ELECTRON-MICROSCOPIC AUTORADIOGRAPHY OF DNA-SYNTHESIZING CELLS OF GROWING SMOOTH MUSCLE TISSUE IN RATS

N. A. Filippova, I. Ya. Kaufman,
Yu. L. Perov, N. T. Raikhlin,
and T. B. Timashkevich

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DNA-synthesizing cells of growing muscle tissue of the stomach after resection of 50% of the fundus, the muscle tissue of the vena cava following disturbance of the venous drainage, and muscle tissue of the appendix after constriction of the ascending colon, were studied in rats by electron-microscopic autoradiography. DNA synthesis was shown to be present in the nuclei of differentiated myocytes of the muscular coat of the stomach and of "activated" myocytes of the stomach and vena cava. Signs of asynchronous DNA synthesis were observed in the nuclei of some smooth muslce cells and fibroblasts.

KEY WORDS: electron-microscopic autoradiography; DNA synthesis; smooth muscle cells; hypertrophy of muscle tissue.

In the presence of growth-stimulating factors (injury or an increase in the functional load) acting on vascular or visceral smooth muscle tissue in sexually mature mammals, DNA synthesis can be revealed by light-optical autoradiography in 5-8% of all cells forming this tissue [2]. However, light microscopy does not reveal the degree of differentiation or the ultrastructural details of DNA-synthesizing leiomyocytes, although this is important for the understanding of the mechanisms of compensatory and reparative growth. This can be done by electron-microscopic autoradiography, which has been used successfully to study the structure of the DNA-synthesizing myocytes of the heart [3]. However, this method has not yet been used to study growing smooth muscle tissue.

It was accordingly decided to study the cells of some types of growing muscle tissues in rats by electron-microscopic autoradiography.

EXPERIMENTAL METHOD

Muscle tissue of the vena cava, stomach, and appendix of noninbred male rats weighing 240-290 g was studied. Growth of the muscle tissue of the vein was induced by constricting the vessel by the method described previously [1]; growth of the muscle tissue of the stomach was stimulated by T. B. Timashkevich's method, by resection of 50% of the fundus of the stomach [4]. Growth of the muscle tissue of the appendix was induced by constricting the ascending colon by means of a thin rubber ligature. The ligature was tied lightly to the end of a catheter 5 mm in diameter, after which the catheter was removed. All the animals (3 in each group, 9 altogether) received an intraperitoneal injection of thymidine-^{3}H (specific activity 7 mCi/mmole) in a dose of 0.5 μCi/g body weight at 10 A.M. on the third to fourth day after the operation (the period of maximal increase in the number of DNA-synthesizing cells). The rats were decapitated 1.5 h after injection of the isotope. Pieces of muscle tissue were fixed in a 2% solution of glutaraldehyde in cacodylate buffer, pH 7.4, postfixed in 1% OsO_4 solution, dehydrated in alcohols of increasing strength, and embedded in a mixture of Araldite and Epon 812.

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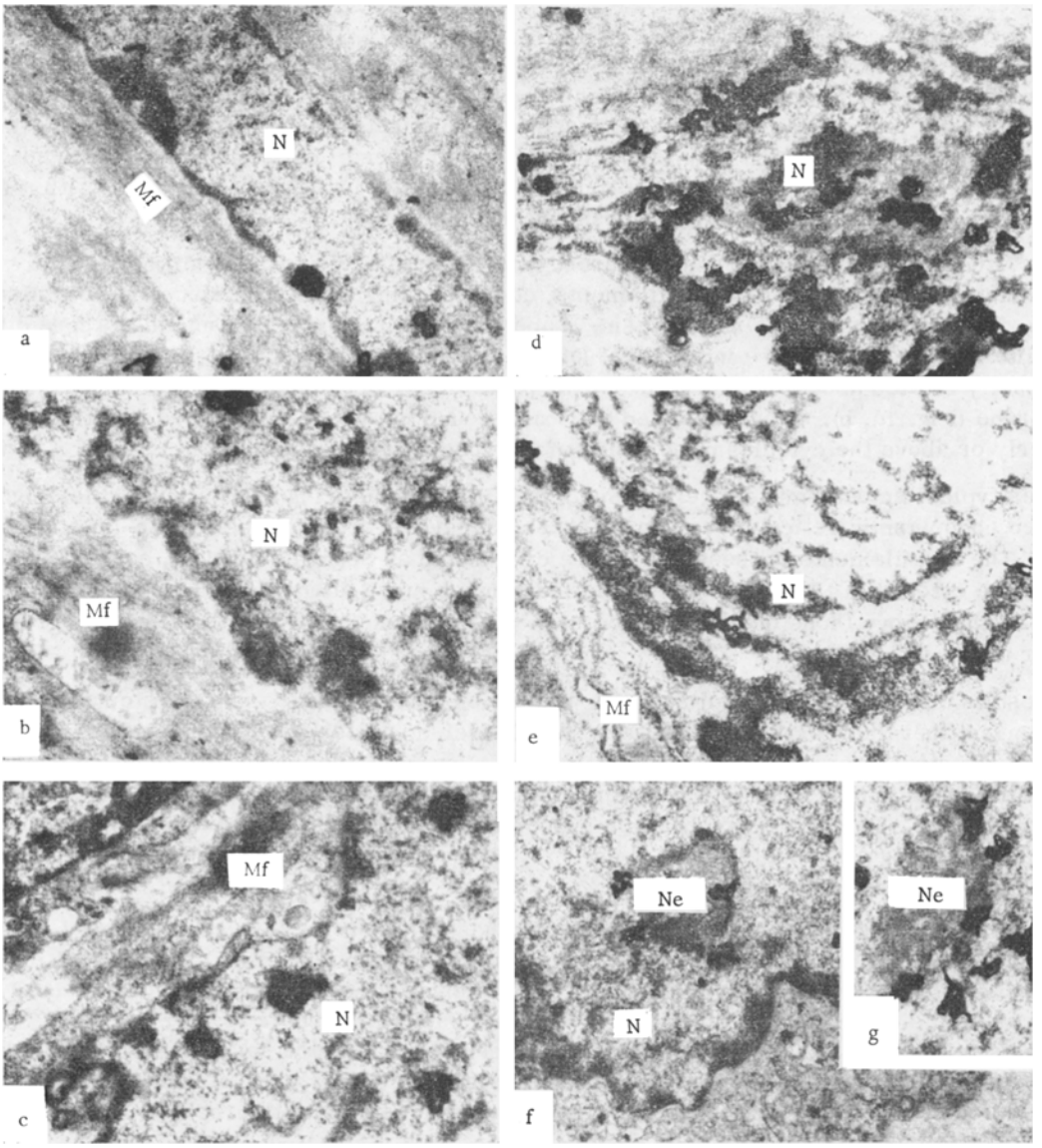

Fig. 1. Electron micrographs of DNA-synthesizing cells of growing muscle tissues of the rat stomach and vena cava. a and b) DNA-synthesizing leiomyocytes of the outer muscle coat of the stomach on the fourth day after resection of 50% of the fundus: bundles of myofilaments are clearly visible in the cytoplasm of the cells, grains of silver corresponding to sites of thymidine-^{3}H incorporation can be seen above the nuclei; a) 9,000 b) 14,300 ×. c) Activated DNA-synthesizing leiomyocyte in the stomach after operation: A group of myofilaments can be seen only in a small area of the peripheral zone of the cytoplasm; 9,000 ×. d and e) Activated DNA-synthesizing leiomyocytes from the vena cava on fourth day after disturbance of venous outflow: condensation of chromatin visible, and on the electron micrograph e grains of silver are located only above certain areas of condensed chromatin (asynchronous DNA synthesis); d) 8,500 ×, e) 14,700 ×. f and g) Asynchronous DNA synthesis in nucleus of an undifferentiated connective-tissue cell (g) and nucleus of a fibroblast (f): in both cases in both cases incorporation of labeled thymidine found in boundary region of nucleolus; f) 15,000 ×, g) 10,600 ×. N) nucleus, Nl) nucleolus; Mf) myofilaments.

Sections 1-2 μ thick were coated with type M (NIIKhimfoto) emulsion and exposed for 30 days at 4°C. Areas of these semithin sections were chosen after development of the autoradiographs and staining for subsequent study by electron-microscopic autoradiography.

Ultrathin sections were cut from the selected areas on the LKB-111 ultramicrotome, transferred to grids, coated with Ilford L-4 emulsion, exposed for 30 days at 4°C, developed in amidol developer, and studied in the JEM-7 electron microscope.

EXPERIMENTAL RESULTS

The greatest variety of cell forms was found among DNA-synthesizing cells in the growing muscle tissue of the stomach. This was mainly because the pieces for investigation were taken close to the line of resection of the organ. Besides DNA-synthesizing leiomyocytes, other types of cells incorporating thymidine-^{3}H into their nuclear structures also were found: leukocytes, fibroblasts, undifferentiated cells with a long oval nucleus and with cytoplasm filled mainly with free ribosomes and polysomes.

DNA-synthesizing leiomyocytes had different types of organelles. Some had the structure of typical differentiated muscle cells. Bundles of myofilaments, corresponding to actin in structure, and also dense bodies were clearly distinguishable in their cytoplasm. Label was observed over the nuclei of these differentiated myocytes quite infrequently and the intensity of labeling was low: 3-4 grains of silver over each nucleus. The label was usually located above the electron-denser parts of the karyoplasm, most frequently close to the nuclear membrane (Fig. 1a, b). It will be noted that background grains of silver were practically never observed extracellularly or above the cytoplasm, evidence of the specificity of labeling.

Besides typical smooth-muscle cells, among the DNA-synthesizing cells activated leiomyocytes with a hypertrophied endoplasmic reticulum and an increased number of free and membrane-bound ribosomes also were found. The myofilaments in these cells were distributed as separate bundles, sometimes only in the processes of the cytoplasm (Fig. 1c). Label was found much more often above the nuclei of DNA-synthesizing activated cells than above those of typical myocytes, and the intensity of their labeling was greater (5-8 grains of silver per nucleus).

The results of the study of serial sections of growing muscle tissue from the stomach, not coated with emulsion, suggest that these activated DNA-synthesizing leiomyocytes were mature cells with injury to their intracellular ultrastructures and with reactive changes. However, the possibility likewise cannot be ruled out that some of the activated DNA-synthesizing smooth muscle cells were precursor cells later differentiating into typical myocytes. This first hypothesis is supported by the results of a study of mitoses in regenerating muscle tissue of the dog stomach [5], which confirm that the structure of the muscle cells can undergo a wide range of phasic and reactive changes.

A group of cells with a high intensity of DNA synthesis in their nuclei was discovered in the growing muscle tissue of the vena cava. Some of these cells were located in the media of the vessel immediately beyond the inner elastic membrane, and they had bundles of filaments in their cytoplasm. The study of serial sections not coated with emulsion showed that these cells were activated leiomyocytes. In most of the DNA-synthesizing cells the media of the vein was located above electron-dense bands in the karyoplasm which had a granulofibrillary structure (Fig. 1d, e). Separate filamentous structures were visible between them. In these respects the structures mentioned in the nuclei closely resemble chromosomes with prophase changes.

In the muscle tissue of the rat appendix no DNA-synthesizing leiomyocytes could be found in the ultrathin sections. The reason was evidently the infrequent and comparatively uniform distribution of these cells in the tissue, as confirmed by autoradiography in semithin sections.

In the writers' view, the results of the study of the ultrastructure of DNA-synthesizing cells in growing muscle tissue of sexually mature animals are evidence of the widespread nature of asynchronous DNA synthesis. For instance, fibroblast-like cells with a high intensity of labeling of the boundary zone of the nucleolus were observed, whereas the rest of the karyoplasm had no label or only solitary grains of silver were present above it (Fig. 1, f, g). In the DNA-synthesizing cells of the media of the vein, individual electron-dense banded regions of the nucleus had a high intensity of labeling, but no silver grains were present above other areas lying alongside.

The investigation thus showed that during exposure of the muscle tissue of sexually mature mammals to growth-stimulating factors, the DNA-synthesizing cells may be leiomyocytes and also fibroblast-like and undifferentiated connective-tissue cells. The intensity of incorporation of thymidine-^{3}H into their nuclei is relatively high. Only negligable DNA synthesis is evidently possible in the nuclei of typical differentiated muscle cells.

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