ULTRASTRUCTURAL CHANGES IN RAT NEUROMUSCULAR SYNAPSES FOLLOWING EXPOSURE TO SPACE FLIGHT FACTORS

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The peripheral neuromuscular apparatus is highly adaptable toward various conditions of function due to the enormous adaptive and compensatory capacity of all its components and their ability to undergo rapid structural changes. The study of the effect of space flight factors, including those which act directly on the neuromuscular system, such as weightlessness and hypokinesia, is therefore of great theoretical and practical interest.

This paper describes the study of the ultrastructure of neuromuscular synapses of striated muscles serving different functions in rats exposed for 7 days during a space flight on the "Kosmos 1667" biosatellite.

EXPERIMENTAL METHODS

Pieces of muscles from the synaptic zone of the soleus, gastrocnemius, and diaphragm muscles were taken for investigation. Material was taken from six rats which had spent 7 days on board the biosatellite and from control animals kept in the animal house (seven Wistar rats, SFP colony). The animals were killed by decapitation 4-8 h after landing. The tissue was prefixed in 4% formaldehyde buffered to pH 7.4 with acetate-Veronal buffer with the addition of iso-osmotic sucrose, followed by washing in cold buffer. Postfixation was carried out for 2 h in cold 1% OsO_4 solution, and the tissue was embedded in Araldite. Ultrathin sections were stained in 15% uranyl acetate solution in methyl alcohol for 5 min and in lead citrate solution by Reynolds' method for 5-10 min.

EXPERIMENTAL RESULTS

The neuromuscular junctions of the soleus (red, postural) muscle include relatively few axon terminals with a well-developed subneural apparatus. The axoplasm in axon terminals has moderate electron density, and a variable number of synaptic vesicles, distributed over the whole area of the terminal. Mitochondria are electron-dense and ribbonlike (Fig. 1a).

Clearing of the axoplasm and destruction of its fragments, sometimes totally, can be identified in individual terminals of the neuromuscular synapses near the presynaptic membrane. The postsynaptic membrane forms multiple deep synaptic folds, sometimes anastomosing with each other, with a clearly demarcated cholinoreceptive zone. The synaptic folds also are preserved even when the axon terminals are completely destroyed.

The main results of the changes in the neuromuscular junction of the soleus muscle of the experimental rats is reduction of the area of synaptic contact due to partial destruction of presynaptic structures (axon terminals). Exposed areas of postsynaptic membrane are found with processes of Schwann cells and fibrous connective tissue elements in contact with them. Near such areas bands of electron-dense basement membrane still remain, an indication of previous destruction. Growing points of axons, surrounded by processes of a Schwann cell also are found.

In the neuromuscular synapses of the gastrocnemius muscle (a mixed muscle of the jumping complex) of the experimental rats the motor end-plates (MEP) are stretched along the muscle

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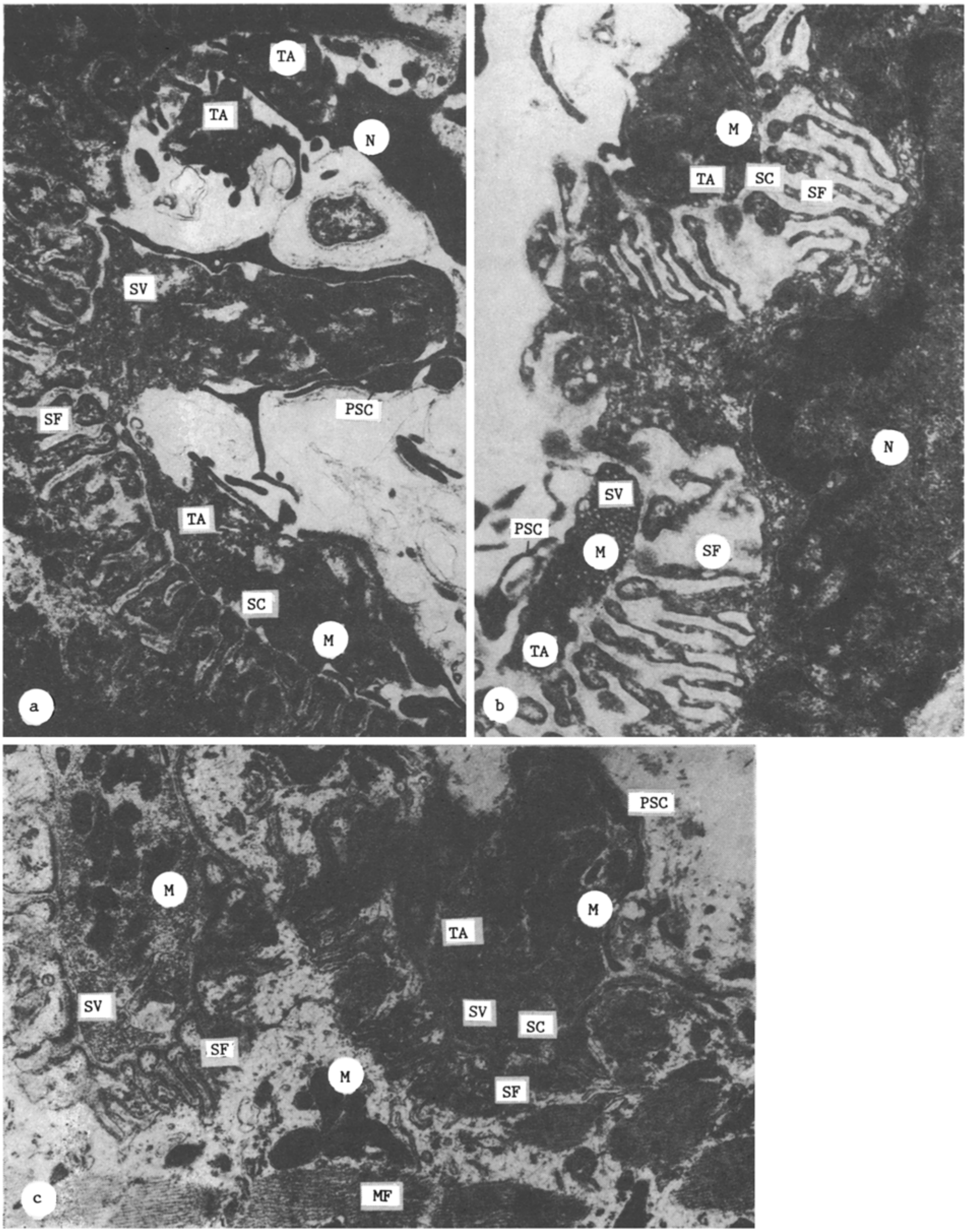

Fig. 1. Neuromuscular synapses of rats after exposure to space flight for 7 days. a) Neuromuscular synapse of soleus muscle: reduction of area of synaptic contact due to destruction of axon terminals (23,000×). b) Neuromuscular synapse of gastrocnemius muscle: many synaptic vesicles visible in axon terminals; synaptic folds somewhat deformed, shortened, and widened, and substance filling them has uneven electron density (37,000×); c) neuromuscular synapse of diaphragm muscle: presynaptic and subsynaptic regions are indistinguishable in organization from normally functioning synapses (20,000×). TA) Axon terminal, M) mitochondria, SV) synaptic vesicles, SF) synaptic folds, SC) synaptic cleft, PSC) processes of Schwann cells, MF) myofibrils, N) nucleus.

fiber and include five or six small axon terminals with axoplasm of average density. Over the whole free area of the terminals there are many synaptic vesicles closely packed together. Mitochondria are few in number. Synaptic folds in most synapses are short and unevenly widened. The ground substance of the cleft and folds is diffusely arranged. A distinct cholinoreceptive zone cannot be identified (Fig. 1b).

In some MEP, destructive changes also are taking place in the axon terminals. Areas of exposed postsynaptic membrane can be seen. At the site of former axon terminals there are processes of Schwann cells, vacuoles, and bundles of parallel bands of basement membrane material. Sometimes new growing axon points are visible in these regions, i.e., there is morphological evidence of an ongoing destructive-regenerative process.

MEP of the diaphragm (a mixed, continuously working muscle) of the experimental rats as a rule consists of two or three axon terminals. Most synapses preserve their normal structural organization, the axon terminals in them have axoplasm of average density, and many round pale synaptic vesicles are present. The mitochondria occupy a central position. No structural changes are found in the synaptic cleft or folds (Fig. 1c). Meanwhile, in some terminals the axoplasm is partially translucent, and in the base of the neuromuscular junctions the synaptic folds are disorganized. Otherwise the synaptic apparatus of the diaphragm is indistinguishable from normal.

When changes in ultrastructure of the neuromuscular synapses of the different muscles under the influence of space flight factors are evaluated, it will be noted that the structural changes taking place in them are based on a destructive-regenerative process. This is most marked in the soleus muscle, less so in the gastrocnemius, and least of all in the diaphragm.

To interpret the changes observed, the study of the ultrastructure of the neuromuscular junction under conditions of experimental and clinical pathology is of great importance, for it enables the rate and time of onset and development of the various kinds of changes in them to be determined with a high degree of reliability [1-3]. In the soleus muscle long-term postnatal plastic reconstruction of the neuromuscular synapses takes place under normal conditions also, due to the increase in weight of the animals in the course of their growth and development [4, 5].

The fact that destructive changes are intensified in the axon terminals may perhaps reflect the absence of load on this muscle under conditions of weightlessness. Meanwhile the hypertrophy of the subneural apparatus which was observed, manifested as an increase in the number of synaptic folds, may be an adaptive response to the reduced secretion of synaptic transmitter. Structural changes in the gastrocnemius muscle leads to changes in the organization of axon terminals and of the subneural region of some synapses, and are also acompanied by reduction of the area of synaptic contacts. These changes can also be linked with reduced function of the muscle due to weightlessness. The structural variability of the synapses found in the diaphragm muscle may be a morphological reflection of the enhanced level of functioning of these synapses after the animals returned to conditions of normal gravitation.

On the whole the scales of the destructive-reparative process taking place in the synaptic apparatus of the muscles studied reflects the varied degree of changes in the functions of these muscles and they are unquestionably reversible.

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