POST-TRAUMATIC DIFFERENTIATION OF SATELLITE CELLS IN SKELETAL MUSCLE

A. V. Volodina and O. M. Pozdnyakov

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During the study of post-traumatic myogenesis we directed our attention to differences in the structural organization of the satellite cells [4], which are usually linked with passage of the cell through particular stages of its life cycle or the morphological and functional state of the muscle fiber [3, 4]. However, this cannot explain all the particular features of the structural organization of the various post-traumatic satellite cells. To shed light on this problem, it was decided to undertake a detailed study of their ultrastructure under different experimental conditions.

EXPERIMENTAL METHODS

Experiments were carried out on noninbred male rats weighing 250-300 g. Trauma was inflicted after preliminary denervation, which is known to stimulate the formation of satellite cells. Under ether anesthesia the medial popliteal nerve innervating the group of muscles of the jumping complex was divided. In five animals, muscles of the jumping complex were traumatized by Cannon's method 6 days after denervation. Material for electron-microscopic investigation also was taken from animals subjected to trauma but without denervation, on the 3rd, 5th, 7th, and 10th days after trauma and also from intact animals. Pieces of the medial head of the gastrocnemius muscle were fixed consecutively in a cold solution of formol-sucrose and 1% buffered OsO_4 solution and embedded in Araldite. The material was analyzed in semithin sections and in the JEM-7A electromicroscope.

EXPERIMENTAL RESULTS

The procedures used, especially trauma preceded by denervation, are accompanied by a marked increase in the number of satellite cells compared with intact muscle. In all the experimental states studied, a sequence of stages of differentiation of satellite cells could be distinguished, culminating in the formation of myoblasts and muscle tubes. When the ultrastructure of satellite cells is characterized, its accurate correlation with the morphological and functional state of the region of the muscle fiber in whose composition they have been identified, must be noted. For instance, in severely injured fibers the satellite cells are characterized by high electron density of their cytoplasm, in which it is difficult to identify the outlines of the nuclei, and the intracellular organelles are virtually impossible to examine. Observations on a large number of such cells in the traumatic focus and of their successive stages of separation from the muscle fiber leads to the conclusion that they are incapable of further differentiation, i.e., that at a certain stage of injury to the muscle fiber, in atrophied fibers with high electron density of the myofibrils, the satellite cells are already nonviable (Fig. 1a).

Starting with the 1st-3rd day after injury to the denervated muscle, a cell population with heterogeneous ultrastructure could be seen beneath the sarcolemma of the muscle fiber, identifiable topographically as satellite cells, i.e., cells located beneath the sarcolemma of the muscle fiber. Some of them were in an undifferentiated state, and they could be classed as "slumbering myoblasts." Undifferentiated satellite cells are found less frequently in injured denervated muscles than in intact muscle. Cells preserved in this functional state are indistinguishable in their ultrastructural organization from those in intact animals. Among post-traumatic satellite cells, the population of which was significantly increased

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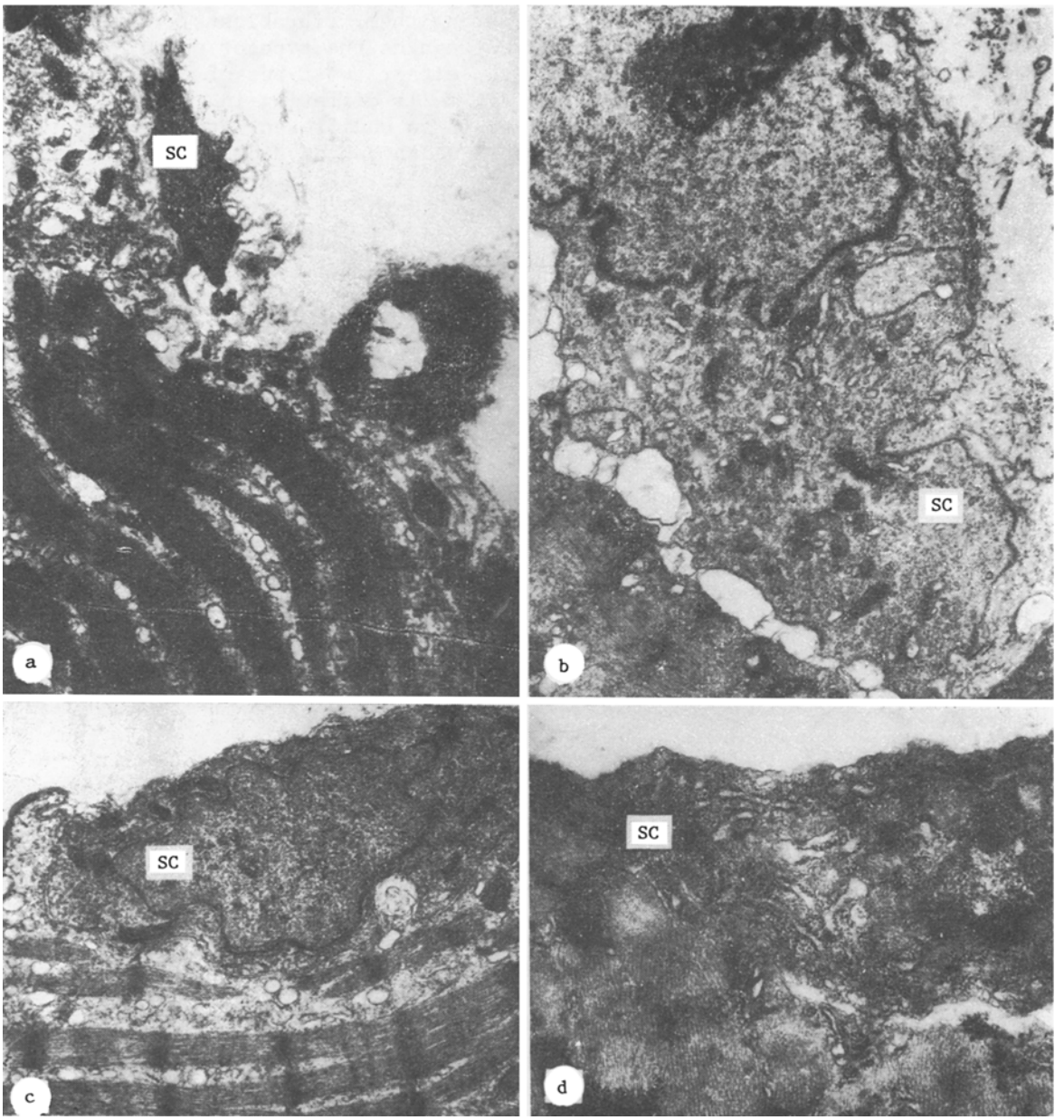

Fig. 1. Post-traumatic satellite cells. a) Degenerative form of satellite cell 15,000×); b) activated satellite cell (30,000×); c) satellite cell with myogenic direction of differentiation: numerous free ribosomes and polysomes visible in the cytoplasm (16,000×); d) satellite cell with fibrogenic direction of differentiation (20,000×). SC) Satellite cell.

on account of differentiation of the "slumbering" satellite cells and the appearance of distinct nucleo-sarcoplasmic regions of the myon (Fig. 1b) two types of cells are found, differing clearly in the organization of their protein-synthesizing structures. Some cells belong to the class actively synthesizing intracellular proteins. The cytoplasm of these cells appears granular, for it is entirely filled with ribosomes and polysomes, free or gathered into rosettes. The lamellar apparatus in these cells is well-defined. They have large nuclei and nucleoli, their karyoplasm contains decondensed chromatin-euchromatin, and their karyolemma forms numerous invaginations. This type of structure is characteristic of young cells and is evidence of activation of protein synthesis. We regarded cells of this type as differentiating in the direction of myoblast formation (Fig. 1c).

In the cytoplasm of the other type of cells (Fig. 1d) the rough endoplasmic reticulum is more richly represented, and its somewhat dilated cisterns occupy the main part of the cell. The rough endoplasmic reticulum is known to be characteristic of cells synthesizing

protein "for export," including secreting cells, among them, fibroblasts. On the basis of the morphological differences between the cells as regards the type of protein synthesis, described above, two hypotheses can be put forward: either two types of cells with myogenic and fibrogenic directions of differentiation, i.e., cells differing in their nature, are present beneath the sarcolemma of the muscle fiber in an undifferentiated state, or the satellite cell is pluripotent and is a cambial cell which can develop in at least two directions. It is impossible to settle this problem finally at the present stage because of the absence of reliable cell markers [5].

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RAT DIAPHRAGM NEUROMUSCULAR JUNCTION IN EXPERIMENTAL HYPOPARATHYROIDISM

V. G. Nikulin, V. S. Smirnova, and O. M. Pozdnyakov

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Calcium ions (Ca^{++}) play an essential role in the regulation of mediator secretion [5]. However, the bulk of the experimental data obtained by the study of the effect of Ca^{++} on synaptic transmission has been obtained in vitro [2], whereas several factors exist that require the introduction of certain corrections into results obtained on isolated nerve-muscle preparations in vitro [4]. The aim of the present investigation was accordingly to study the ultrastructure of the neuromuscular junction in experimental endogenous hypocalcemia induced by partial destruction of the parathyroid glands.

EXPERIMENTAL METHODS

Experiments were carried out on noninbred albino rats weighing 180 g. Under ether anesthesia the parathyroid glands were partially destroyed by means of a thermocautery. The effectiveness of the operation was assessed by determining the plasma calcium level before and after the operation. The diaphragm was fixed in vivo by injecting a cold solution of formol-sucrose into the peritoneal and pleural cavities of the anesthetized animal. After removal of the synaptic zone from the muscle, the tissue was dehydrated in ethanol and acetone and embedded in Araldite.

Ultrathin sections were cut on the LKB III Ultratome, stained by Reynolds' method, and examined in the JEM-7a electron microscope. The electron micrographs were subjected to morphometric analysis with the aid of the Leitz ASM semiautomatic image analyzer. The number of synaptic vesicles was counted and their perimeter measured. The perimeter of the vesicles was calculated relative to the equivalent volume of a sphere.

EXPERIMENTAL RESULTS

The most marked ultrastructural changes in the neuromuscular junction were observed on the 7th day after the operation. The cellular structure of the synapse was unchanged this

Laboratory of Experimental Pathomorphology and Laboratory of General Pathology of the Nervous System, Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.)

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