BIOCHEMICAL, MORPHOLOGICAL, AND FUNCTIONAL CHANGES IN THE MYOCARDIUM OF DOGS WITH OCCLUSION OF THE TERMINAL AORTA

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Embolism of the main arteries of the limbs is often complicated by the development of acute cardiovascular failure and myocardial infarction, leading to death of the patient [1]. However, the pathophysiological mechanisms of this complication are not sufficiently clear. The connection between the metabolic and morphological-functional state of the moycardium in the event of acute occlusion of the terminal part of the aorta is not completely known. Yet the study of these problems is very important because it could lead to the development of pathogenetic correction of the disturbances observed. We know that one of the most important points of application in the mechanisms of regulation of cardiac activity is a change in the cyclic nucleotide concentration in cells of the myocardium [6]. The role of direct participants in the processes of contraction and relaxation of heart muscle is ascribed to cyclic nucleotides [8]. One of the most intensively studied aspects of contractile processes in the myocardium is the potassium-sodium balance.

The aim of this investigation was to study the relationship between concentrations of cyclic nucleotides, potassium, sodium, chlorides, and inorganic phosphorus in the myocardium and the morphological and functional state of the heart muscle in experiments on dogs with a model of acute occlusion of the terminal part of the aorta.

EXPERIMENTAL METHOD

Altogether 48 experiments were carried out on mongrel dogs of both sexes, in which acute arterial occlusion was created [2]. Trimeperidine, in a dose of 1 ml of a 1% solution/kg body weight was used for premedication. Myocardial contractility was assessed by determining the intensity of functioning of structures [3], the time-tension index, and the index of contractility [9] before and 6 and 12 h after occlusion. Euthanasia was induced by intravenous injection of hexobarbital. Homogenates were prepared from the myocardium of both ventricles after ischemia of the lower limbs for 3, 6, and 12 h, and concentrations of the following substances were measured in them: cAMP and cGMP by a radiological method using standard kits (from Amersham International, England), sodium and potassium ions — on the "NaK Analyzer-902" (Corning, USA), with selective electrodes, chloride ions by an electrochemical method on the "Chloride Analyzer-925" (Corning, USA), and inorganic phosphorus on an "Impact" automatic blood analyzer (Corning, USA), using kits from the same firm. Activity of succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), NAD-diaphorase (NAD), phosphorylase, and myosin ATPase

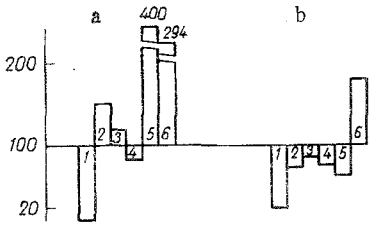

Fig. 1. Concentrations (in % of values after 3 h) of cAMP (1), cGMP (2), sodium (3), potassium (4), chlorides (5), and inorganic phosphorus (6) in myocardium of right (a) and left (b) ventricles.

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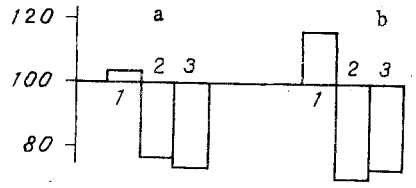

Fig. 2. Parameters of myocardial contractility (in % of control) 6 h (a) and 12 h (b) after occlusion of the terminal aorta. 1) Intensity of functioning of structures; 2) time-tension index per cycle; 3) contractility index.

was determined by the usual methods [5] in frozen sections through the walls of the left and right ventricles, the ventricular septum, and the left posterior papillary muscle. Activity of the dehydrogenases was assessed qualitatively on a "Microvideomat" television image analyzer (Opton, West Germany), controlled by a "Wang 720C" computer (USA) by a special program [4]. Paraffin sections through the myocardium were stained with hematoxylin and eosin and by Goldner's trichrome method, and were also studied in polarized light. Lesions in the myocardium were revealed by the methods of Regaud and Lie.

EXPERIMENTAL RESULTS

Investigation of homogenates of the left ventricular myocardium (Fig. 1b) revealed a decrease in concentrations of cyclic nucleotides, sodium, potassium, and chloride, and an increase in the inorganic phosphorus concentration at all stages. These changes progressed with an increase in the duration of occlusion of the terminal aorta. A decrease in concentrations of cAMP and potassium and chloride ions was observed in the myocardium of the right ventricle, whereas the cGMP and sodium concentrations rose here, unlike in the myocardium of the left ventricle (Fig. 1a). The inorganic phosphorus level in the myocardium of the right ventricle rose with an increase in severity of ischemic damage to the lower limbs.

Morphological investigation of the heart muscle revealed interstitial edema even in the early stages of the experiment (Fig. 3b), and it was most marked in the subendocardial layers of both ventricles. Separate lesions of the cardiomyocytes, characterized by increased eosinophilia of their sarcoplasm, fuchsinorrhagia when stained by Lie's method, and intensive staining by Regaud's method, were located in these same parts of the heart. In polarized light, increased anisotropy of the A-disks was observed, the disks were closer together, and segmental contractural lesions also were present (Fig. 3d). With an increase in duration of the experiment, the myocardial lesions became larger and more frequent. Changes in dehydrogenase activity in the cardiomyocytes should be noted. In all parts of the heart studied there was a significant increase in SDN (by 20%) and NAD (by 53%) activity, and LDH activity was increased (Fig. 3a) in the cardiomyocytes of the left and right ventricles (by 11 and 21%, respectively) in the early stages of the experiment. However, LDH activity in the ventricular septum was then reduced by 10%, and in the papillary muscle by 26%. An increase in the severity of the ischemic damage to the lower limbs was accompanied by a fall in the activity of all enzymes studied in the cardiomyocytes: SDH by 26%, NAD by 41% (Fig. 3f), and LDH by 63%. Single cardiomyocytes in which virtually no activity of phosphorylase (Fig. 3c), ATPase (Fig. 3e), or dehydrogenases could be detected, also were found in the preparations. Contractural lesions were located mainly in the papillary muscles and subendocardially.

The biochemical and morpholigical changes thus revealed were accompanied by disturbances of myocardial function (Fig. 2), manifested by a progressive decrease in the rate of formation and cleavage of actin-moysin bonds, and also by a change in the intensity of tension developed by the heart.

The experimental results revealed changes in myocardial metabolism in response to occlusion of the terminal part of the aorta, and evidently due to hypoxia. Evidence of myocardial hypoxia was given by changes in activity of the dehydrogenases studied, especially LDH. In fact, some increase in the activity of this enzyme in the early stages of the experiment indicated intensification of glycolysis, a characteristic feature of the initial period of ischemia [7]. Lowering of dehydrogenase activity after occlusion of the terminal portion of the aorta for 12 h illustrates the more profound changes in cellular metabolism and, in particular, in energy metabolism. Lowering of the cAMP level in the heart muscle, on the one hand, was the result of a decrease in the production of ATP — the precursor of the nucleotide, on the one hand, and on the other it was the result of disturbance of the integrity of the cardiomyocyte membranes, with outflow of cAMP from the cells. A definite contribution to the formation of injury to heart muscle was made by the ionic imbalance in the myocardium; changes were observed, moreover, not only in the absolute concentrations of the ions, but also in their relative concentrations. This effect was seen more demonstratively in the myo-

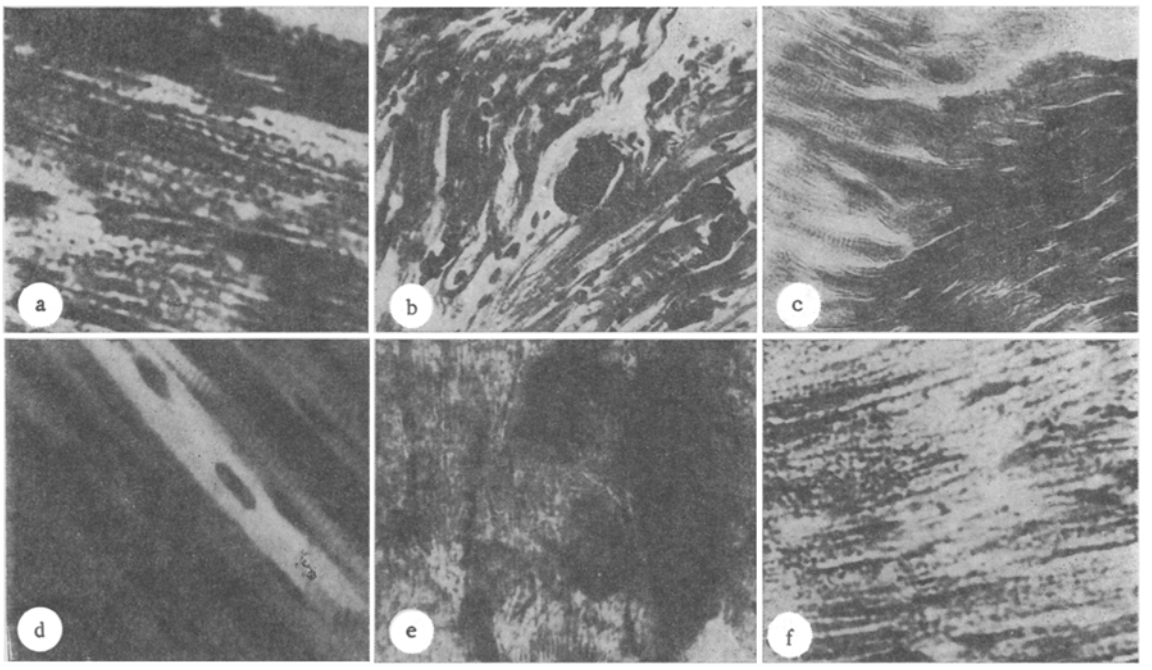

Fig. 3. Morphological changes in the myocardium associated with acute ischemia of the limbs: a) increased LDH activity in cardiomyocytes after 3 h of ischemia. Nitro-CT, 600 ×; b) edema of myocardial stroma, congestion, aggregation of erythrocytes in small vessels (6 h of ischemia). Goldner's trichrome stain, 256 ×; c) focal reduction in phosphorylase activity in cardiomyocytes after 12 h of ischemia. Takeushi's stain, 256 ×; d) contractural lesions of cardiomyocytes (12 h of ischemia). Hematoxylin and eosin. Polarized light. 400 ×; e) lowering of myosin ATPase activity in cardiomyocytes, activity preserved in wall of arteriole (6 h of ischemia). Stained by method of Padycula and Flerman, 400 ×; f) decrease in NAD-diaphorase activity in cardiomyocytes (12 h of ischemia). Stained by Hess's reaction, 600 ×.

cardium of the left ventricle, where loss of potassium, sodium, and chloride ions was found. This synchronization of the changes in ion concentrations leads to a disturbance of the electrolyte balance in the cardiomyocytes and, as a result of this, to changes in electrophysiological processes in the heart muscle. Hypoxic changes in energy metabolism, disturbance of the working of the adenylate cyclase system, and the ionic imbalance lead to disturbance of myocardial functional activity, and this conclusion is confirmed by the progressive decline in ATPase activity in the cardiomyocytes and a decrease in the velocity of formation and cleavage of actin-myosin bonds.

Occlusion of the terminal part of the aorta thus causes injury to heart muscle; the severity of the damage to the cardiomyocytes and also the number of lesions in the myocardium are directly proportional to the duration of ischemic damage to the lower limbs, or in other words, the more severe the ischemic limb damage, the more severe the damage to the heart muscle.

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AFFERENTATION OF THE HEART IN SOME MYOCARDIOPATHIES

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KEY WORDS: heart, sinus node; afferentiation; ischemia; myocardial necrosis

Judging by the relative paucity of data in the literature [2-4, 6, 10], the role of the spinal afferent system of the heart is extremely important, for it is through it that information about the most important changes in functional state of the heart muscle must reach the regulating systems of the CNS both during formal activity and when various pathogenic agents act on the myocardium. In a previous investigation [1] the writers studied the representation of the spinal afferent system of the heart in the cerebral cortex and certain deep brain structures.

In the present investigation, which is a continuation of previous studies, changes in the information capacity of the spinal afferent system of the heart were examined under normal conditions and during the development of certain pathological states of the myocardium (reversible ischemia, necrosis).

EXPERIMENTAL METHOD

Several series of acute experiments were carried out on 18 mature cats under chloralose and with muscle relaxation. The principal electrophysiological method of investigation was the evoked potentials (EP) method; EP were recorded in the rostral zones of the cortex. Stimulation (square pulses, 0.3 msec, 5-10 mA, frequency not more than 0.3 Hz) was applied to the zone of the sinus node (SN) of the heart. The design of the bipolar stimulating electrodes also enabled solutions of the corresponding substances to be applied directly to the region of stimulation. Recording, averaging, and statistical analysis (by Student's test) of the basic parameters of EP were carried out on a coherent averager (Neuroaverager, from OTE-Biomedica) and an Iskra-1256 computer system. The level of significance of differences between means was taken to be $P \leqslant 0.05$. As a rule, at the end of the experiments, pieces of tissue from the region of the sinus node were taken from the animal's heart, after treatment by the standard method, ultrathin sections were cut from them for examination in the electron microscope.

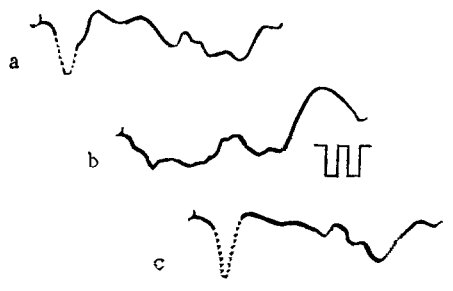

Fig. 1. Changes in cortical EP recorded during stimulation of SN in the normal heart (a), 2 min after injection of 0.1 ml of a 10% solution of neutral formalin into the myocardium (b), and 70 min after injection of formalin (c). Coherent averaging method was used (n = 20). Calibration: 60 μV, 20 msec.

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