ULTRASTRUCTURAL CHANGES IN THE SPINAL CORD PRODUCED BY TETANUS TOXIN

G. N. Kryzhanovskii,* V. N. Grafova, V. P. Tumanov, and B. V. Vtyurin

UDC 616.981.551-092.9-07:616.832-091.8-07

In local tetanus induced in albino rats by injection of toxin (0.1 MLD) into the muscles of the hind limb, ultrastructural changes indicating marked functional strain on the cell were observed in the motoneurons of the lumbosacral enlargement on the side of injection of the toxin. Most synapses on the bodies of the motoneurons had an increased number of elongated synaptic vesicles and features of an inactive state. Signs of pericellular edema were present. These changes could be detected on the third day after injection of the toxin, but they were most marked on the 10th day; they were absent on the opposite side. Increased electrical activity (EA) in the muscles, indicating considerable functional activity of the motoneurons, and the clinical picture of local tetanus (muscular rigidity), which were clearly visible on the third day, still persisted at the maximal level on the 10th day. On the 20th day these ultrastructural changes were virtually undetectable, but the increased EA and muscular rigidity still persisted, although they were weaker. Normal EA was restored and the contracture disappeared much later.

Tetanus is an example of molecular pathology [3], and for that reason the search for ultrastructural changes which can reflect the pathogenic processes at the subcellular level is of great importance.

The object of the present investigation was to make an electron-microscopic analysis of components of the spinal reflex apparatus in local tetanus.

Few electron-microscopic studies have been made of the CNS in tetanus [14, 15, 18], and their results are contradictory.

EXPERIMENTAL METHOD

Albino rats were used. Local poisoning of the spinal cord with associated local tetanus was induced by injection of tetanus toxin (0.1-0.2 MLD) into the muscles of the left hind limb. Under these conditions the tetanus toxin traveled along the regional nerves to the anterior horns of the lumbosacral segments of the spinal cord on the side of injection [3]. The toxin was injected at several points into the muscles of the thigh and leg so that a more uniform distribution of toxin in the spinal cord was obtained (along several nerves). The tests were carried out on the 3rd, 5th, and 20th days and later after injection of the toxin. The controls consisted of intact animals and the muscles of the right hind limb of the experimental rats for the electrophysiological investigation and the right side of the lumbosacral enlargement for the electron-microscopic investigation. Muscle potentials were recorded by coaxial electrodes on a "Disa" electromyograph.

Before the material for electron-microscopic investigation was taken, the animals were perfused with 2.5% glutaraldehyde solution in phosphate buffer, pH 7.4. Pieces shaped like small columns were excised

*Corresponding Member, Academy of Medical Sciences of the USSR.

Laboratory of Pathophysiology of Toxico-infections, Institute of Normal and Pathological Anatomy, A. A. Vyshnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 75, No. 5, pp. 100-106, May, 1973. Original article submitted April 23, 1972.

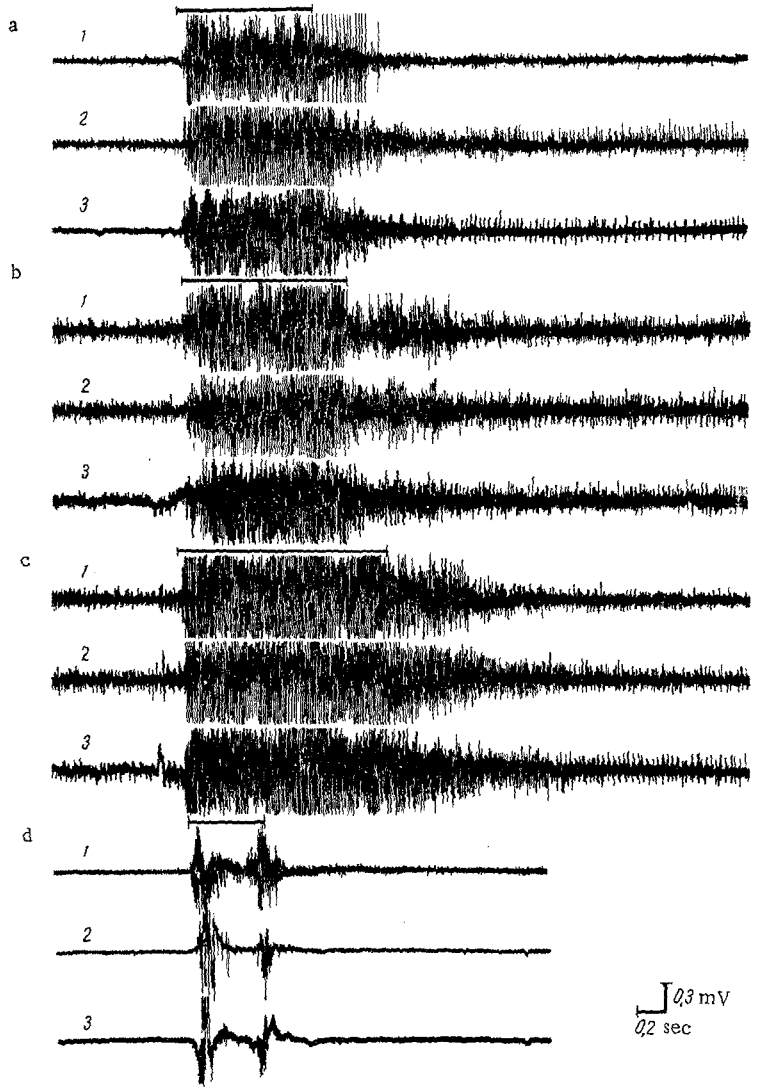

Fig. 1. Electrical activity (EA) in muscles of hind limbs in local tetanus: 1) EA in posterior group of thigh muscles, 2) in anterior group of leg muscles, and 3) in gastrocnemius muscle 3 (a), 5 (b), and 10 (c) days after injection of tetanus toxin (0.1 MLD) into above-mentioned muscles of left hind limb; d) EA in analogous muscles of opposite limb recorded on 10th day after injection of toxin. Spontaneous EA and bursts of EA provoked by application of stimuli (squeezing the foot) to affected (a, b, c) and opposite (D) limbs. Moments of stimulation marked by horizontal line.

from the anterior horns of the lumbosacral enlargement of the spinal cord, prefixed with OsO_4, and embedded in Araldite; sections were examined in the IÉM-100V electron microscope.

EXPERIMENTAL RESULTS

A marked increase in electrical activity (EA) characteristic of local tetanus [3], consisting of a strong burst of activity in response to the application of provocative stimulation, with a long after-effect and a raised spontaneous ground (Fig. 1), was observed in the muscles of the affected limb. These changes in EA were observed 3 days after injection of the toxin and recorded on the 5th and 10th days. The severity of the clinical manifestations of local tetanus (muscular rigidity and fixation of the joints) reached its maximum on the 3rd-5th day and then remained unchanged for 10 days.

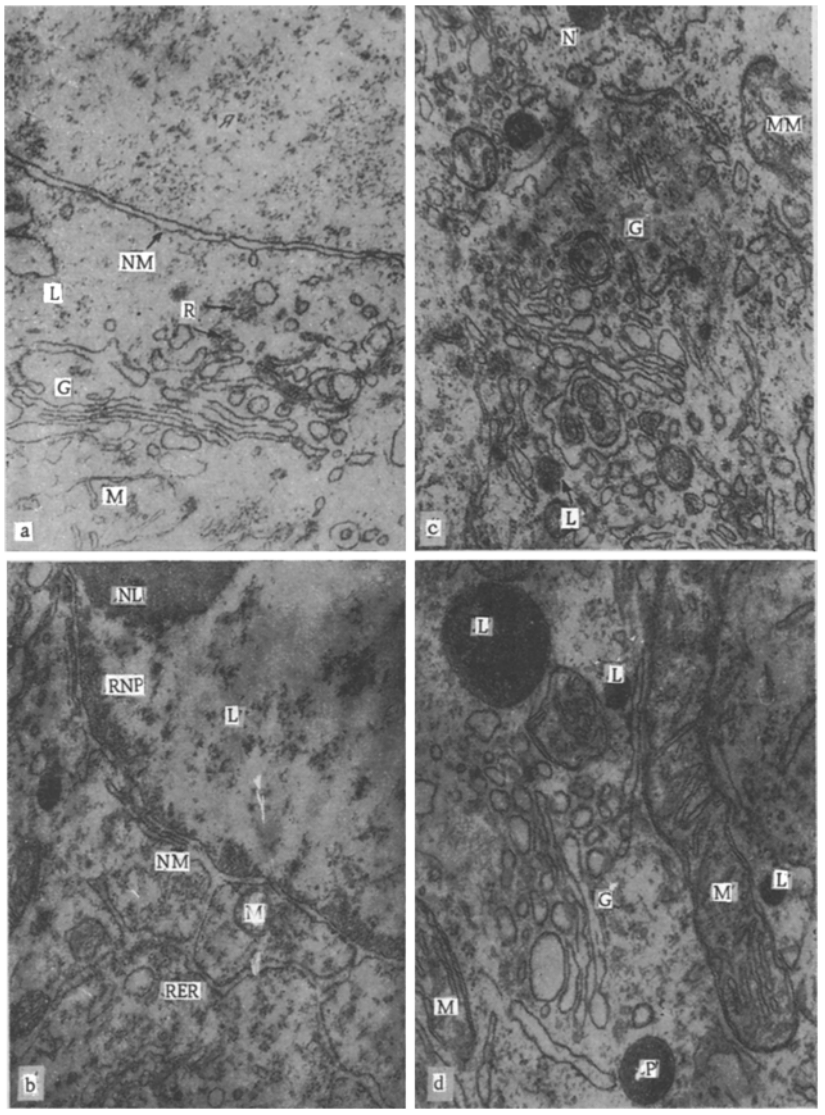

Fig. 2. Ultrastructural changes in spinal motoneurons in local tetanus: a) motoneuron with large nucleus poor in chromatin (N); nuclear membranes (NM) clearly outlined; cytoplasm contains few ribosomes (R) and polysomes (P); hypertrophy of Golgi apparatus (G); mitochondria (M) with translucent matrix (× 65,000); b) motoneuron with pale nucleus (N) and chromatin (RNP) on inner membrane (NM), ectopia of nucleolus (Nl). RER) Round endoplasmic reticulum (× 45,000); c) Golgi apparatus (G) on motoneuron. Hypertrophy and hyperplasia of cistern and tubules, increase in number of lysosomes (× 45,000); d) Golgi apparatus (G), lengthened mitochondrion (M) with pale matrix, (L) lysozomes (× 70,000).

After 20 days or later weakening of the rigidity and normalization of the increased EA of the muscles of the affected limb were observed.

Results of the electron-microscopic investigation of the spinal motoneuron of the control animals and motoneurons of the experimental animals on the side opposite to injection of the toxin showed a clear picture of the architectonics of the organelles, the ultrastructure of which continued to exhibit regular shapes. The nucleus had a high content of chromatin, as a rule with a central nucleolus. Most motoneurons were in a state of normochromia; their mitochondria had regular cristae and a fairly dense matrix. Two main types of synaptic boutons could be observed in the neuropil: The boutons of type 1 contained agranular vesicles with a circular outline measuring about 250-350Å, those of type 2 contained flat, elongated agranular vesicles, with a mean width of 300-500Å. Synaptic boutons with flat vesicles as a rule contained a few round vesicles,

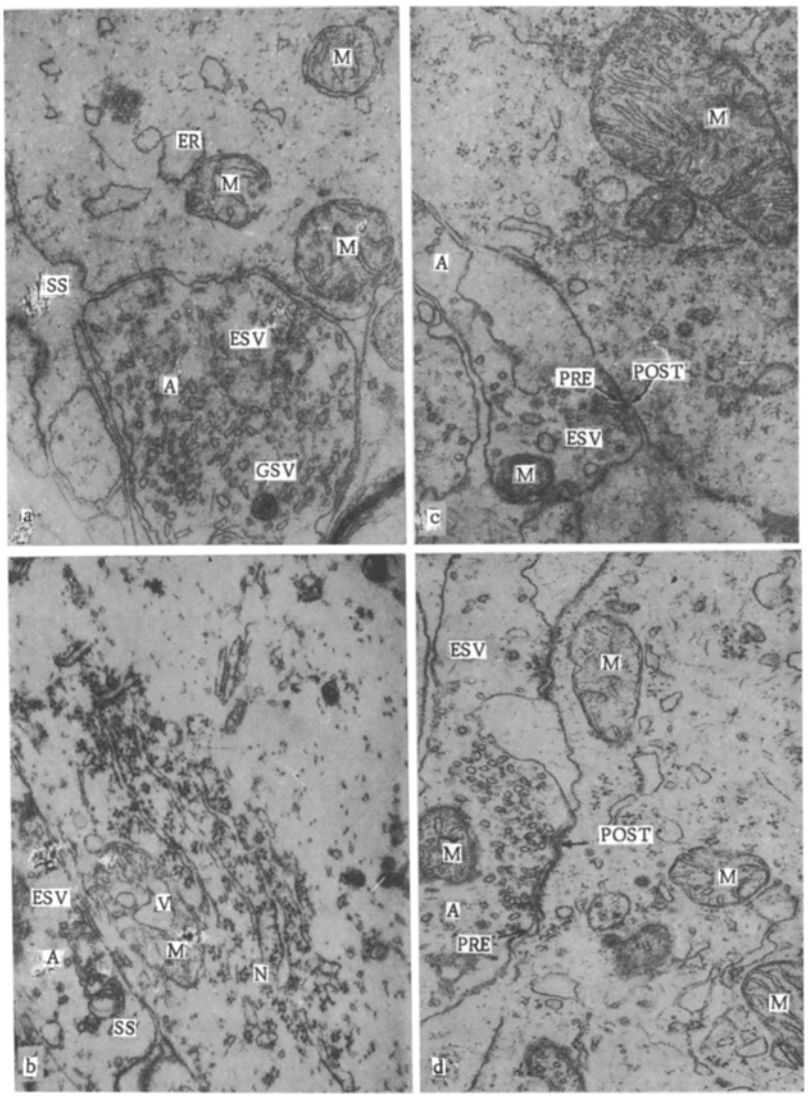

Fig. 3. Ultrastructure of axosomatic synapses on spinal motoneurons in local tetanus: a) Axosomatic synapse in "passive" state; presynaptic terminal (A) contains numerous flat, elongated agranular vesicles (ESV), a few granular synaptic vesicles (GSV), synaptic space (SS) in inactive state; dilated tubules of endoplasmic reticulum (ER) in cytoplasm of motoneuron, × 50,000; b) flat elongated agranular vesicles in presynaptic terminal, synaptic space in inactive state; synapse located in part of motoneuron body where there is a swollen mitochondrion (M) containing a vacuole (V), N) Nissl's body (× 45,000); C) pericellular edema, detachment of presynaptic terminal from body of motoneuron whose cytoplasm is denuded of ribosomes and polysomes; presynaptic terminal contains flat, elongated agranular vesicles. Synaptic space (arrows) slightly widened, presynaptic membrane (PRE) condensed and thickened in active zone. POST) Postynaptic membrane, × 45,000; d) pericellular edema, detachment of presynaptic terminal rich in flat, elongated synaptic vesicles (ESV) (× 45,000).

whereas no flat vesicles were observed in the type 1 boutons. These two types of boutons contained a very few granular synaptic vesicles measuring 500-700 A.

The types of boutons described above were about equally numerous.

Investigation of the spinal motoneurons of the experimental animals on the side of injection of the tetanus toxin showed changes affecting both the body of the neuron and its components and also the synapses located on it. These changes were detectable as early as on the first day after injection of the toxin, but they were most clearly visible on the 10th day. At this time most of the motoneurons had a swollen nucleus, almost free from chromatin, but with clearly defined layers of the nuclear membrane (Fig. 2a). The distribution of nuclear chromatin on the surface of the inner nuclear membrane could frequently be seen. As a rule the nucleolus was shifted toward the inner nuclear membrane (Fig. 2b). The cytoplasm of the motoneurons contained considerably fewer ribosomes, both free and attached to membranes of the endoplasmic reticulum. This latter structure consisted of several dilated vacuoles and cisterns. The Golgi apparatus was hyperplastic. An increase in the number of lysosomes was observed both in the cytoplasm and in the zone of the Golgi apparatus. The mitochondria in some neurons were swollen and their matrix was translucent but in others the changes were less marked (Fig. 2c, d). As a rule the axosomatic boutons (Fig. 3) contained flat, elongated, agranular synaptic vesicles with a few round, agranular synaptic vesicles; sometimes solitary granular vesicles up to 700 Å in diameter could be seen (Fig. 3a). The boutons described above occupied the greater part of the body surface of the motoneurons. The axosomatic contacts had very few active zones, and axosomatic synapses in a "passive" state were frequently seen (Fig. 3a). The presynaptic terminals of these synapses contained an increased number of elongated vesicles. In the postsynaptic zone, in the cytoplasm of the motoneuron, mitochondria in a swollen state (Fig. 3b) could be seen, and some zones of the cytoplasm were denuded of ribosomes and polysomes. The synaptic space varied in width from normal (150-200 Å) to 300 Å. The membrane of the presynaptic ending was frequently somewhat thickened (80-90 Å; Fig. 3c). Mitochondria with a fairly dense matrix were present in the presynaptic region (Fig. 3c, d). Signs of pericellular edema (Fig. 3a, c) and swelling of the processes of the glial cells were frequently visible around most motoneurons.

The results of these investigations thus showed that in local tetanus no gross ultrastructural injuries are observed. The changes found in the motoneurons show that these cells were in a state of considerable functional stress. These results agree with those of electrophysiological investigations showing that at these times after administration of tetanus toxin there is a marked increase in EA of the muscles, indicating equally considerable activity of the motoneurons. The changes observed in the motoneurons are not specific in character, for similar changes can be observed in other states [1, 5, 6, 13].

No gross morphological changes were observed in the presynaptic apparatus of the axosomatic synapses. A curious feature was the presence of comparatively many elongated synaptic vesicles and the relative increase in number of synapses with oblong synaptic space were observed in these synapses more frequently than usual. Since axosomatic synapses of this type with elongated synaptic vesicles are considered [9, 12, 16, 17] to be inhibitory, the patterns observed probably reflect blocking of the activity of inhibitory synapses, characteristic of the action of tetanus toxin [3, 7, 8, 10, 11]. In this connection the results of the writers' previous investigations [4] can be cited, when the number of presynaptic vesicles in the presynaptic terminals was considerably increased during disturbance of the liberation of mediator by the presynaptic apparatus of the neuromuscular system of the diaphragm after administration of a large dose of toxin. The results now described also agree with those of an investigation [2] showing that in local tetanus there is a statistically significant decrease in the number of argentophilic axosomatic synapses.

The signs of pericellular edema were evidently secondary in character, for they occurred chiefly in the later stages of the pathological process. It should also be noted that processes of the glial cells frequently entered the synaptic space, with the consequent detachment of the presynaptic parts of the synapse from the postsynaptic. Both these phenomena may play a supplementary pathogenetic role in the disturbance of synaptic conduction.

The fact that the ultrastructural changes described above are no longer visible on the 20th day of the experiment is interesting and deserves special analysis; meanwhile the EA in the muscles persists at a high level even after this period and does not return to normal until the 60th-70th day.

LITERATURE CITED

1. B. V. Vtyurin and V. P. Tumanov, Byull. Éksperim. Biol. i Med., No. 10, 108 (1971).
2. Yu. Ya. Geinisman, M. V. D'yakonova, and G. N. Kryzhanovskii, Byull. Éksperim. Biol. i Med., No. 11, 71 (1967).
3. G. N. Kryzhanovskii, Tetanus [in Russian], Moscow (1966).
4. G. N. Kryzhanovskii, O. N. Pozdnyakov, M. V. D'yakonova, et al., Byull. Éksperim. Biol. i Med., No. 12, 27 (1971).
5. A. A. Manina, Folia Morph., 20, 38 (1972).
6. D. S. Sarkisov and B. V. Vtyurin, Electron Microscopy of Destructive and Regenerative Intracellular Processes [in Russian], Moscow (1967).
7. Yu. S. Sverdlov, Fiziol. Zh. SSSR, No. 8, 941 (1960).
8. Yu. S. Sverdlov, Neirofiziologiya, No. 1, 25 (1969).
9. J. C. Eccles, The Physiology of Synapses, Berlin (1964).
10. V. B. Brooks, D. R. Curtis, and J. C. Eccles, J. Physiol. (London), 135, 655 (1957).
11. D. R. Curtis and W. C. de Groat, Brain Res., 10, 203 (1968).
12. E. G. Gray, J. Anat., 97, 101 (1963).
13. A. B. Novikoff, in: The Cell, New York (1961), p. 299.
14. C. Peracchia, Lab. Invest., 15, 479 (1966).
15. C. Peracchia, Path. et Biol., 15, 132 (1967).
16. K. Uzhizono, cited in: Structure and Function of Inhibitory Neuronal Mechanisms, Oxford (1968), p. 21.
17. J. C. Yates and R. D. Yates, J. Ultrastruct. Res., 16, 382 (1966).