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ELECTRON-MICROSCOPIC STUDY OF DISTURBANCES OF CARDIOMYOCYTE MEMBRANE PERMEABILITY IN IMMUNE CARDIOPATHY

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Previous investigations [1, 3, 4] showed that focal immune injury to the heart, produced by intracoronary injection of anticardial cytotoxic serum (ACS) is accompanied by considerable changes in the contractile apparatus of cardiomyocytes: contracture, fragmentation, and rupture of the myofibrils. These changes were found in the early period of development of immune cardiopathy, namely during the first 5-7 min.

In the modern view [5-7] disturbances of the myocardial contractile system of contracture type are based on an excessive inflow of calcium into the sarcoplasm of the cardiomyocytes as a result of disturbance of ionic permeability of the sarcolemma.

Since anticardial antibodies can be fixed on the sarcolemma of cardiomyocytes in the subsarcolemmal position also [10], it might be supposed that damage to cardiomyocyte mem-

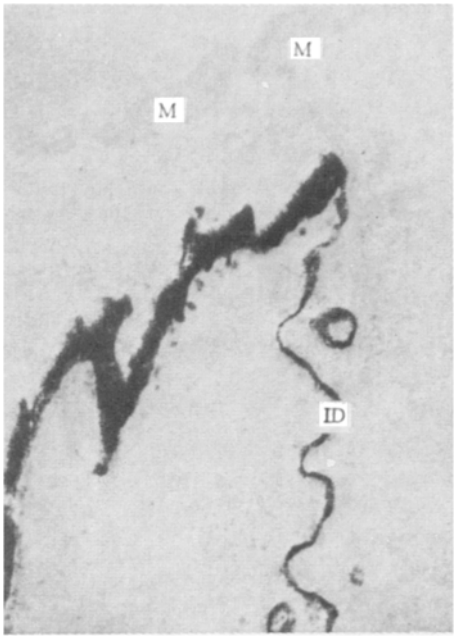

Fig. 1. Ultrastructure of intact area of myocardium. Colloidal lanthanum outlines intercalated disk (ID) and intercellular space. M) Mitochondria. 18,000 ×.

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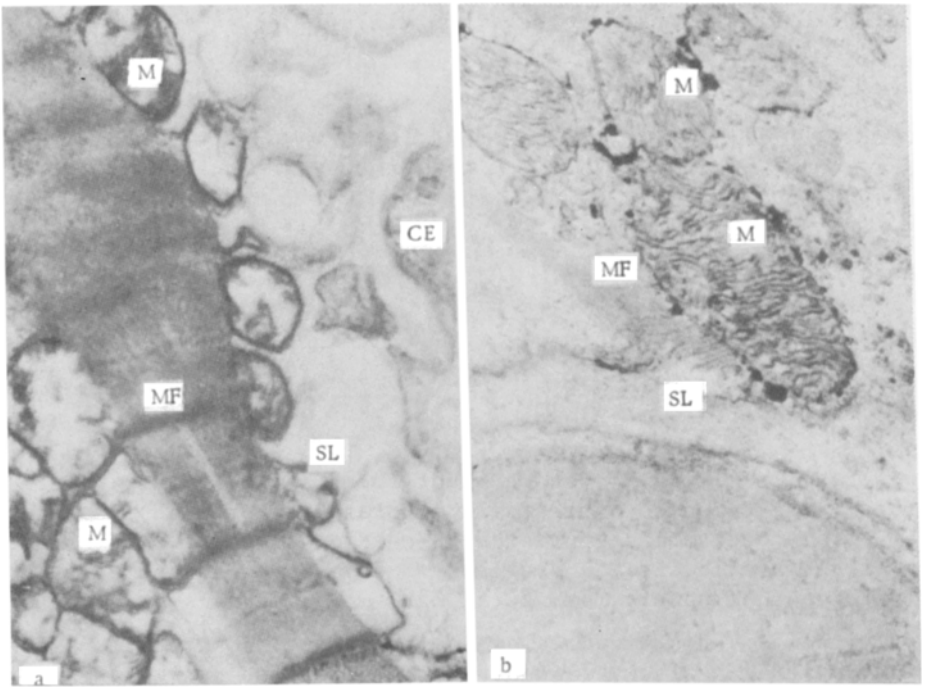

Fig. 2. Ultrastructure of injured area of myocardium 5 min after intracoronary injection of ACS. a) Irregular contraction of myofibrils (MF), vacuolation of mitochondria (M). 12,000 ×; b) colloidal lanthanum on mitochrondrial membranes. 18,000 ×. SL) Sarcolemma, CE) capillary endothelium.

branes and the resulting uncontrollable inflow of calcium are the immediate causes of damage to the contractile system of the myocardium of immune genesis observed in our previous experiments.

In the investigation described below permeability of the cardiomyocyte sarcolemma was studied with the aid of an electron-microscopic tracer — colloidal lanthanum; this method enables the earliest disturbances of membrane permeability to be detected.

EXPERIMENTAL METHOD

Experiments were carried out on 20 mongrel dogs weighing 15-20 kg anesthetized with chloralose and urethane (0.07 and 0.7 g/kg respectively). Immune cardiopathy was produced by injection of 1-2 ml of ACS into one of the branches of the left coronary artery (circumflex or descending); the ACS was obtained from rabbits immunized beforehand with the supernatant fraction of canine heart homogenate [3].

There were three series of experiments. In series I and II morphological investigations of the heart were carried out 5 and 60 min respectively after intracoronary injection of ACS. In series III the effect of intravenous injection of the antihistamine drug phencarol* (0.5 mg/kg) 10-15 min before intracoronary injection of ACS on the morphological changes was investigated. Myocardial tissue was taken from the injured (zone of injection of ACS) and uninjured regions of the left ventricle 60 min after injection of the ACS. The myocardium was fixed with colloidal lanthanum by the method in [11], quickly dehydrated in alcohols of rising concentration, and embedded in Araldite. Ultrathin sections were examined in the JEM-7A electron microscope.

EXPERIMENTAL RESULTS

The experiments showed that granules of colloidal lanthanum were located extracellularly in the intact (uninjured) areas of myocardium of the left ventricle, outlining the outer layers of the cardiomyocyte sarcolemma, the T system, and the intercalated disks (Fig. 1), evidence of integrity of the sarcolemma of cardiomyocytes located at a distance from the fo-

*Quinuclidyl-3-diphenylcarbinol.

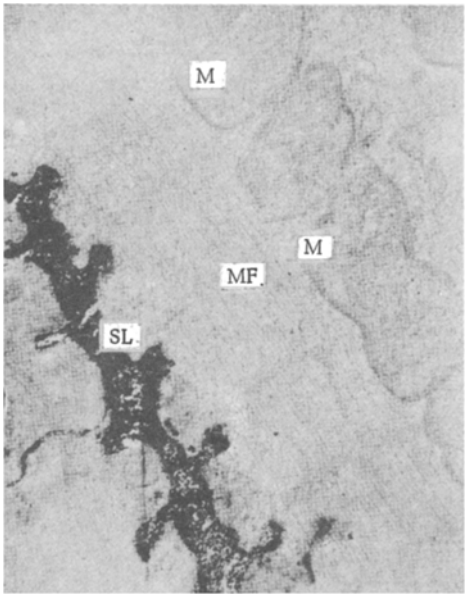

Fig. 3. Cardiomyocytes from region of injection of ACS (1 h after injection) with phencarol premedication. Colloidal lanthanum in intercellular space on outer side of sarcolemma. 18,000 ×. Legend as to Fig. 2.

cus of immune injury, and in agreement with data published previously [9] on distribution of lanthanum in the normal myocardium. In the same areas of myocardium in the early period after injection of ACS, fixation of anticardial antibodies or significant changes in the contractile apparatus and organelles of the muscle cells were not found.

Pathological foci containing cardiomyocytes with considerable structural changes were found in areas of myocardium of the left ventricle exposed to the primary action of antibodies injected into the coronary artery in the experiments of series I (Fig. 2). Areas of overcontraction of myofibrils, alternating with areas of overstretching and even rupture, vacuolation of the mitochondria, and deformation of their cristae were observed. At the same time particles of colloidal lanthanum were found to have penetrated into the sarcoplasm in these same areas of myocardium 3-5 min after the beginning of the reaction (Fig. 2a, b). Lanthanum particles were located mainly on the outer membranes, but in a few cardiomyocytes they were also seen on the inner membranes of the mitochrondria, evidence of the presence of permeability defects in the outer membranes. Injury to the outer mitochrondrial membranes takes place only when they are in contact with an excess of calcium. The results thus indicate that the immune reaction taking place in the heart gives rise to considerable disturbances of permeability of the sarcolemma, as a result of which calcium evidently flows uncontrollably inside the cardiomyocytes, causing destructive changes in their organelles characteristic of primary calcium lesions of the heart. In the later stages (1-1.5 h after injection of ACS) the number of modified cardiomyocytes was considerably increased; the pattern of injury, moreover, became more varied. Besides signs of calcium lesions (contractures, vacuolation of the mitochondria) signs of ischemic damage to the cardiomyocytes were observed: a decrease in the number of glycogen granules, swelling of the mitochrondria, margination of the nuclear chromatin, relaxation of the sarcomeres, and intracellular edema. Most of the cardiomyocytes in the region of immune injury to the left ventricle contained colloidal lanthanum granules.

The increase with time in the number of cardiomyocytes with signs of calcium injury was evidently linked with the unevenness of damage to the sarcolemma in individual cells in the course of development of the immune reaction in the heart. A few minutes after injection of ACS defects sufficient to allow passage of colloidal lanthanum particles (mean radius 2 nm [8]) appeared in the sarcolemma of those cardiomyocytes that were exposed to more severe immune challenge; defects appeared in the sarcolemma of less severely injured cells later and marked changes in the cells were observed after 1-1.5 h.

A massive disturbance of permeability of the sarcolemma in the focus of antibody fixation and subsequent changes in the cardiomyocytes are evidently among the main causes of the reduced contractility of an area of the left ventricular myocardium in local immune cardiopathy.

Preliminary injection of the antihistamine drug phencarol considerably prevented structural changes of immune genesis in the myocardial cells. In the area of primary action of anticardial antibodies (the area of injury) the cardiomyocytes mainly preserved their normal ultrastructure 1.5 h after the beginning of the immune reaction in the heart. Perivascular and intracellular edema, so characteristic of immune injury to the heart, was sharply reduced. Only solitary injured cardiomyocytes were observed. The reaction with colloidal lanthanum showed that its distribution was mainly extracellular (Fig. 3).

Immune attack on the myocardium is thus accompanied by disturbance of ionic permeability of the cardiomyocyte sarcolemma, leading to the development of injuries to the contractile apparatus and organelles of the heart muscle cells typical of "calcium" necroses. A characteristic feature of immune cardiopathy compared with other forms of pathology of the heart (acute myocardial ischemia, catecholamine necroses) is the unusually rapid onset of disturbance of membrane permeability, as revealed by penetration of colloidal lanthanum into the sarcoplasm actually during the first minutes of the reaction. The concrete mechanisms of such a rapid disturbance of membrane permeability will be investigated later. The data given in this paper are evidence in support of the view that factors involved in the pathochemical phase of the immune reaction, notably histamine, play an essential role in the development of these disturbances.

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