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ULTRASTRUCTURAL SIGNS OF HEART FAILURE AND ITS CORRECTION AFTER ASPHYXIA

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KEY WORDS: resuscitation; myocardium; ultrastructure; pathogenetic therapy

Restoration of adequate cardiac activity after clinical death is largely responsible for the final outcome of resuscitation [4]. Functional heart failure developing in the recovering period leads to a disturbance of the systematic circulation and calls for professional measures to protect the myocardium. Accordingly, in this study on a model of clinical death from mechanical asphyxia, our aim was an experimental study of the ulrastructural signs of heart failure and the effectiveness of drugs with antihypoxic, membrane-stabilizing, and protease-inhibiting action during resuscitation.

EXPERIMENTAL METHOD

Experiments were carried out on 60 albino rats weighing 170-200 g, six of which served as the control. All the experiments were carried out under ether anesthesia. Clinical death was induced by compressing the intubation tube, and subsequent resuscitation effected by indirect cardiac massage and artificial ventilation of the lungs. The drugs were injected intraperitoneally during the resuscitation measures in the following doses: gutimin (guanylthiourea) 100 mg/kg, sodium hydroxybutyate 150 mg/kg, prednisolone 0.3 mg/kg, and contrykal 2000 antitrypsin units (ATU)/kg. Nine rats, subjected to asphyxia, were not treated with these drugs. The myocardium was taken for electron-microscopic study 1, 5, 6, and 24 h after resuscitation. Fixation was carried out in a 4% solution of praformaldehyde and the material was embedded in Epon-Araldite. Ultrathin sections were studied in the EVM-100 AK electron microscope.

EXPERIMENTAL RESULTS

Widespread destructive changes were found in the myocardium of rats not receiving the drugs in the postresuscitation period. Toward 1.5 h after resuscitation, profuse intracullar edema developed, leading to disintegration of organelles and isolation of the sarcotubular system. Denudation of the cytoplasmic matrix was observed, due to the almost total disappearance of glycogen granules. The greatest changes affected the contractile apparatus of the cardiomyocytes, as we observed also on a different model of clinical death [2], and was expressed as widespread microlysis and relaxation of the myofibrils. The active lytic process led to a reduction in thickness and fragmentation of the myofibrils and was accompanied by the appearance of morphologically intact mitochondria and a few polysomes in the areas of lysis. A uniform widening of the pace of many intercalated disks and moderate dystrophic changes in the vascular endothelium were observed.

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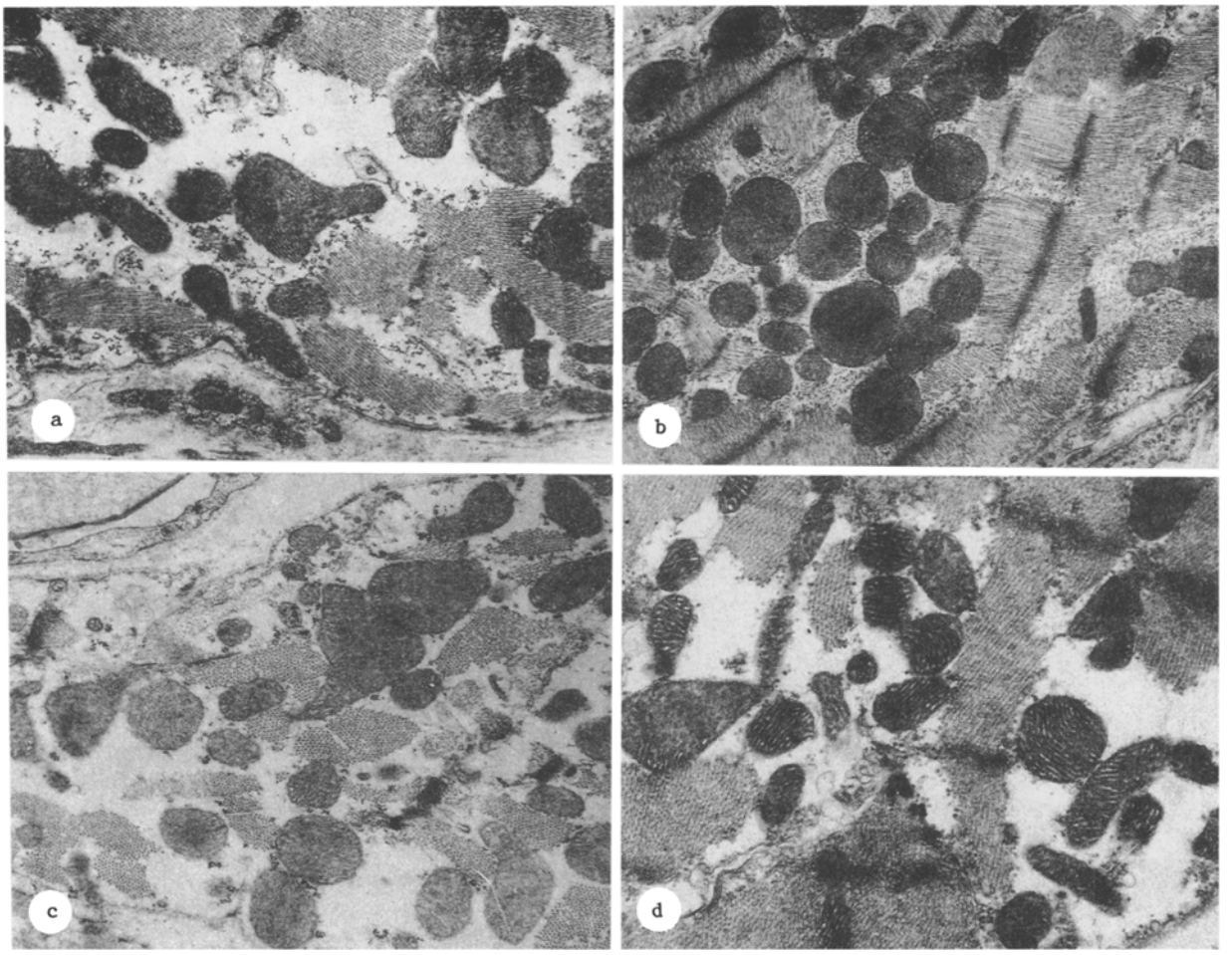

Fig. 1. Myocardial ultrastructure 24 h after resuscitation without the use of drugs (a) and using contrykal (b), gutimin (c), and prednisolone (d). a) Microlysis of myofibrils, focal intracellular edema. 18,000 ×. b) Concentration of mitochondria and cytogranules in focus of regeneration, normalization of cardiomyocyte ultrastructure. 14,000 ×. c) Moderate intracellular edema, disintegration of organelles. 15,000 ×. d) Denudation of cytoplasmic matrix, focal edema, lysis of myofibrils. 18,000 ×.

Ultrastructural pathological changes in the myocardium were even more marked 6 h after resuscitation. This was true especially of the myofibrils which, because of widespread lysis, were partly dissolved and fragmented. The thin relaxed myofibrils were separated and lay in the profusely edematous cytoplasmic matrix. Foci of lysis often affected a very large volume of the cell. This often led to complete liquefaction of the myofibrils in the region of the intercalated disk, disturbing coordinated contractile activity of the cardiomyocytes. The rare collections of polysomes and the slow recovery of integrity of the myofibrils were evidence of a weak intracellular regenerative response. The energy-forming apparatus of the cells was not greatly affected: most mitochondria preserved their usual structure, and only in a few of them was fragmentation of randomly arranged disks and rupture of the outer membrane discovered. As a result of the considerable decrease in the content of condensed chromatin, the cardiomyocyte nuclei were translucent.

Stabilization of destructive processes and partial normalization of the ultrastructure of the myocardial cells were observed 24 h after resuscitation, evidence of activation of intracellular regeneration. Intracellular edema was moderately developed and focal in character. Areas of lysis of the myofibrils were filled with mitochondria, some of which were newly formed and small, and also with cytogranules (Fig. 1a). Focal relaxation of the myofibrils was preserved. The number of secondary lysosomes was appreciably increased. Changes in the myocardium observed during the first 24 h after resuscitation constitute the structural basis of functional heart failure, observed in the postresuscitation period [1].

The study of the effect of drugs on the state of the heart after clinical death showed that contrykal had the strongest protective action on the myocardium. This was shown by diminution of destructive changes in the cardiomyocytes and activation of intracellular repair processes. In particular, foci of lysis and microlysis of the myofibrils were smaller and less frequent than in animals of the previous groups. The intensity of intracellular edema was reduced in the early postresuscitation period, but it was virtually absent 24 h after resuscitation. Relaxation of the myofibrils was not observed, and in conjunction with the more limited spread of foci of their lysis, the possibility of adequate contractile activity of the cardiomyocytes was ensured. In the damage areas of the cells many polysomes, tubules of the rough endoplasic reticulum, and small, newly formed mitochondria appeared, evidence of marked activation of intracellular regenerative processes (Fig. 1b). These processes were intensified in the endothelium of the microcirculatory bed also, thereby leading to a improvement of transvascular exchange. Contrykal is a protease inhibitor. Its protective effect is evidently due to partial inactivation of proteolytic enzymes, and this emphasizes the important role of these enzymes in the pathogenesis of destructive changes in the myocardium in the postresuscitation period. One probable cause of activation of proteases, leading to destruction of the contractile apparatus, is an excessive inflow of calcium into the cardiomyocytes during reoxygenation [3, 8-10].

After administration of gutimin and sodium hydroxybutyrate, the positive effect was small. During the first day after resuscitation, against the background of destructive changes in the myofibrils, only a decrease in the intracellular edema in the cardiomyocytes was noted (Fig. 1c). These preparations are known to have an antihypoxic action. Gutimin, in particular, increases mitochondrial oxygen utilization and enhances the coupling of oxidative phosphorylation, which leads to stimulation of ATP synthesis and to a reduction in the intensity of lipid peroxidation [5]. These factors are perhaps not the dominant features in the pathogenesis of myocardial damage, more especially because the energy-forming apparatus of the cardiomyocytes suffers negligible damage.

The use of prednisolone led to increased severity of the ultrastructural disturbances of the myocardium during the 24 h after resuscitation. Active lysis with liquefaction of the myofibrils, denudation of the cell matrix, contracture, and relaxation of the contractile apparatus were observed. Rosettes of polysomes, and figures of protofibril assembly on ribosomes in areas of lysis were seen less frequently than in the group of animals not receiving the drugs (Fig. 1d). The probable reason for this is evidently a side effect of prednisolone, namely delayed syntehsis and more rapid breakdown of protein, slowing of regenerative proceses, and reduction of capillary permeability [6]. The membrane-stabilizing action of prednisolone, reducing the outflow of hydrolases from the lysosomes, proved ineffective for preventing myocardiocyte damage. Together with our data on the absence of lysosomes in foci of lysis and on their preservation in other areas, this is evidence of the unimportant role of cardiomyocyte lysosomes in the development of intracellular destructive changes. Evidently other sources of hydrolases then lysosomes must exist in myocardial cells [7].

The investigations showed that a combination of destructive chagnes, most marked during the first 6 h after resuscitation, develops in the myocardium in the postresuscitation period. The dominant factor is damage to the contractile system of the cardiomyocytes against the background of intracellular edema, whereas the energy forming system suffers negligible damage. Changes discovered in the myocardium are evidently largely due to hyperactivity of proteolytic enzymes, inhibition of which by means of contrykal during resuscitation measures has a protective effect. The use of gutimin and sodium hydroxybutyrate during resuscitation is less effective for preventing destructive changes in the myocardium.

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ULTRASTRUCTURAL AND ULTRACYTOCHEMICAL CHANGES IN THE LUNGS IN DIABETIC RATS

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KEY WORDS: diabetes mellitus; ultrastructure of lungs; glycocalyx.

Experimental evidence has been obtained that lipid metabolism in the lungs is regulated by insulin, and also that the ability of the lungs to oxidize glucose and to incorporate ^{3}H-leucine into protein is inhibited in diabetes mellitus [11]. Specific receptors for insulin were identified for the first time in membrane structures of normal rat lungs in [10]. Insulin interacts quickly with these receptors, saturation being reversible and depending on time and temperature. Since lipid and protein synthesis in the lung tissue is mainly associated with type II pneumocytes [3, 7], changes are found in these cells in rats with streptozotocin-induced diabetes [13]. However, no chronic experimental investigation of the lungs has been undertaken on animals with experimental diabetes.

The aim of this investigation was to study the time course of ultrastructural changes in the lungs and the state of the glycocalyx of the pneumocytes in experimental streptozotocin-induced diabetes.

EXPERIMENTAL METHOD

Experiments were carried out on 64 male and female rats of mixed lines weighing 180-250 g. Streptozotocin was dissolved in physiological saline and given as a single intraperitoneal injection in a does of 90-100 mg/kg. Six intact rats served as the control. The onset of diabetes was recorded on the basis of glycosuria, polyuria, and polydipsia. Glucose in the urine was determined by the paper strip method (Labstix, Dextrostix).

Of the 64 rats, 39 with diabetes died in the first 2 weeks: most of them showed signs of focal pneumonia. In six rats the signs of diabetes disappeared at the end of the 2nd week and they were eliminated from the experiment. Tests were carried out on the remaining 19 rats with developed diabetes. The rats were killed after 4, 7, 15, 30, and 60 days. Under ether anesthia thoracotomy was peformed, the right bronchus was clamped, a catheter was inserted into the left bronchus, and a solution of 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) was injected into the lung. The right lung was used for ultracytochemical detection of the glycocalyx: a catheter was inserted into the right bronchus of the same rat and a 2.5% solution of glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3), in which ruthenium red was dissolved in a concentration of 1 mg/ml, was injected into the lung. After fixation for 1 h, subsequent treatment followed Luft's formula (Geyer, 1974).

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