ROLE OF INJURY IN THE APPEARANCE OF "ACTIVATED" SMOOTH MUSCLE CELLS

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In experiments on rats the ultrastructure of the muscle tissue of the inferior vena cava after disturbance of the outflow of blood, of the muscular coat of the stomach after resection of 50% of its fundus, and of the muscular coat of the cecum after constriction of its ascending portion was studied. "Activation" of smooth muscles was shown to reflect the phase of injury to the ultrastructure of the cells, followed by processes of intracellular regeneration. Analysis of the ratio between DNA-synthesizing and "activated" cells showed the local origin of the latter from differentiated myocytes.

KEY WORDS: *smooth muscle cells; ultrastructure; injury; intracellular regeneration.*

"Activated," "modified," or "myointimal" [4, 7] smooth muscle cells are the names given to myocytes in the walls of the blood vessels of sexually mature mammals whose structure differs from that of differentiated cells: A sharply increased number of myofilaments in these cells is combined with an increase in volume of the endoplasmic reticulum and of the lamellar complex. Insufficient attention has been paid to the study of the origin of these cells. It is likewise not clear how injuries are reflected in the ultrastructure of differentiated leiomyocytes, and the present investigation was carried out to study this problem.

EXPERIMENTAL METHOD

Noninbred male rats weighing 240-290 g were used. Pieces of muscle tissue for electron-microscopic investigation were taken from the muscle coat of the stomach after resection of 50% of the fundus (Timashkevich's model [2]), the muscle coat of the cecum after constriction of the ascending portion of the large intestine by means of a rubber ligature on a 5-mm catheter, and the wall of the inferior vena cava after constriction of its lumen with a silk ligature [1]. The pieces of muscle tissue of the stomach were excised immediately next to the site of resection, i.e., in a region of intensive mechanical injury; pieces of the muscle coat of the cecum were taken at a distance of 1-2 cm away from the ligature, and pieces of vein at a distance of 0.1-0.5 cm from the point of constriction. The rats were decapitated. Material taken for investigation was fixed in a buffered solution of glutaraldehyde or formaldehyde (pH 7.4) and then postfixed in a 1% buffered solution of osmium tetroxide, and then embedded in Durcupan AMC or a mixture of Araldite and Epon 812. Using glass knives, sections 1-2 μ thick were cut from the fragments of material embedded in epoxide resin, and the sites for electron-microscopic investigation were chosen in semithin sections; the number of mitotically dividing cells was determined. Semithin sections also were used for the preparation of histoautoradiographs. Ultrathin sections were cut in the LKB-III microtome, examined in the HU-11ES microscope, and photographed. The volume of cytoplasm occupied by filaments was calculated on the electron micrographs by Weibel's point volumetric method [6]. Altogether muscle tissue from 22 experimental and 6 control rats was studied; material from 12 animals (4 for each model) was taken on the 3rd to 4th day after the operation, and 1 h before sacrifice the animals were given an intraperitoneal injection of [^{3}H]thymidine (0.3 μCi/g, specific

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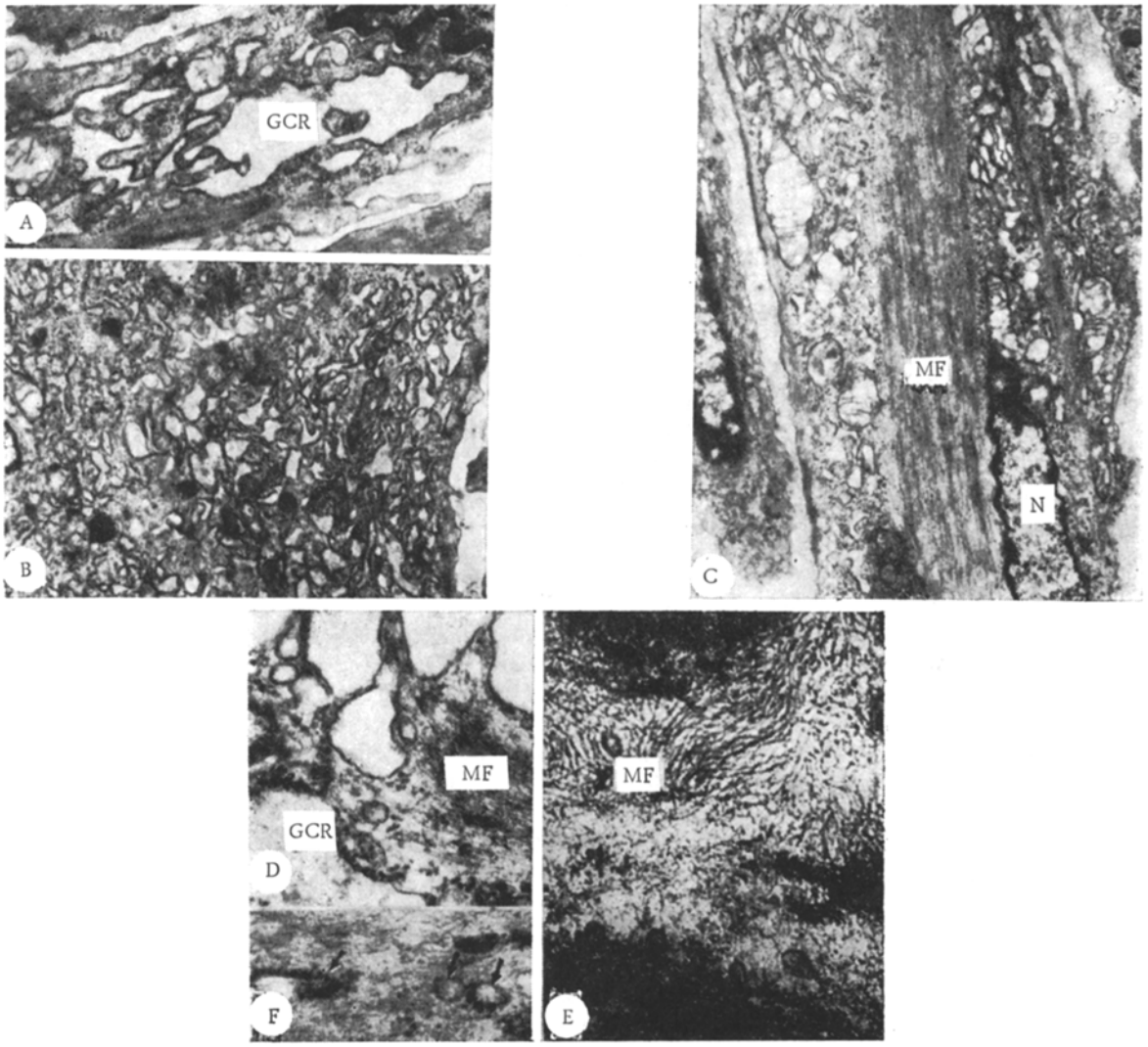

Fig. 1. Ultrastructure of "activated" smooth muscle cells of rats. A) Fragment of myocyte of hypertrophied muscle tissue of cecum with greatly dilated tubules of cytoplasmic reticulum; B) muscle cell of inferior vena cava with "frothy" appearance; C) myocyte of outer muscle coat of stomach with reduced number of myofilaments and with injury to individual mitochondria; D) fragment of myocyte from stomach with greatly dilated structures of lamellar complex and tubules of cytoplasmic reticulum; E) newly formed bundle of "coarse" filaments (filaments 100 Å in diameter) in muscle cell from vein; F) ring-shaped polysomes (arrows) in peripheral part of cytoplasm of muscle cell of stomach. A-D, F) 3rd to 4th day after operation; E) 7th day after constriction of vein. N) Nucleus; MF) myofilaments; GCR) granular cytoplasmic reticulum. Magnification: A) 4600×; B) 3200×; C) 8100×; D) 28,000×; E) 32,000×; F) 39,000×.

activity 12.7 Ci/mmole). The number of DNA-synthesizing muscle cells was counted in histoautoradiographs prepared as described previously [3] and the topography of these cells was compared with the topography of "activated" myocytes in ultrathin sections cut from identical fragments. Material was taken from 10 rats on which the above operations had been performed at later stages after the operation (from 7 to 30 days).

EXPERIMENTAL RESULTS

Few DNA-synthesizing cells with the character of smooth muscle cells were found in histoautoradiographs of muscle tissue of the stomach and veins 3-4 days after the operation: 1 or 2 to every 20-30 unlabeled myocytes in the area of the section. No mitotically dividing cells were found. DNA-synthesizing cells were found less frequently still in the muscle

tissue of the cecum: 0-1 in every 30 cells; no mitoses likewise were found. Meanwhile, examination of ultrathin sections through the muscle tissue of the stomach at the same time after the operation showed that "activated" smooth muscle cells formed large clusters each containing 10 to 20 cells in the immediate vicinity of the zone of maximal mechanical injury, as was revealed by the closeness of these cells to the zone of necrosis (cell debris). A short distance from the line of resection the number of "activated" leiomyocytes fell considerably and the muscle tissue was formed by comparatively typical (differentiated) cells.

Practically all the cells of the tunica media at this period of the experiments were "activated" in appearance. Similar cells also were seen inside the inner elastic membrane.

"Activated" myocytes were rare in the muscle tissue of the cecum, where their number was comparable with the number of DNA-synthesizing myocytes.

In all types of growing muscle tissue the "activated" muscle cells had a similar ultrastructure (Fig. 1A, B, D). In particular, injury to the mitochondria, in some of which focal destruction of the cristae (Fig. 1C) and translucency of the matrix were observed, whereas in others there was marked osmiophilia of the matrix, was characteristic; the cytoplasmic reticulum had an increased number of tubules, which were irregularly dilated and frequently empty, or contained finely granular contents (Fig. 1A, D) or amorphous condensates of average electron-optical density. These amorphous formations also were seen in the dilated saccules of the lamellar complex or they lay freely in the cytoplasm. Everywhere close to the plasmalemma and in the depths of the cytoplasm of the smooth muscles many vesicles of different shapes and sizes could be seen. A particularly characteristic feature of the "activated" leiomyocytes was a decrease in the number of myofilaments (Fig. 1A-C), which could sometimes be found only in some of the processes of the cell. For instance, whereas filaments were found in 78 ± 4.0% of the volume of the cytoplasm in the myocytes from the vein of the intact animals, on the 3rd day after disturbance of the outflow of blood they were discovered in only 42 ± 1.8% of that volume ($P < 0.01$). Meanwhile, the volume occupied by vesicular structures increased from 1.5 ± 0.5 to 8.0 ± 0.8% ($P < 0.01$). On account of this sharp increase in the size of the vesicular structures and dilatation of the tubules and cisterns of the cytoplasmic reticulum and saccules of the lamellar complex, some cells became "frothy" in appearance (Fig. 1B).

It is important to note that a "gradient" of ultrastructural changes could be identified in the leiomyocytes of the stomach depending on the location of the cells relative to the line of resection. For instance, side by side with a zone of death of the cells (cell debris) monocytes could be seen in a state of necrobiosis, with the "activated" structure of their cytoplasm. In some cells either coarse condensation of chromatin in the form of single strongly osmiophilic conglomerate or a marked decrease in the volume of the nucleus with an asymmetrical widening of the space between the two nuclear membranes was observed. At some distance from these cells sharply "activated" myocytes with the appearance of "frothy" cells were present, while still further from the zone of injury there were cells which were "intermediate" between the strongly "activated" and differentiated cells (Fig. 1C).

Besides these changes in the ultrastructure of the "activated" muscle cells, features undoubtedly indicating regenerative processes in the cell were already apparent on the 3rd to 4th day after the operation: hypertrophy of the nucleoli, an increase in the number of free and membrane-bound ribosomes. On the 6th to 7th day restoration of the contractile filaments began: In the peripheral zones of the gastric myocytes ring-shaped polysomes could be seen among the filaments (Fig. 1F), where their presence was evidently associated with regeneration of the filaments, as in the myocardium during its hyperfunction [5]. However, both in the muscles of the stomach and the muscles of the vein under these circumstances the regenerating myofilaments frequently pursued an irregular course in different directions, and together with thin actin filaments coarser and thicker filaments were found (diameter 10 nm, Fig. 1E). In the 2nd to 3rd week after the operation smooth muscle cells of the organs studied lost their "activated" appearance and their cytoplasm was densely filled with myofilaments which, in the myocytes of the vein, for example, occupied 83 ± 3.6% of the volume of the cytoplasm at that period.

The investigations showed a clear parallel between the intensity of injury to the muscle tissue and the intensity of the processes of "activation" of the leiomyocytes; these processes were most marked in regions of severe mechanical injury to the muscle tissue of the stomach and they were very ill-defined in the cecum. In the wall of the vein the intensity

of "activation" of the myocytes was approximately the same as in the resected stomach. The number of "activated" muscle cells and their topography are evidence of their local origin from preexisting differentiated myocytes, for the intensity of proliferative processes on the 3rd to 4th day after the operation in the muscle tissue of the stomach and vein was such that it cannot explain the appearance of large collections of "activated" cells by proliferation of undifferentiated precursors.

The dynamics of the changes in ultrastructure of the myocytes in the stomach, veins, and cecum described above suggests that "activation" processes reflect the phage of regeneration—restoration as a response of the ultrastructures of the myocytes to injury.

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