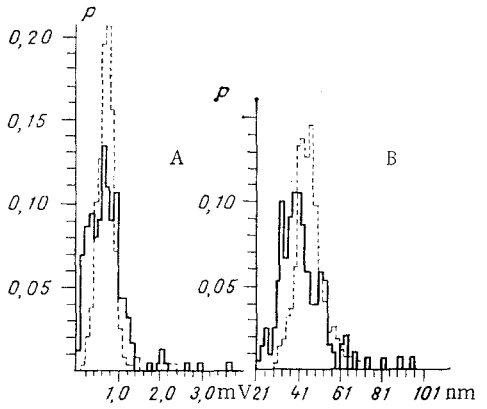

Fig. 1. Changes in character of distribution of MEPPs by amplitude and of synaptic vesicles by size in neuromuscular junctions when secretion is disturbed by TT. Frequency histograms (number of observations over 200 in each) of MEPP amplitudes (A) and diameters of synaptic vesicles (B) for intact (broken line) and poisoned (continuous line) neuromuscular junctions. Abscissa: A) amplitude of MEPP (in mV), B) diameters (circumferences equivalent to profiles of synaptic vesicles, in nm); ordinate, relative frequency.

The dimensions of SV on the electron micrograph and the amplitudes of MEPPs on the traces were measured by means of a semiautomatic Leitz A. C. M. image analysis system. The total number of SV in the terminal was estimated from their mean number per unit volume, reckoning that in electron micrographs of ultrathin sections (60-70 nm) all vesicles within the volume of that particular section of the terminal are visible. The volume of the terminal was calculated on the assumption that it formed three spheres with diameter equivalent to the cross-section of the synaptic junction visible in the section (values obtained by measuring all sections were averaged).

EXPERIMENTAL RESULTS

Under the influence of TT the monomodal character of the curve of distribution of SV diameters and MEPP amplitudes characteristic of the normal situation were transformed into polymodal, often with the appearance of a distinct mode for lower values (Fig. 1). Under normal conditions a monomodal character of the histograms was observed in eight of ten cases for MEPPs and in ten of 12 cases for SV, whereas under the influence of TT polymodal histograms were the majority: six of seven for MEPPs and seven of nine for SV. A significant ($P = 0.01$) increase also was observed in the coefficients of variation of MEPP amplitudes (from 0.33 ± 0.05 to 0.51 ± 0.04, dimensionless) and of SV diameters (from 0.13 ± 0.01 to 0.24 ± 0.01, dimensionless).

Under the influence of TT the total number of SV per terminal increased significantly ($P = 0.05$) from $1.69 \times 10^4 \pm 0.33\ 10^4$ ($n = 10$) to $2.68\ 10^4 \pm 0.64 \times 10^4$ ($n = 9$); i.e., by about 59%, with the mean volume of the terminal unchanged: $(5.08 \pm 0.52) \times 10^9$ and $(5.30 \pm 0.63) \times 10^9$ nm^3.

The observed increase in the number of SV in images of the poisoned terminal could be due to stabilization of the presynaptic membrane under the influence of TT, preventing fixation artefacts, and in that case it would reflect the living situation characteristic of the normal state. Activation of transmitter liberation by the intact terminal observed during fixation of the neuromuscular junction with formaldehyde is known to be substantially in excess of that found during fixation after poisoning [1]. However, assessment of the number of "quanta" discharged under these circumstances shows that this fact alone is insufficient to explain the difference in the degree of filling of the intact and "tetanized" terminals with vesicles. Assuming that the preliminary action of TT on the presynaptic membrane blocks the passage of "quanta" of transmitter through it, as is shown by inhibition of synaptic activity in the poisoned synapse (the frequency of MEPP was reduced by more

than tenfold), it can be concluded that after poisoning true accumulation of transmitter takes place. This has already been demonstrated by the fact that liberation of transmitter, when inhibited by toxin, can be reactivated through the modulator components of secretion regulation, which are evidently based on the sodium pump [3]. It is interesting to note, however, that during the activating action of ouabain (in concentrations of 0.1 and 1.0 mM) the level of liberation of transmitter from the poisoned terminal, reflecting its operative reserves, is higher than that under normal conditions by approximately the same degree (by 42 and 68% respectively) as the number of synaptic vesicles [6].

At the same time, to judge from the shift of the SV and MEPP histograms, not only the liberation of quanta of transmitter, but also the "quantization" process itself, the standardization of the portions of transmitter to be released, is impaired directly or indirectly through the influence of TT. It may be that the effects of the toxin are mediated through calcium ions, the concentration of which in the cytoplasm is regulated not only by exocytosis of transmitter from the vesicles, but also by their formation. As regards the formation of quanta of transmitter, just as of its exocytosis during fusion of the vesicle with the presynaptic membrane, the analogous situation may possibly be modeled in experiments on liposomes when the factors determining the constancy of the final size of liposomal vesicles on fusion one with the other are investigated [11]. It should be noted that the process of fusion of the vesicles and their formation depend on calcium ions and the osmotic gradient, i.e., on the same factors whose regulating influences on transmitter secretion is depressed by TT [3, 5].

The estimates for parameters of MEPP and SV thus obtained and analysis of their relationships for different states of the synaptic transmitter secretion apparatus in the neuromuscular junction thus revealed certain structural-functional correlations and a shift of those correlations when secretion was disturbed, and which can be explained sufficiently without the risk of contradiction only in terms of the quantum-vesicular hypothesis. For instance, the character of disturbance of standardization of quanta (amplitudes of MEPPs) found under the influence of TT corresponds to heterogeneity of the SV pool in the poisoned terminal. Finally, the accumulation of SV in them and the corresponding increase in the operative reserves are in good agreement with the hypothetical mechanism of inhibition of secretion — by a disturbance of exocytosis of quanta of the transmitter. The results of this investigation thus evidently support the view that SV play an essential role in determination of the quantum of transmitter for secretion.

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