EFFECT OF DEXAMETHASONE ON NEUROMUSCULAR SYNAPSE ULTRASTRUCUTRE

A. N. Studitskii, T. P. Seene, and M. M. Umnova

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The neuromuscular synapse is the region of contact between the axon of a neuron and a muscle fiber. A presynaptic part, represented by the axon terminal, and a postsynaptic zone, represented by the sarcoplasmic part of the muscle fiber, separated by the synaptic cleft, can be distinguished in its structure [10]. In mammals the neuromuscular synapse is bush shaped. This similarity is determined by the postneuronal folds, which the sarcolemma of the muscle fiber forms around the axon terminal. A characteristic feature of the presynaptic part of cholinergic synapses is the presence of numerous pale synaptic vesicles with a smooth surface, containing bound acetylcholine [2, 8]. The presence of these coated vesicles explains the process of recycling of the membrane of the secretory vesicles [3, 8]. In the last decade evidence has been published of the influence of glucocorticoids on neuromuscular synapses [6, 11]. One of the complications of prolonged steroid therapy is muscular weakness, the possible causes of which are catabolic processes induced in muscles by adrenocortical hormones [7, 13, 14]. Data have been obtained which show that the action of glucocorticoids is dose-dependent. Electrophysiological studies have shown that the use of glucocorticoids in small doses improves neuromuscular transmission, probably due to release of acetylcholine from nerve endings. Adminsitration of the steroid in large doses reduces the reliability of neuromuscular transmission due to its possible effect on postsynaptic structures [6, 11].

This paper describes an investigation of neuromuscular synapses at the ultrastructural level after administration of the synthetic glucocorticoid dexamethasone (DM).

EXPERIMENTAL METHODS

Adult male Wistar albino rats weighing 250 g received intraperitoneal injections of DM in a dose of 100 μg/100 g body weight daily for 10 days. Since the structure of synapses is often linked with the type of muscle fiber [12], pieces of muscles for the investigation were excised from regions of the quadriceps femoris muscle of the experimental animals which appeared visually to be white and red. Muscle fibers with a glycolytic type of metabolism (fast white) and an oxidative-glycolytic metabolism (fast red) were distingiushed in the composition of the test muscle. Material was fixed with a 2.5% solution of glutaraldehyde in 0.1 M phosphate buffer, and then postfixed with a 1% solution of OsO_4 in the same buffer.

EXPERIMENTAL RESULTS

The study of ultrathin sections of white muscle fibers from the experimental animals received injections of DM showed that the presynaptic part of neuromuscular synapses is extended oval-shaped and filled with many synaptic vesicles 30-60 nm in diameter. Single vesicles about 100 nm in diameter are found. The vesicles are distributed freely in the axoplasm, but some are adjacent to the presynaptic membrane. Only a few coated vesicles are present. In the presynaptic part of the ending proliferation of mitochondria by division along the cristae and by budding of small daughter mitochondria with single cristae from large

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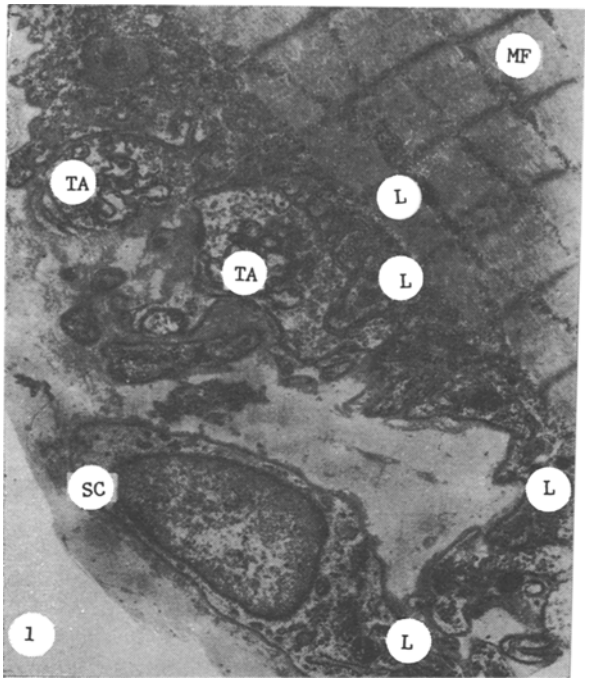

Fig. 1. Neuromuscular synapses on white muscle fiber (10,000×). TA) Axon terminal, MF) muscle fiber, L) lysosome-like structures, SC) Schwann cell.

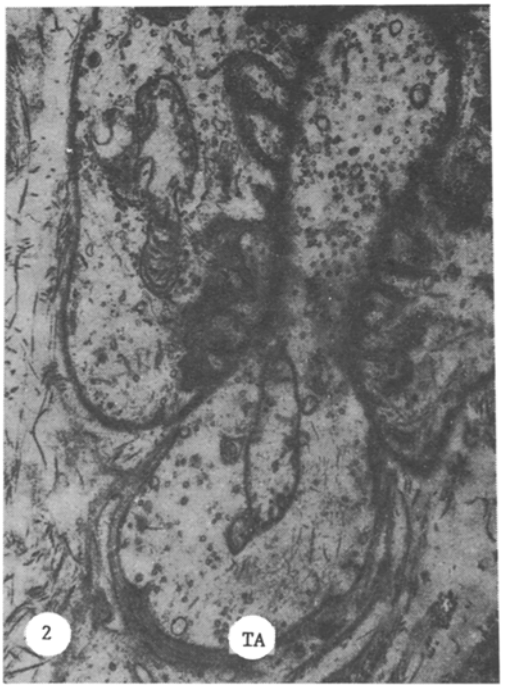

Fig. 2. Large synaptic vesicles in an axon terminal (13,750×).

organelles is observed. The mitochondria are swollen in appearance with a translucent matrix and a few cristae. A characteristic feature of the postsynaptic zone is the presence of numerous small particles of lysosome type. Lysosome-like structures sometimes form chains in the postsynaptic zone. Large lysosomes containing granular material also are found here (Fig. 1). Sometimes destruction of protofibrils and disturbance of the integrity of the sarcomeres are observed in the postsynaptic zone. Secondary lysosomes with heterogeneous

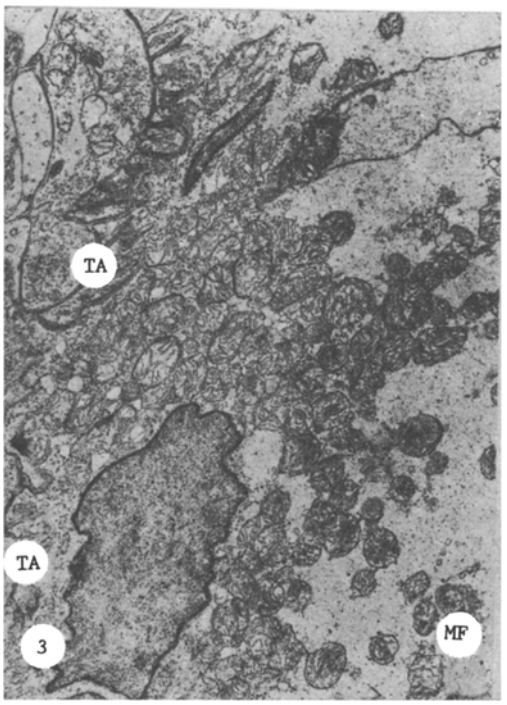

Fig. 3. Neuromuscular synapse on red muscle fiber of rat quadriceps femoris muscle (6600×).

structure are found in the interfibrillary sarcoplasm. Cytolysosomes are visible in the Schwann cells in the region of a well defined Golgi complex. Analysis of the experimental material shows that a characteristic feature of the postsynaptic region of neuromuscular synapses of white muscle fibers is the presence of only a few organelles. In areas where no muscle nuclei are present in the lane of the section, the postsynaptic zone is represented by a narrow layer of subsarcolemmal sarcoplasm, containing single mitochondria, a Golgi complex, separate vesicles, and numerous glycogen granules. In the region of the muscle nuclei the postsynaptic zone widens. Shallow postneuronal folds, with few branches, are arranged perpendicularly to the muscle fiber and almost reach the myofibril.

Besides small synaptic vesicles and coated vesicles, large vesicles with a smooth surface, 100 nm or more in diameter, also are found in the presynaptic part of the neuromuscular synapses on red muscle fibers (Fig. 2). Sometimes these vesicles or vacuoles lie adjacent to the presynaptic membrane. Small myelin figures may be present in individual terminals. Synaptic vesicles are formed in the synaptic cleft. Occasionally widening of the synaptic cleft is observed because of invagination of the presynaptic membrane inside the terminal, and in that region fibrillary structures and vesicles may be seen. Large mitochondria with a pale matrix, and often with numerous cristae, are present in the plane of the section in nearly all terminals. Neurofilaments are visible in many terminals. Few synaptic vesicles are found in terminals containing many mitochondria and filaments. After administration of DM many mitochondria located in the postsynaptic zone have a translucent matrix, some of them undergo vacuolar degeneration, and the space between the outer and inner membranes in individual mitochondria is widened (Fig. 3). Dilated tubules of the rough sarcoplasmic reticulum are visible in the postsynaptic region, and ribosomes are absent in some parts of it. In this zone there is much free sarcoplasm because of destruction of some organelles, including myofibrils. Neuromuscular synapses of red muscle fibers contain many organelles in the postsynaptic region, short postneuronal folds, dividing dichotomously at the ends, are arranged around the terminals, and the shape of the terminals varies.

The response of the neuromuscular synapses of white and red muscle fibers to administration of DM is thus basically similar. At the ultrastructural level changes are observed in the pre- and postsynaptic zones of the synapses. Large synaptic vesicles 100 nm or more in diameter are often found in the presynaptic part. They are found more often in synapses of red muscle fibers. The presence of large vesicles in the terminals is linked with the

absence of secretory activity of the nerve endings and they are regarded as neurotransmitter depots [3]. Meanwhile the appearance of large synaptic vesicles has been noted during regeneration of axons [4], and after administration of DM some investigators have observed lengthening of terminals and enlargement of motor nerve endings at the light-optical level [15]. We consider that in the present experiments the existence of large vesicles and a few coated vesicles in the synapses can evidently be connected with depression of functional activity of the nerve endings due to injection of DM. Migration of functionally unidentified vesicles into the synaptic cleft may perhaps be evidence of a change in permeability of the presynaptic membrane. Widening of the synaptic cleft probably disturbs neuromuscular transmission. DM has a dual action on the mitochondrial apparatus, causing destruction of some organelles and stimulating their proliferation both by the formation of new mitochondria and by enlargement of existing forms, partially, it seems, on account of edema [1]. In the postsynaptic region (and this can be seen particularly clearly in white muscle fibers) there are many morphologically identifiable lysosomes and derivatives of the lysosomal system. Investigators who have studied other tissue systems in vitro observed activation of lysosomes in response to addition of glucocorticoids to the incubation medium [5].

Changes observed in the present investigation in pre- and postsynaptic structures of neuromuscular synapses in combination with destruction of myofibrils, observed in response to administration of DM to rats [9], may possibly determine one cause of steroid myopathy. It can also be concluded that the structure of neuromuscular synapses is largely determined by the functional profile of the muscle fiber. Synapses differ clearly also in the ultrastructural organization of the postsynaptic zone in experimental animals, but it is more difficult to identify them by the shape of the terminals and the character of branching of the postsynaptic folds.

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