ULTRASTRUCTURAL STUDY OF ANTIISCHEMIC PROTECTION OF THE NORMAL AND HYPERTROPHIED MYOCARDIUM BY CREATINE PHOSPHATE

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Recent research has shown that creatine phosphate (CP) has a marked protective action on ischemic cardiomyocytes. For example, in vitro studies using free hand slices [9, 10] from papillary muscles of minipigs and dogs have demonstrated the ability of CP not only to protect the pool of high-energy phosphates, but also to prevent the formation of evident defects in the sarcolemma at a critically low ATP level in ischemic tissue [7, 8]. On the basis of these findings CP can be regarded as a promising agent for lengthening the storage life of a donor's heart for transplantation, and also to lengthen the possible periods of cardioplegic arrest during operations on the "dry" heart.

However, this does not allow the data thus obtained to be extrapolated sufficiently objectively to clinical conditions. This is true at least for those cases in which protection of the ischemic heart takes place against the background of hypertrophy caused by rheumatic heart disease. The aim of this investigation was to study the effect of total ischemia on the myocardial ultrastructure of the hypertrophied heart and also the protective action of CP under these conditions.

EXPERIMENTAL METHOD

Large mongrel dogs weighing 14-22 kg were used as experimental animals. The operation was performed under general intravenous phenobarbital anesthesia (40 mg/kg) with artificial ventilation and monitoring of the pH and gas composition of the blood. Blood pressure was recorded, No. 5 catheters (USCI, USA) were introduced into the left ventricle, common carotid artery, and external jugular vein, and a type MFV-1200 electromagnetic blood flowmeter (Nihon Kohden, Japan) was applied to the ascending arch of the aorta.

After the physiological parameters had been recorded in the control animals (group 1) the operation wound was sutured and a ligature made of sterile tape was applied to the ascending arch of the aorta of the remaining dogs (group 2), the degree of stenosis of the aorta being regulated by measuring the systolic pressure gradient between the left ventricle and aorta, which reached 31-42 mm Hg [5, 6].

Animals of both groups (four dogs in each group) were kept in cages under normal conditions for 10-12 months. At the end of that time a further operation was performed on the dogs as described above, and the physiological parameters of cardiac activity were recorded again. The aorta was compressed at its base, and a standard crystalloid cardioplegic solution injected into the root of the aorta at room temperature, into two animals of group 1 and two animals of group 2; a crystalloid cardioplegic solution containing CP was injected into the same number of animals in each group. The arrested heart was quickly removed from the thorax, weighed, and placed in tightly closed cellophane bags, which were immersed in water kept at a constant temperature of 37°C.

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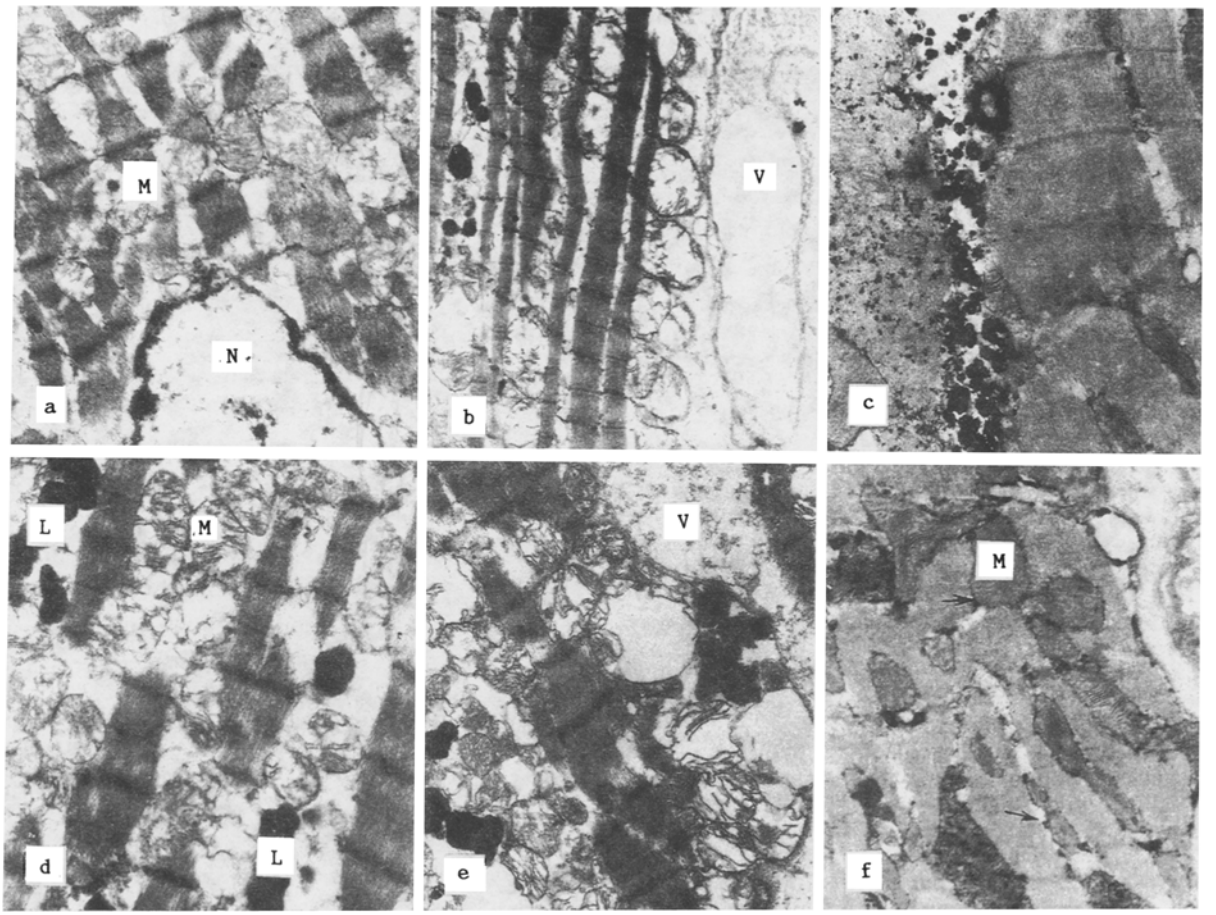

Fig. 1. Ultrastructure of normal and hypertrophied myocardium at different stages of normothermic conservation of the heart. a, b, c) Animals of group 1: a) cardiomyocytes after 90 min of total ischemia, protection without CP, aggregation and margination of chromatin in nucleus (N), electron-dense residues in empty matrix of mitochondria (M); b) cardiomyocytes after 120 min of total ischemia, protection without CP, vacuoles (V) present in subsarcolemmal zone and intercellular space; c) cardiomyocytes after 120 min of total ischemia, protection with CP, fixation with lanthanum, particles of tracer visible in upper cell. d, e, f) Animals of group 2: d) cardiomyocytes after 60 min of total ischemia, protection without CP, sarcomeres uniformly contracted, mitochondrial matrix translucent, most cristae reduced, secondary lysosomes (L) visible; e) cardiomyocytes after 60 min of total ischemia, protection without CP, signs of edema, large vacuoles, and fragments of membranes present in intercellular spaces; f) cardiomyocytes after 90 min of total ischemia, protection with CP, fixation with lanthanum, no lanthanum present in cell on right, but is visible in neighboring cell on outer surface of mitochondria (arrow). Magnification: a, d) 5000, b, e) 4000, c, f) 10,000.

Tissue samples for electron microscopy and biochemical analysis were taken from the anterior wall of the left ventricle immediately after weighing of the heart, and 60, 90, and 120 min after the beginning of thermostating. Tissue for electron microscopy was fixed with glutaraldehyde and osmic acid by the routine method or with the addition of colloidal lanthanum [10].

EXPERIMENTAL RESULTS

The relative weight of the left ventricle of dogs with experimental aortic stenosis (group 2) was 40% greater than that of animals of group 1, evidence that they had developed myocardial hypertrophy.

Analysis of the ultrastructure of the myocardium confirmed the development of features of hypertrophy in the dogs of group 2. The volume of the cardiomyocytes was increased, many small mitochondria were formed at the periphery of the cells, many secondary lysosomes appeared in the sarcoplasm, as described previously by other workers during myocardial hypertrophy [1, 2, 3].

Signs of distinct and irreversible damage to cells in the animals of group 1, on incubation of the hearts without addition of CP, were found at the 90th minute. They consisted of coarse aggregation and margination of the nuclear chromatin, emptying of the sarcoplasmic matrix and matrix of the mitochondria, the appearance of electron-dense homogeneous residues in the mitochondria, partial separation of the sarcolemma, and fragmentation of the plasmalemma. All the sarcomeres were in a state of uniform contraction (Fig. 1a). All these changes were described previously as absolute signs of irreversible ischemic damage to the myocardium [4]. After 120 min, signs of intercellular edema were added to the changes described above: vacuoles and membrane fragments appeared in the intercellular space (Fig. 1b). When the heart was incubated after arrest with the addition of CP, signs of aggregation and margination of the nuclear chromatin also were observed after 90 min, although they were less marked. Some glycogen was preserved in the sarcoplasmic matrix, and no electron-dense residues were found in the slightly swollen mitochondria. No visible defects were present likewise in the sarcolemma. A similar picture also was found after 120 min of incubation, and only by the use of colloidal lanthanum could the initial defects be detected in the sarcolemma of some cells: lanthanum penetrated into these cells and was distributed as a finely dispersed residue above the myofibrils (Fig. 1c).

The development of evident irreversible changes in dogs with hypertrophied hearts when arrested without the use of CP was observed as early as the 60th minute of incubation. Ultrastructural features of irreversible damage were the same as in the animals of group 1 at the 90th minute of incubation, the only difference being that a certain number of large secondary lysosomes was present constantly in the sarcolemma (Fig. 1d). Signs of intercellular edema, with the appearance of vacuoles and membrane fragments in the intercellular spaces, were observed in only some of the animals (Fig. 1e), although this was characteristic of the animals of group 1 after 120 min of incubation without addition of CP. These differences were evidently linked with the unequal degree of development of myocardial hypertrophy in different animals. After 90 min of incubation ultrastructural changes characteristic of the 120th minute in the control group without addition of CP were observed in all the animals of group 2.

The use of CP prevented the development of irreversible changes in the cardiomyocytes at the 60th minute of incubation in the hypertrophied hearts. Only some degree of swelling of the mitochondria was noted. The use of colloidal lanthanum also demonstrated absence of irreversible changes: particles of the tracer did not penetrate into the sarcoplasmic matrix and were located entirely on the outer side of the sarcolemma. After incubation of these hearts for 90 min the development of ultrastructural changes could not be observed by the use of routine fixation, but with colloidal lanthanum the presence of early defects in the sarcolemma was noted in some cells (Fig. 1g).

The investigation showed that irreversible changes began to appear in the animals of group 1, without the use of CP, at the 90th minute of normothermic incubation, in full agreement with data in the literature [4, 9]. This indicates that the methods we used were adequate.

Myocardial hypertrophy reduces the resistance of the heart to ischemic damage: irreversible changes in the hypertrophied myocardium developed sooner during normothermic incubation than in intact tissue. CP exhibits its protective properties also on the hypertrophied myocardium, but its use does not always enable differences between the hypertrophied and intact myocardium to be eliminated.

Thus although the details of the mechanism of the protective action of exogenous CP on the hypertrophied myocardium have been inadequately studied, the results of the present investigation justify the recommendation of the use of this substance for conserving the heart both for clinical transplantation of the organ and for protection of the hypertrophied heart during cardioplegic arrest in the course of operations involving the use of an artificial circulation.

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FUNCTIONAL MORPHOLOGY OF GASTRIN-PRODUCING CELLS IN DIFFERENT PHASES OF PERIODIC GASTRIC ACTIVITY IN DOGS

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Periodic activity (PA) of the gastrointestinal tract is one of the most demonstrative rhythms of the body [1-3]. An essential role in the neurohumoral mechanisms of regulation of alternation of the phases of PA, namely a period of relative rest (PR) and a period of work (PW), is played by the peptide component [3, 4, 7]. Data on changes in the blood gastrin concentration (BGC) during alternation of the phases of PA are highly contradictory [2, 4], although a circadian rhythm of BGC has been found in man and in animals [5, 8, 10].

We studied the ultrastructure of gastrin-producing cells of the antral mucosa of the canine stomach in different phases of PA, in the fasting state.

EXPERIMENTAL METHOD

Material for electron-microscopic and morphometric investigation was taken from 10 mongrel dogs with fistulas of the body and pyloric part of the stomach. Alternation of the phases of PA was studied by recording gastric motor activity on the drum of a kymograph by a combined hydraulic and pneumatic transmission system.

Food stimulation consisted of feeding the dogs with minced meat, and 20 min later biopsy specimens were taken from the mucosa of the antral part of the stomach.

Material taken in the middle of PR and PW, and also 20 min after food stimulation was fixed in a 4% solution of paraformaldehyde in Hanks' buffer (pH 7.4), cooled to 4°C, postfixed with OsO_4, dehydrated, and embedded in a mixture of Epon and Araldite resins. Ultrathin sections were stained with lead citrate and uranyl acetate, examined in the JEM-100CX microscope, and photographed under magnification of 8000. Morphometry of the negatives was carried out on a semiautomatic "MOP-Videoplan" image analyzer ("Reichert," Austria). The number of secretory granules (SG) per square micron area of the cytoplasm of a gastrin-producing (GPC), the area of each type of SG, the maximal diameter and relative area of each type of SG,

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