EFFECT OF POTASSIUM OROTATE ON ULTRASTRUCTURE OF HEART MUSCLE CELLS IN EXPERIMENTAL MYOCARDIAL INFARCTION

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Changes produced in the ultrastructure of the ventricular muscle cells of the heart by potassium orotate in normal rabbits and in rabbits with experimental myocardial infarction were studied. On the 7th day of administration of potassium orotate considerable accumulation of glycogen and activation of the muscle cell nuclei were observed. The number of nucleoli in the nucleus was increased and binuclear muscle cells were found. The decrease in the glycogen content and marked increase in elements of the rough endoplasmic reticulum and membranous structures, and the increased density of the matrix of the mitochondria observed on the 14th day of potassium orotate administration are the ultrastructural reflection of activation of the synthetic function of the muscle cells. The glycogen accumulation effect perhaps lies at the basis of the beneficial action of potassium orotate on the clinical course of myocardial infarction during the first days of disease.

It was shown previously that orotic acid accelerates the healing of necrotic foci in experimental myocardial infarctions [3], increases the contractile power of the fatigued heart muscle [2], and has a marked positive effect on the clinical course of myocardial infarction [4, 5, 9]. Meanwhile, the mechanism of action of this compound and the point of its action in the muscle cell have not yet been adequately explained.

The object of this investigation was to study the effect of potassium orotate on the fine structure of the heart muscle cells in experimental myocardial infarction.

EXPERIMENTAL METHOD

Experiments were carried out on 30 rabbits weighing 2.5-3 kg, divided into four groups: 1) intact animals, 2) normal animals receiving potassium orotate, 3) rabbits with experimental myocardial infarction, and 4) rabbits with experimental infarction receiving potassium orotate. The compound was given by mouth in a dose of 200 mg/kg for 6 or 13 days. The animals were sacrificed on the 7th and 14th days, respectively. Myocardial infarction was produced by ligation of the descending branch of the left coronary artery at the level of the lower border of the left auricle. Tissue was taken from the left ventricle of the animals of groups 1 and 2 for electron-microscopic investigation. In the animals of groups 3 and 4 the zone near the infarct (2-3 mm from its visible border) was studied, for its stage largely determines the course and prognosis of the myocardial infarct [6].

The material was fixed by Caulfield's method. The tissue was embedded in Araldite. Sections were stained with uranyl acetate and lead acetate by Reynolds' method. Electron micrographs were prepared on the UÉVM-100-V microscope with magnifications of 9000 to 40,000×.

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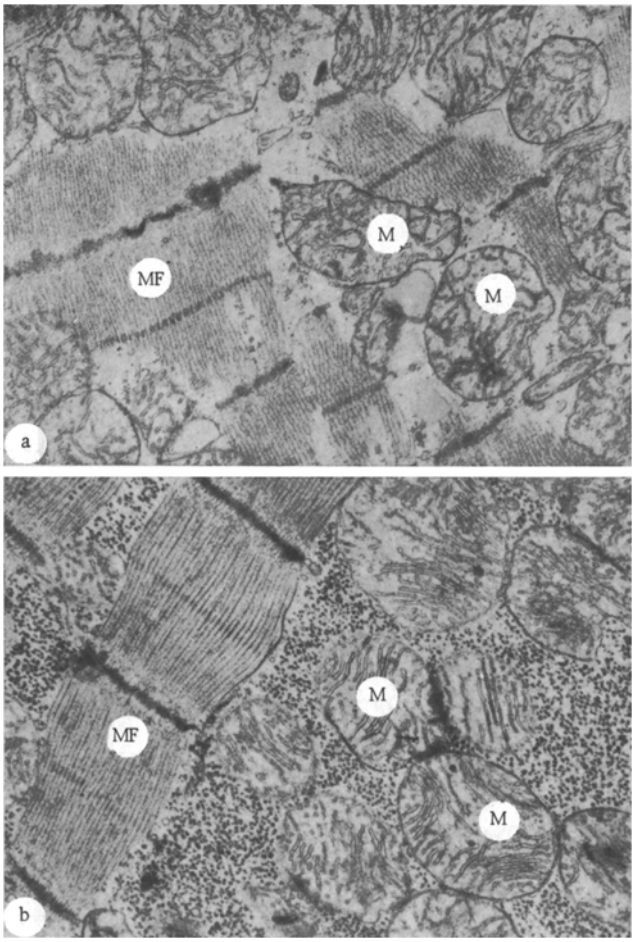

Fig. 1. Myocardium in the zone of the rabbit heart near the infarct on the 7th day after ligation of the coronary artery: a) control: swelling of mitochondria, edema of cytoplasm of muscle cell (21,000×); b) administration of potassium orotate; collection of glycogen granules (22,000×). M) Mitochondria, MF) myofibrils.

EXPERIMENTAL RESULTS

The electron-microscopic study of the myocardium of healthy animals receiving potassium orotate (group 2) showed a significant increase in the glycogen content in the muscle cells compared with normal. Glycogen granules measuring 400-500 Å, round or polygonal in shape, were diffusely arranged between the mitochondria and myofibrils. Frequently they formed curved and branch chains consisting of 8-20 granules. In some muscle fibers particularly large collections of glycogen granules were observed beneath the sarcolemma. The appearance of oval osmiophilic partials, bounded by a single membrane and resembling Palade's bodies in the atria in their structure, was particularly characteristic in the myocytes of the ventricles. Sometimes droplets of lipids 0.3-0.5 μ in diameter were seen in the muscle cells. The structure of the myofibrils and nucleus was indistinguishable from normal. The mitochondria had the typical structure and a very dense, finely granular matrix.

Electron-microscopic investigation of the muscle cells of the zone near the infarct in the animals of group 3 on the 7th day revealed considerable edema of the muscle cells, separation of the myofibrils, and disturbance of structure of the mitochondria. The mitochondria were enlarged, irregular in shape, and swollen (Fig. 1a). Their matrix was translucent and disorganized, vacuoles were present, and the cristae were fragmented.

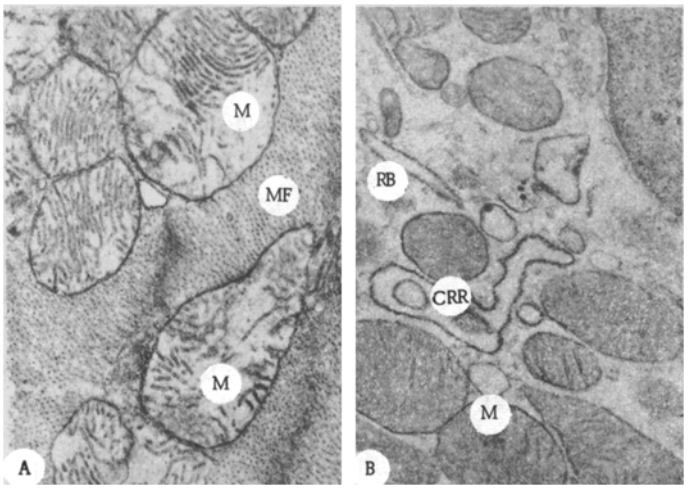

Fig. 2. Myocardium of zone of rabbit's heart near infarct on 14th day after ligation of the coronary artery: A) control, moderate swelling of mitochondria (20,000×); B) administration of potassium orotate, widened cisterns of rough reticulum (CRR), mitochondria (M) with finely granular, dense matrix, ribosomes (RB), 25,000×.

In the animals of group 4 (with myocardial infarction and receiving potassium orotate) no marked edema of the muscle cells was observed on the 7th day of the experiment, as was constantly found in the experiments without potassium orotate. The general structural plan of the muscle cell was undisturbed, despite swelling of the mitochondria and widening of the spaces of the sarcoplasmic reticulum. Characteristically the mitochondrial cristae were regular in their arrangement. The mitochondria were always round or oval in shape. Against the background of the swollen mitochondria there was a marked increase in the glycogen content between the myofibrils and, in particular, beneath the sarcolemma (Fig. 1b). Sometimes the glycogen granules formed dense irregularly shaped structures measuring 0.4-0.6 μ. Palade's bodies were frequently seen in the cytoplasm. One the 7th day, two, three, or more nucleoli were observed in the nuclei of the muscle cells. The nucleolus was always clearly distinguishable, very dense, finely granular in structure, and shaped like two overlapping loops. In some cases two nuclei were found in the myocardial muscle cells. The cytoplasm of these cells was loose and contained numerous membranes; the space of 12-14 μ between the nucleoli was filled with glycogen and mitochondria.

On the 14th day after ligation of the coronary artery a decrease in the edema of the muscle cells was observed in the control animals by comparison with the 7th day of the experiment. Meanwhile the intermyofibrillary spaces were widened, and the mitochondria were moderately swollen and irregular in shape (Fig. 2A).

In the animals receiving potassium orotate, on the 14th day a marked decrease in the glycogen content and an increase in the number of tubules and cisterns of the rough reticulum compared with the 7th day of the experiment were observed in the muscle cell. Often in the perinuclear zone dilated and branching tubules of the sarcotubular system with a single row of ribosomes, attached to the membranes, could be seen (Fig. 2B). Polysomes and vesicular structures were found in the cytoplasm. The mitochondria were of the usual structure and contained from 15 to 20 cristae, while their matrix was finely granular and dense. Sometimes there was a tendency toward division of the mitochondria, by the formation of a constriction band. Usually the nucleus had a clearly defined membrane, the typical structure, and one nucleolus. In general these muscle cells were similar in structure to the cells of the normal myocardium in a state of increased functional activity.

Comparison of the results shows that on the 7th day of administration of potassium orotate glycogen was accumulating and the nuclei of the muscle cell were activated. The number of nucleoli in the nucleus was increased and some binuclear muscle cells were present. The decrease in the glycogen content and the marked increase in the number of elements of the rough reticulum and membranous structures observed

by the 14th day, together with restoration of the normal structure of the mitochondria and condensation of their matrix are the essentials of the ultrastructural picture of activation of the synthetic function of the muscle cells.

These results demonstrate the biphasic character of the action of potassium orotate: an initial change in the carbohydrate metabolism with accumulation of glycogen is followed by synthesis of proteins in the heart muscle cells.

Orotic acid is a natural metabolite in uridine biosynthesis and it is a precursor of the pyrimidine bases of the nuclei acids [11]. In the course of several biochemical conversions orotic acid can be converted into uridine monophosphate, which in turn can participate in the formation of uridine diphosphate and triphosphate.

The observed increase in the glycogen content evidently took place under the influence of uridine diphosphate formed in the muscle cell [12]. It has been shown that uridine coenzymes play an active part in carbohydrate metabolism and, in particular, in the various reactions of transfer of the glycosyl residue, leading to synthesis and polymerization of sugars [11]. Takeuchi and Sasaki [12] confirmed these observations by electron-histochemical methods. The possibility cannot be ruled out that it is the glycogen accumulation, accompanied by activation of energy metabolism, which accounts for the rapid increase in amplitude of contraction of the muscle fibers under the influence of potassium orotate perfusion [2, 10].

The effect of glycogen accumulation possibly lies at the basis of the beneficial action of potassium orotate on the clinical course of myocardial infarction during the first days of the disease, through economy in the consumption of oxygen in the system of oxidative phosphorylation and stabilization of the free oxidation system [1]. Destruction of the mitochondria in the acute stage of ischemia accompanied by disturbance of aerobic energy formation can evidently be compensated by the accumulation of glycogen and a temporary predominance of the anaerobic pathway of oxidation.

The increase in the number of nuclei, some of them containing two or three nucleoli, hyperplasia of elements of the rough reticulum, division of the mitochondria, and also the increase in the number of polysomes and smooth-membranous vacuolar formations are all features reflecting activation of intracellular regeneration [8] in the zone around the infarct. An important role in these regenerative processes undoubtedly belongs to uridine monophosphate and triphosphate, of which orotic acid is the precursor. The ribonucleic acids thus formed contribute to a high level of protein synthesis in the cytoplasm of the muscle cells. An increase in the concentration of nucleic acids and in the intensity of protein synthesis under the influence of potassium orotate has been described by several workers [7, 13].

The electron-microscopic study of the fine structure of the myocardium in normal animals and the myocardium around the infarct after administration of potassium orotate thus demonstrated the unique action of this compound. The process was found to take place in definite stages: accumulation of glycogen by the 7th day and an increase in the level of protein synthesis by the 14th day of the experiment.

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