CHANGES IN THE ULTRASTRUCTURE OF THE NEUROMUSCULAR SYNAPSE PRODUCED BY TETANUS TOXIN

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The ultrastructure of neuromuscular synapses in the diaphragm was investigated in experiments on rats 3-5 h after injection of $2 \cdot 10^5$ MLD tetanus toxin into the muscle in situ. Changes were found mainly in the axon terminals. The most significant change was a marked increase in the number of synaptic vesicles. Meanwhile in some terminals there was an increase in the number of mitochondria and in the density of their matrix. In some synapses there were destructuve changes in the components of the terminals, including the presynaptic membrane, evidently as a result of further development of the toxic process. It is concluded that damage may occur to the presynaptic membrane through the action of tetanus toxin.

The action of tetanus toxin on the neuromuscular synapse leads to a marked decrease in its spontaneous activity [1, 3, 4]. Electron-microscopic investigations of damage to neuromuscular synapses and the effects of stimulation of the motor nerve supplying the poisoned diaphragm have led to the hypothesis that inhibition of the spontaneous secretion of mediator takes place under these circumstances as a result of a disturbance of its liberation through the presynaptic membrane and not as a result of exhaustion of its reserves.

This paper describes the results of an ultrastructural analysis of the neuromuscular synapse in the rat diaphragm when damaged by tetanus toxin, and an interpretation is given of these results to enable an evaluation to be made of the dynamics of the resulting changes.

EXPERIMENTAL METHOD

Male August rats weighing 100-120 g were used. Between 3 and 5 h before the material was taken the animals received an injection of $2 \cdot 10^5$ MLD tetanus toxin into the diaphragm. The method of injecting the toxin, the choice of the dose and the period of exposure, together with the results of an electrophysiological investigation, will be found elsewhere [3].

The diaphragm muscle was fixed in situ by injecting buffered formol-sucrose solution into the thorax and abdomen of the rat anesthetized with ether. Ten minutes later pieces excised from the synaptic zone of the diaphragm were placed in cold formol-sucrose for 1-1.5 h, rinsed, fixed in buffered osmium tetroxide solution for 1 h, dehydrated, and embedded in Araldite. Sections negatively stained with uranyl acetate and lead citrate were examined in the JEM-7A electron microscope.

The poisoned diaphragm muscle was fixed in situ in the resting state (unilateral division of the phrenic nerve 20 min before fixation) and also against the background of respiration. Neuromuscular synapses from the diaphragm of intact rats, fixed in the same way, served as the control.

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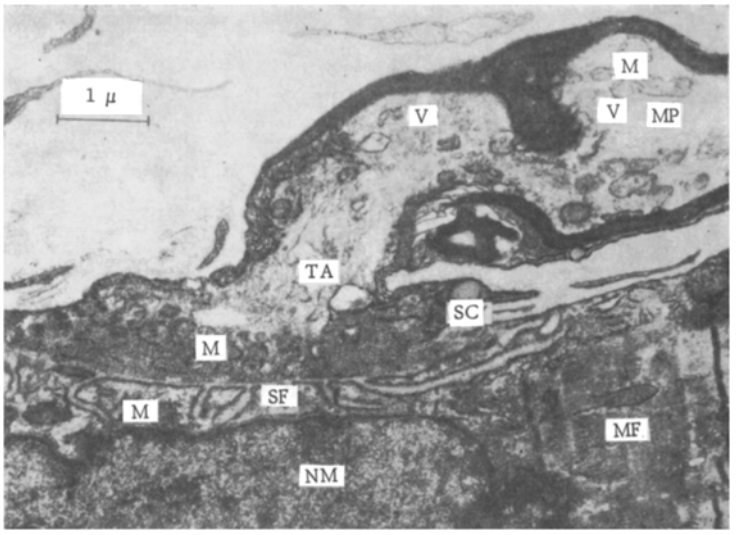

Fig. 1. Neuromuscular synapse from diaphragm of a rat poisoned with tetanus toxin, fixed 20 min after division of the phrenic nerve. TA) axon terminal; MP) myelinized preterminal; MF) muscle fiber; SC) process of Schwann cell; NM) nucleus of muscle fiber; V) vesicles in myelinated preterminal; M) mitochondria; SF) synaptic folds.

EXPERIMENTAL RESULTS AND DISCUSSION

As was pointed out earlier [3], the general structural plan and, in particular, the mutual relations of the cells, are undisturbed in neuromuscular synapses subjected to the action of tetanus toxin (Fig. 1). However, changes in the subcellular structures, mainly in the presynaptic part, are found in them.

In most synapses investigated the axoplasmic membrane, including the presynaptic membrane, was clearly defined throughout its extent. By contrast with the control, large vesicular structures are found fairly frequently not only in the axon terminals, but also in unmyelinated preterminals, where they sometimes formed chains, and even in the myelinated part of the axon as clusters (Fig. 1). In axon terminals of resting synapses the synaptic vesicles were very densely arranged and as a rule occupied all the free space (Figs. 1 and 2A). The overwhelming majority of them were of the usual shape, size, and electron density. Longer and compound vesicles and short tubular structures also were seen.

One difference in synapses fixed without preliminary division of the nerve was a rather smaller increase in the number of vesicles which were uniformly distributed in the terminal. The number of lengthened and compound vesicles in the terminals was relatively large (Fig. 2B). Some synaptic vesicles, including some of the lengthened ones, were in contact with the presynaptic membrane ("contact" vesicles). However, despite the marked increase in the total number of vesicles, there was no evident increase in the number of contact vesicles.

Mitochondria in the axon terminals were more numerous (Fig. 2A, B). Most were located near the central axis of the terminals, where they formed compact clusters and frequently touched each other with their outer membranes. The matrix of the mitochondria had increased electron density so that they stood out clearly against the background of the synaptic vesicles. Among the mitochondria there were many filamentous forms, some of them branching. Mitochondria also were more numerous in the preterminals and in the cross sections of the myelinated axons (Fig. 1). The outer membranes of the dense mitochondria were indistinct in some places. Often in these areas pictures interpreted as migration of vesicles from the mitochondria were observed. In some mitochondria vesicular structures could be distinguished against the background of the electron-dense matrix (Fig. 3).

The synaptic space was uniform in width and it included areas with diminished electron density. In the synaptic folds circular electron-dense structures were sometimes found (Fig. 2B). The postsynaptic membrane had its usual structure throughout its extent. In the organelles of the end-plate of the synapse

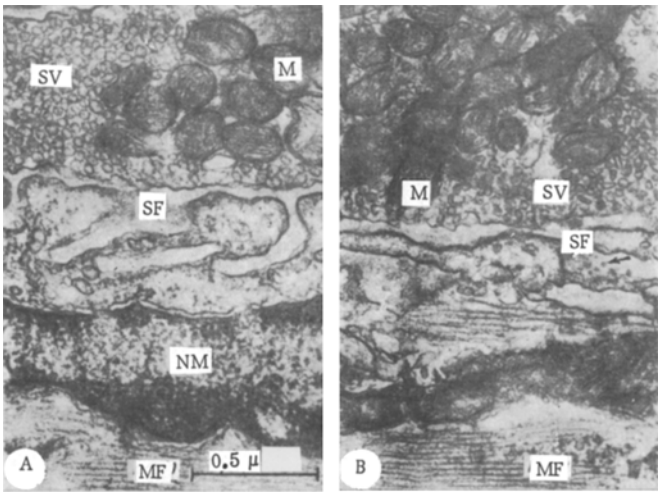

Fig. 2. Area of neuromuscular synapse from diaphragm of a rat poisoned with tetanus toxin, fixed 20 min after division of phrenic nerve (A) and against the background of respiration (B). SV) Synaptic vesicles in axon terminal; M) mitochondria in axon terminal; MF) myofibrils; arrows denote dense inclusions in synaptic folds. Remainder of legend as in Fig. 1.

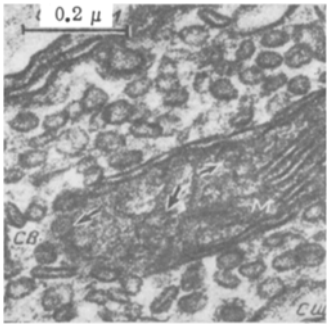

Fig. 3. Area of axon terminal from rat diaphragm poisoned with tetanus toxin. M) mitochondrion; SV) synaptic vesicles; SS) synaptic space; arrows denote vesicular structures in mitochondrion.

and in the myofibrils no special features could be distinguished. Furthermore, in some synapses whose terminals also were filled with synaptic vesicles, the mitochondria showed signs of degeneration, including swelling, partial or complete translucency of the matrix, and destruction of the cristae (Fig. 1). The axoplasm of these terminals was dark, granular, and vacuolated. The presynaptic membrane was weakly stained and indistinctly outlined against the background of the dense axoplasm and adjacent synaptic vesicles. The synaptic space was uniform in width but the electron density of its material was slightly greater at the periphery near the pre- and postsynaptic membranes.

Finally, in the terminals of some synapses which also were filled with vesicular structures and contained a larger number of mitochondria, which were in various stages of degeneration, the synaptic vesicles in close contact with each other were deformed and their membranes were unevenly stained. The presynaptic membrane also was not stained throughout its extent. Sometimes the terminals consisted of conglomerations of indistinct and irregularly shaped vesicles and modified mitochondria, which appeared not to be separated from the synaptic space and were irregular in width and electron density. Only an increase in the electron density of the matrix and in the number of granules in the mitochondria was found in the organelles of the end-plate and contractile apparatus of the muscle fibers.

To identify the initial component of the pathogenic effects of the toxin it is very useful to try to estimate the dynamics of development of the changes observed, which is determined both by the time elapsing after injection of the toxin and by the distance of the synapses examined from the site of injection.

This description of the ultrastructure of the neuromuscular synapses shows that the effects of tetanus toxin extend principally to presynaptic structures. The uniformity of the course of the developing lesion is shown by the fact that even severely altered terminals, their function evidently ended, demonstrate the characteristic features of tetanus poisoning: a marked increase in the number of synaptic vesicles and mitochondria.

The most interesting neuromuscular synapses, in the writers' opinion, were those in which it was not yet possible to find degenerative changes, for it was they which evidently were responsible for the inhibition of synaptic activity revealed by electrophysiological investigation [1, 3, 4]. The increased number of synaptic vesicles in the axon terminals of these synapses can be explained by a decrease in consumption of the mediator as the result of the action of tetanus toxin. However, some features (the appearance of vesicles in unmyelinated and myelinated preterminals, the state of the mitochondrial apparatus) indicate that increased formation of mediator or, more exactly, of vesicles takes place simultaneously, and in any case, the character of the metabolic processes in the terminals is changed [5]. As the cause of the decrease in spontaneous synaptic secretions it is therefore postulated that the liberation of mediator from the presynaptic structures is disturbed. No special features could be discovered in the interaction between the synaptic vesicles and presynaptic membrane. However, when the changes in the presynaptic structures caused by the action of the toxin were far advanced, the presynaptic membrane was irregularly stained or it even could not be detected. It can accordingly be concluded that the presynaptic membrane is one of the structures selectively attacked by tetanus toxin and that in the earlier stages of action of the toxin it also may be damaged to some extent.

The relatively frequent finding of ultrastructural pictures which some workers [2] interpret as the formation of synaptic vesicles from mitochondria on the electron micrographs of the damaged germinals is interesting. Since contact between synaptic vesicles and the outer membrane of the mitochondria is frequently observed in the terminals of neuromuscular synapses it seems likely that it is an essential stage in the formation of synaptic vesicles connected with the consumption of energy. The writers consider that pictures simulating the formation of vesicles from mitochondria occur when contact takes place in areas in which the outer membrane of the mitochondria is destroyed or is ill defined, as was the case in the present investigation (Fig. 3). So far as the discovery of vesicular structures inside the mitochondria is concerned, they may arise as a result of fragmentation of the cristae.

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