ELECTRON-MICROSCOPIC STUDY OF THE EFFECT OF ELECTRICAL STIMULATION OF BONE REGENERATION ON REPARATIVE OSTEOGENESIS

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KEY WORDS: reparative osteogenesis; regenerating bone tissue; electrical stimulation.

In recent years various types of electrical stimulation of reparative osteogenesis have been used increasingly often in an attempt to activate reparative regeneration of bone tissue. Theoretical and practical problems associated with the stimulating action of this factor are being intensively studied [1-7, 10]. However, the question of the effect of a weak electric current on osteogenesis and on the cells of regenerating bone tissue has not yet been studied.

The object of this investigation was to study changes in the state of the cells of regenerating bone tissue under the influence of a weak pulsed electric current, with particular reference to cell differentiation, changes in the state of the intracellular organelles, and changes in the ground substance of regenerating bone at the submicroscopic level.

EXPERIMENTAL METHOD

Resection of a fragment measuring 0.5 cm from the middle third of the radial diaphysis of rabbits was used as the model of trauma. The operation was performed under local anesthesia (20 ml of 0.5% procaine solution). Holes were drilled in each of the fragments and a platinum electrode was inserted into each of them at right angles to the long axis of the bone. The end of one electrode was placed in the gap between the fragments. The other ends of the electrodes were exteriorized and regeneration of bone was stimulated by passing a pulsed electric current through them. The total period of observation was 3 weeks. Details of the electrical stimulation technique were described previously [2, 3]. To study changes taking place in the regenerating bone tissue an electron-microscopic investigation was made of different fragments of regenerating bone. To monitor the process of reparative osteogenesis, histological preparations stained with hematoxylin and eosin and by Van Gieson's method were studied. Material for electron-microscopic investigation was fixed in a 2.5% solution of glutaraldehyde in cacodylate buffer, pH 7.4, and postfixed in osmic acid as described in [8]. Ultrathin sections were cut on the LKB-4800 Ultratome and studied in the JEM-7A electron microscope.

EXPERIMENTAL RESULTS

Histological investigation on the 7th day after trauma revealed an organizing hematoma in the gap between the fragments in both control and experimental animals, with the development of fibrocellular and, in some places, osteogenic tissue. On electron-microscopic investigation the region of the gap in the control preparations was filled chiefly by large groups of erythrocytes and fibrin threads, against the background of which single collagen fibrils could be seen. In some parts of the defect a few fibroblastic cells in various stages of differentiation were arranged among the fibrin masses and collagen fibrils. After electrical stimulation the region of the defect between the fragments was filled with osteogenic tissue, consisting of fibroblastic and osteoblastic cells lying in the developed fibrillary matrix. Some of them were undifferentiated fibroblasts, in the cytoplasm of which there were a few flattened tubules of the rough endoplasmic reticulum. Others were mature, differentiated fibroblastic cells with a developed system of tubules of the endoplasmic reticulum and a lamellar complex. The mitochondria were of different shapes with a translucent matrix and with internal cristae. The nucleus was oval in shape, with an invaginated nuclear membrane. The nucleoli were large and normal in structure.

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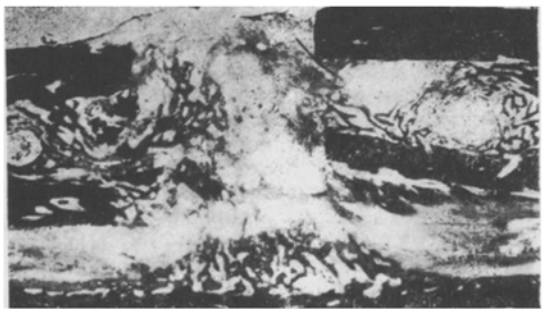

Fig. 1

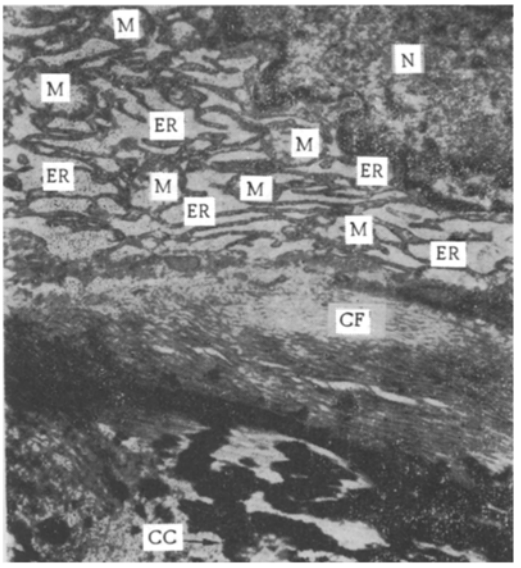

Fig. 2

Fig. 1. Regenerating bone on 14th day after operation and stimulation by pulsed electric current. Hematoxylin and eosin, 8×.

Fig. 2. Osteoblasts on newly formed bony trabecula on 14th day after operation and stimulation by pulsed electric current. N) Nucleus; M) mitochondria; ER) endoplasmic reticulum; CF) collagen fibrils; CC) calcium crystals; 7500×.

Intensive development of structures of the ergastoplasm and lamellar complex also was observed in the perinuclear zone of osteoblastic cells. Hypertrophy and hyperplasia observed in the cell organelles could be connected with the fact that at this period of regeneration the cells are involved in processes aimed at the formation of ground substance.

Histological examination 2 weeks after trauma showed that the bone defect in the experimental group was half-filled (Fig. 1) with spongy bone tissue, located near one of the fragments. The other half was filled mainly with hyaline cartilage, on the basis of which bone tissue formation could be observed. In the control series a zone of newly formed bone tissue, similar in character and maturity to the newly formed bone in the preparations of the experimental group, could be detected near one of the fragments. Areas of endochondral bone formation also were present. Newly formed bone tissue filled one-third of the gap between the fragments. The rest of the gap was filled chiefly with fibrous and cartilage tissue, whereas near the newly formed bone it was filled with fibrocellular osteogenic tissue.

Electron-microscopic investigation showed an increase in size of cell masses of osteoblastic type in the regenerating tissue of animals subjected to electrical stimulation. The osteoblasts were large polygonal cells surrounded by wide layers of collagen fibrils (Fig. 2). Their nucleus was situated eccentrically. Their cytoplasmic membrane gave off processes. Hypertrophy and hyperplasia of structures of the ergastoplasm and lamellar complex were observed. Sometimes small lysosome-like bodies were found in the region of the lamellar complex. Mitochondria were fairly numerous. They were small in size, with a moderately dense matrix. Mitochondria in osteoblasts, which were distributed on the surface of the bony trabeculae and showed a tendency to be immured in the ground substance, were large and had a transparent matrix. Small groups of hydroxyapatite crystals were observed along the fibrils, which they masked. The osteoblasts gradually began to be immured in the calcified osteoid.

The other half of the regenerating tissue consisted of hyaline cartilage. Chondroblasts were located in the uncalcified cartilage among the collagen fibrils. In the zone of calcified cartilage hypertrophied chondrocytes were seen. In their external appearance they resembled vesicular cartilage cells. This morphological appearance can be explained by the presence of widely dilated vacuoles of the hypertrophied lamellar complex. These vacuoles were filled with a substance of moderate electron density, evidently mucoprotein in nature. The vacuoles opened on the surface of the cytoplasmic membrane.

In the control animals at this time most of the defect was filled with fibrocellular tissue, consisting of fibroblastic and osteoblastic cells (Fig. 3). In small areas of cartilage tissue chondroblasts and chondrocytes

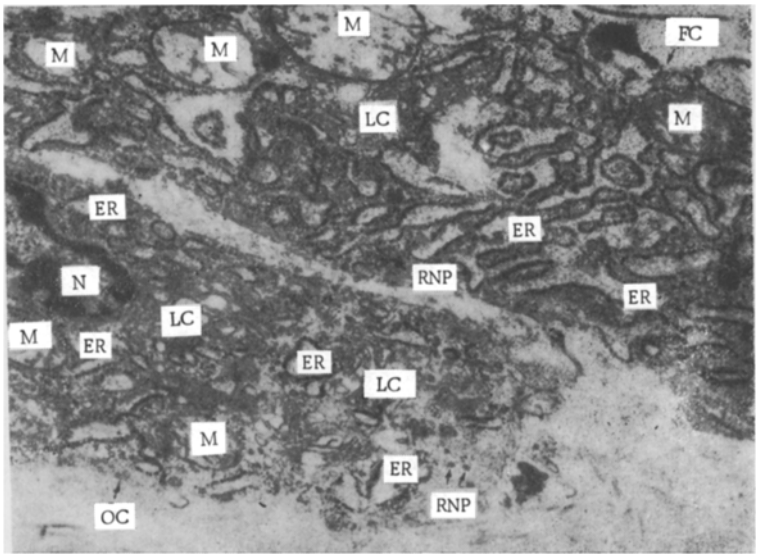

Fig. 3. Fibroblast and osteoblast on 14th day in regenerating bone tissue of animals of control group. RNP) Ribonucleoproteins; LC) lamellar complex; FC) fibroblastic cell; OC) osteoblastic cell. Remainder of legend as to Fig. 2; 30,000×.

were present. The structure of the areas of newly formed bone tissue was identical with that in the experimental series.

The bone defect in the experimental animals 3 weeks after trauma was filled mainly with spongy bone tissue, mainly fibrous and lamellar. In some places newly formed osteons could be identified.

In the control group the bone defect was only half filled with newly formed bone tissue, which was identical in character and maturity with the bone tissue of the callus in the experimental group of rabbits. It was located near the fragments. Areas of cartilage were identified nearer the center of the defect. Signs of endochondral ossification were observed. In the central part of the bone defect extensive foci of proliferation of fibrous tissue were seen.

The submicroscopic characteristics of the osteoblasts during stimulation were the same as in the bone defect after 14 days. However, the ultrastructure of the mitochondria differed even in the same cell. Some mitochondria had a darker matrix with numerous cristae, others appeared to be swollen. At this stage of regeneration intensive mineralization of the collagen fibrils with the formation of bony trabeculae was observed. The collagen fibrils were mature, with clearly defined cross-striation. Many osteoblasts located on trabeculae were surrounded on all sides by mineralized ground substance, and were converted into osteocytes.

In the regenerating bone in the control at this period newly formed bone tissue identical in ultrastructure with that described above was observed. At the same time there were large areas of hyaline cartilage and fibrous tissue consisting of fibroblastic cells and collagen fibrils.

It can thus be seen that the undifferentiated mesenchymal cells of the regenerating bone underwent more rapid processes of cell proliferation and differentiation under the influence of electrical stimulation. The increase in the intensity of cell proliferation and of mitosis in the zone of action of the electric field observed previously in tissue culture experiments [9] was thus confirmed to some extent by the results of the present investigation. In addition, compared with the control, earlier differentiation of cells characteristic of osteogenesis was observed: under these conditions it was expressed as increased hypertrophy and hyperplasia of the intracellular organelles and intensification of the secretory activity of the cells, i.e., the production of large quantities of protein of collagen type. As a result of all the changes mentioned above, the defect between the fragments was filled more rapidly with newly formed bone tissue.

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INTERACTION OF STEROID HORMONES WITH HEPATOCYTES AND THEIR PLASMA MEMBRANES

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The plasma membranes of target cells play an important role in the mechanism of the biological signal of the steroid hormones, for they are the primary systems for "recognition," binding, and incorporation of the molecules of these compounds.

Interaction between members of three groups of steroid hormones (corticosterone, estradiol, and testosterone) with rat liver cells and their plasma membranes was investigated. The choice of hormones (estradiol and testosterone, with no affinity for the liver, corticosterone with affinity for the liver) and of test objects was determined by the need to undertake a comparative (correlative) analysis of the response of the membranous structures of the liver to these steroids, for it is in these structures that the "preference systems" are found, at least for corticosteroids [3].

EXPERIMENTAL METHOD

Plasma membranes (PM) were isolated from the liver of female albino rats by the method of Dorling and Le Page [6] with modifications. The yield of PM was 1-1.2 mg protein/g tissue. The degree of purification of PM was verified electron-microscopically and on the basis of the increase in specific activity of PM marker enzymes compared with the homogenate (Table 1).

Isolated hepatocytes were obtained by the method of Kanaeva et al. [2] with modifications. The yield of cells from 5-6 g liver was 30% of the total number of cells, viz. 300-500 million. The protein content was 0.6-0.8 mg/million cells. The viability of the hepatocytes was estimated by staining with trypan blue (the number of viable cells was 90-95%) and by polarographic monitoring of their respiratory activity. Lipids for lysosome formation were isolated by Folch's method from egg yolk. A suspension of lipids for liposome formation was sonicated on an MSE (Sweden) ultrasonic disperser. Liposomes contained cholesterol and phosphatidylcholine in the ratio of 1:1 by weight.

Isolated hepatocytes, PM, and liposomes were incubated with labeled steroid hormones (corticosterone, estradiol, or testosterone, from the Radiochemical Centre, Amersham, England) in concentrations of the latter of between 0.5 and 50 nM for 30 sec or 3, 10, or 30 min. The incubation temperature was 37°C for cells and 20°C for PM and liposomes. Incubation of the cells was interrupted by filtration of the suspension through a

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