ULTRASTRUCTURAL AND FUNCTIONAL CHANGES IN THE MYOCARDIUM AND ITS VESSELS AFTER MASSIVE BLOOD LOSS

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Blood loss is a threatening complication after wounding of the heart and large vessels. The effectiveness of measures to prevent it is largely dependent on a proper concept of the morphological and functional features of the cardiovascular system in circulatory hypoxia. Despite much research into this question, the early changes in the heart muscle [3, 5, 8, 9] and its vessels has not yet been studied in detail, nor has any explanation been given of certain forms of destruction of cardiomyocytes [4, 6], which take into consideration the state of Ca^{++} ion transport at the cell membrane level.

The aim of the present investigation was accordingly to assess changes in ultrastructure of the cardiomyocytes and of vessels of the microcirculatory system and to compare them with changes in transmembrane Ca^{++} transport in the early posthemorrhagic period.

EXPERIMENTAL METHOD

Experiments were carried out on the hearts of 14 mongrel dogs. Massive blood loss was simulated under intravenous thiopental sodium anesthesia (0.5 g/kg) with controlled artificial respiration with the RO-6 apparatus. Bleeding (20-30 ml/kg body weight) was carried out from the femoral artery for 10-15 min. The anterior wall of the left ventricle was excised from the contracting heart 60 min after bleeding (7 of the 14 experiments) and in the control animals immediately after thoracotomy. One piece of the anterior wall (1 × 1 × 5 mm) was immersed in a 2% solution of glutaraldehyde in cacodylate buffer (pH 7.4). Blocks of heart muscle for electron microscopy were fixed in a 1% solution of oxmium tetroxide for 60 min and then embedded in Araldite. Ultrathin sections were cut on the LKB-88 Ultrotome. Sections were examined in the JEM-100B microscope. Fractions of sarcolemma were isolated from the second fragment of the left ventricle [7]. cAMP-stimulated Ca^{++} transport was studied in medium consisting of 40 mM Tris-HCl (pH 6.8), 5 mM $MgCl_2$, 100 mM KCl, 5 mM Na oxalate, 100 μM $CaCl_2$, and containing 0.05 μCi ^{45}Ca, 5 mM ATP, 30 μg protein, with or without 10^{-6} M cAMP and 0.2 mg/ml of protein kinase, obtained by the method in [10]. Aliquots were filtered through "Millipore" filters. The concentration of oxalate-bound Ca^{++} in the sarcolemma was determined as the difference between the radioactivity of the original reagent mixture and of the filtrate.

EXPERIMENTAL RESULTS

Examination of the electron micrographs revealed focal injury to the cardiomyocytes. In some areas they preserved their external structure, but in others they were separated. It will be clear from Fig. 1 that the sarcomeres in them were partially or completely detached. So-called "contraction bands" could be seen in the region of the Z-membranes. The basement membrane appeared reduced in thickness, and in some areas it was torn. The sarcolemma was heterogeneous: sometimes it appeared in the form of a clear line, sometimes it became broken or indistinct. In the region of the intercalated disks the sarcolemma as a rule was uninjured. Osmiophilic desmosome-like formations were concentrated in it at various distances. Few nexuses were seen at the level of the intercalated disks, evidently because the intercellular

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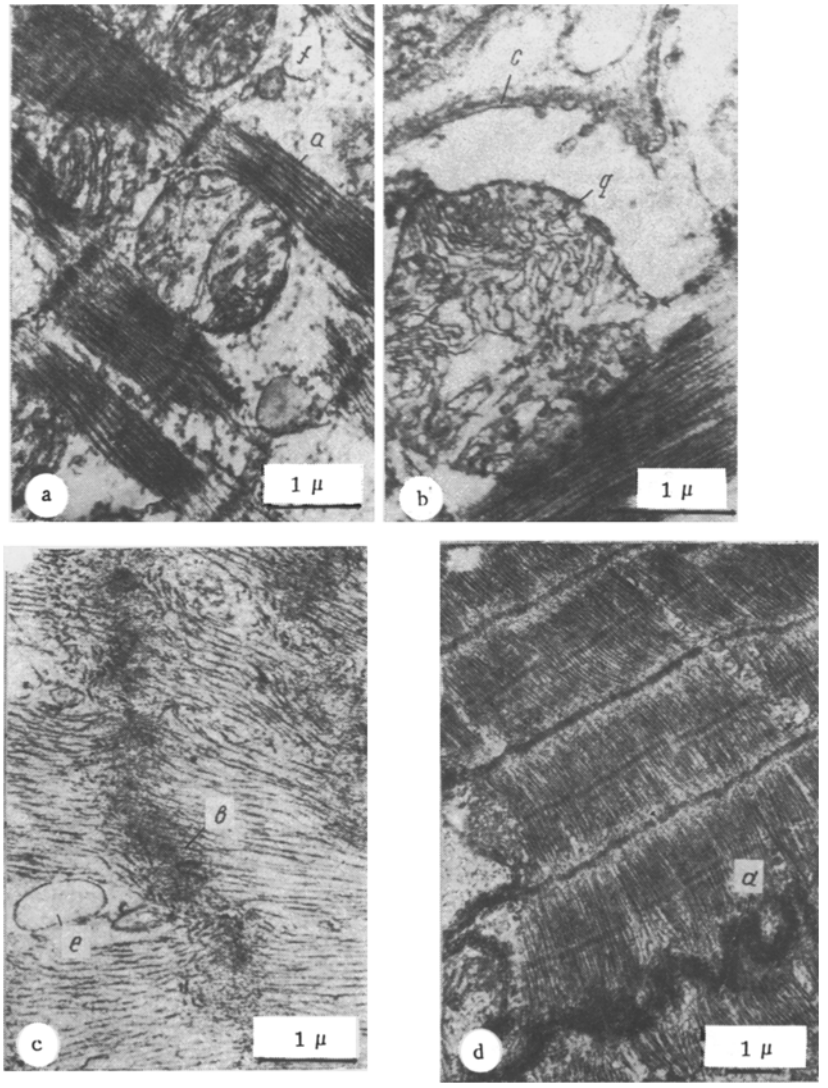

Fig. 1. Electron micrograph of cardiomyocytes after massive blood loss. [Notations here and in Fig. 2 not defined in Russian original — Publisher.]

space was wider than in the control. In the region of individual sarcomeres it was convoluted in character, forming round swellings. A similar picture was observed in the T system. The rough sarcoplasmic reticulum was wider in the perinuclear zone than normally. Most mitochondria were disoriented in arrangement, their cristae torn, and their matrix translucent. As these results show, the structure of the membranes, myofibrils, and sarcoplasmic reticulum of the cardiomyocytes underwent profound changes in the early posthemorrhagic period. It must be emphasized that the picture described above developed against the background of initial signs of disturbance of the microcirculation. Evidence of these was given by the numerous constrictive or widely dilated capillaries, the marked edema of the endotheliocytes in the wall of the venous portion of the capillaries and in the postcapillaries, and widening of the junctions between the endotheliocytes and the high transport activity of the pinocytotic apparatus (Fig. 2).

It follows from the above account that uncompensated blood loss during the first hour caused insufficiency of the coronary circulation. Disturbance of the microcirculation was evidently accompanied by reduction of the absorption pressure gradient in the venous part of the capillary system, and by a fall in metabolic activity. This was supported by the appearance of extra- and intracellular edema of the myocardium.

Changes observed in the structural components of the myocardium correlated with the characteristics of Ca^{++} ion transport, determined by cAMP-dependent phosphorylation, through the membrane of the sarcoplasmic reticulum [1] and through the sarcolemma. This mechanism, as we know, is responsible for removing the excess of Ca from the cytosol and, consequently, it participates in regulation of the force of the cardiac contractions. When its action in vitro is evaluated, it is evident that the basal (unstimulated) level of Ca^{++} accumulation in

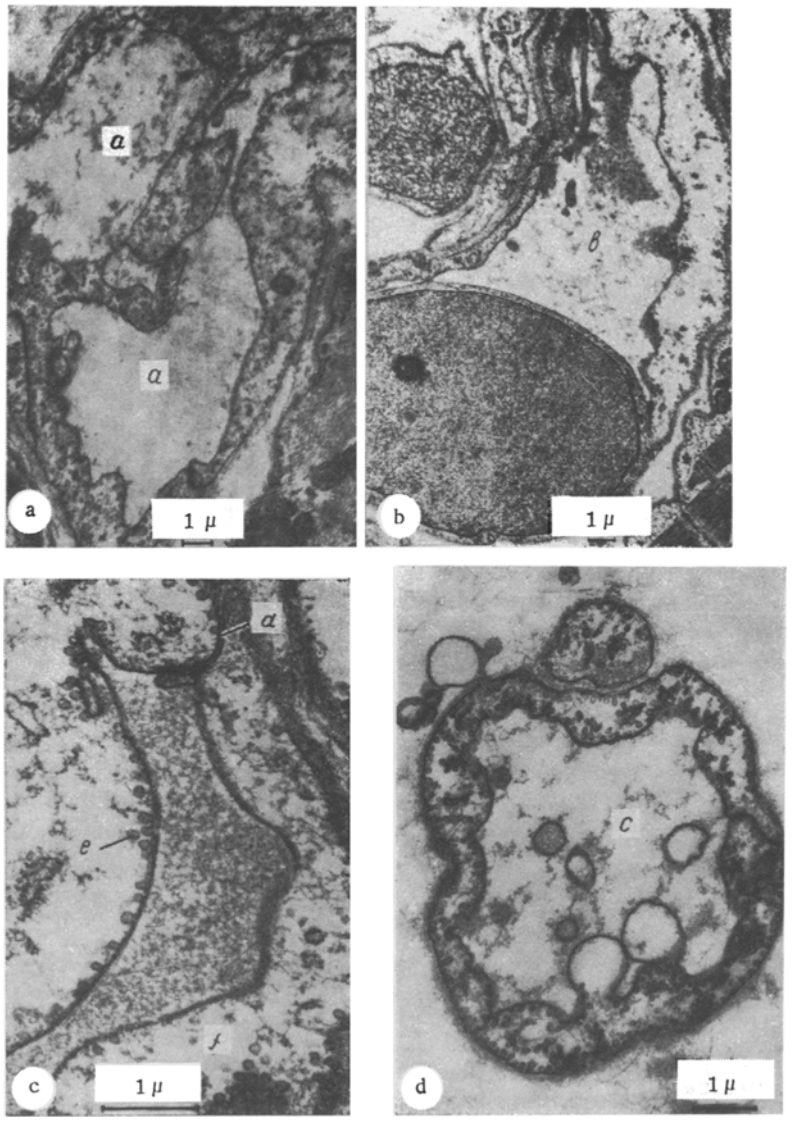

Fig. 2. Electron micrograph of vessels of microcirculatory system of the myocardium after massive blood loss.

preparations of intact sarcolemmal membranes, after incubation for 4 min, averaged 16.56 ± 1.33 nmoles ^{45}Ca/mg protein. Incubation of the sarcolemma for 5 min with 10^{-6} M cAMP ("endogenous" phosphorylation) led to activation of Ca^{++} transport by 1.6 times, whereas the addition of protein kinase to the incubation medium along with cAMP ("exogenous" phosphorylation) increased the Ca^{+} accumulation by 2.3 times compared with the basal level. In preparations of sarcolemma isolated from the myocardium of the experimental animals, the basal level of Ca^{++} accumulation after incubation for 4 min was virtually equal to the control, namely 14.84 ± 1.27 nmoles ^{45}Ca/mg protein. Addition of 10^{-6} M cAMP to the incubation medium caused an increase in the rate of Ca transport by about 1.4 times, i.e., rather less than in intact membranes. Addition of both cAMP and protein kinase to the incubation medium led to equalization of the level of Ca accumulation compared with the corresponding level on the undamaged sarcolemmal membrane. The disturbance of cAMP-dependent regulation of the Ca-transporting capacity of the myocardial sarcolemma thus revealed cannot evidently be explained by a change in the cyclic nucleotide level in the heart muscle when in a state of hypoxia, for it has been shown that the cAMP concentration in the myocardium does not fall significantly during circulatory hypoxia [2].

It can be postulated that the reduction of Ca exchange activity by means of the cAMP-dependent transport mechanism was due to disturbance of the ability of the protein kinase holoenzyme to dissociate its tetramer into the regulatory dimer and the free phosphorylating catalytic subunit. As a result of this the excess Ca^{++} concentration in the sarcoplasm prob-

ably caused sharp contractions of the cardiomyocyte protofibrils with the formation of contraction bands, and with detachment from the Z-membranes.

The results of these experiments thus show that 1 h after massive blood loss ultrastructural disturbances arise in the heart muscle, together with reduction of cAMP-dependent regulation of the Ca-transporting capacity of the cardiomyocyte sarcolemma. In this connection, modulation of the action potential of the cardiomyocytes by injections of the catalytic subunit is an interesting development in the abolition of the consequences of blood loss.

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ULTRASTRUCTURE OF SMOOTH MUSCLE CELLS OF THE FEMORAL ARTERY IN RATS EXPOSED TO VIBRATION

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The earliest changes after exposure to vibration in man and animals are observed in the nervous and cardiovascular systems. The character and severity of the circulatory disturbance depend on the parameters of vibration: frequency, amplitude, and duration. Long-term exposure to mechanical oscillations with a frequency below 40 Hz leads to changes of different kinds: not only spastic, but frequently also spastic-atonic and atonic states of the arteries, whereas exposure to high-frequency vibration (40-100 Hz) leads to spasm of the small and large arteries and arterioles [2, 5, 7, 12].

Despite much progress in the study of the functional state of the vascular wall, virtually no attempt has been made to study the morphology of blood vessels in vibration pathology. Accordingly the aim of the present investigation was to analyze the ultrastructure of smooth-muscle cells (SMC) of the femoral artery of experimental animals exposed in the long term to general high-frequency vibration.

EXPERIMENTAL METHOD

The experimental group consisted of eight noninbred female albino rats weighing 140-160 g, which were exposed (3 h daily) for 10 weeks to the action of general vertical vibration with a frequency of 100 Hz and an amplitude of 0.5-0.8 mm. Eight rats of the corresponding sex and

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