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STATE OF THE SARCOLEMMA OF SUBENDOCARDIAL PURKINJE CELLS IN THE LATE STAGE OF EXPERIMENTAL MYOCARDIAL INFRACTION IN DOGS

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Morphological studies have shown that occlusion of the left descending coronary artery in dogs gives rise to necrosis in the myocardium of the left ventricle; cell death takes place primarily in the subendocardial region of the myocardium, after which the process of injury spreads toward the epicardium. Between 6 and 24 h after the beginning of ischemia, the infarct as a rule becomes transmural, i.e., most of the myocytes in the zone of ischemia have suffered irreversible injury [12]. In this late stage of experimental infarction persistent disturbances of rhythm develop, due to the activity of subendocardial Purkinje fibers located in the zone of ischemia [5, 9, 14]. Despite the fact that after occlusion of the coronary artery the earliest and most profound changes arise in the region of the endocardium, the subendocardial elements of the conducting system preserve their viability and act as sources of arrhythmias in the late stage of experimental myocardial infarction.

The subendocardial Purkinje fibers isolated from the zone of ischemia in the late stage of infarction are known not to have undergone any significant changes [6].

The object of this investigation was to study the ultrastructural features of the subendocardial Purkinje fibers in the zone of ischemia, paying special attention to the state of the sarcolemma, changes in the structure of which may be responsible both for the development of arrhythmias and for the high sensitivity of the Purkinje cells to antiarrhythmic drugs in the late stage of experimental myocardial infarction [4].

EXPERIMENTAL METHOD

Experiments were carried out on three mongrel dogs of both sexes weighing 10-15 kg. Under pentobarbital anesthia (35 mg/kg, intravenously) a myocardial infarct was induced by two-stage occlusion of the left descending coronary artery [7]. The dogs were reanesthetized 24 h after ligation of the coronary artery (100 mg/kg chloralose, intravenously) and the chest opened; the heart was quickly removed, the left ventricle was cut out and placed epicardium uppermost in oxygenated Tyrode's solution containing (in mM): NaCl 130, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 0.6, $NaHCO_3$ 20, glucose 5, pH 7.4; the temperature of the solution was 20°C. Material for electron-microscopic investigation was taken from the most

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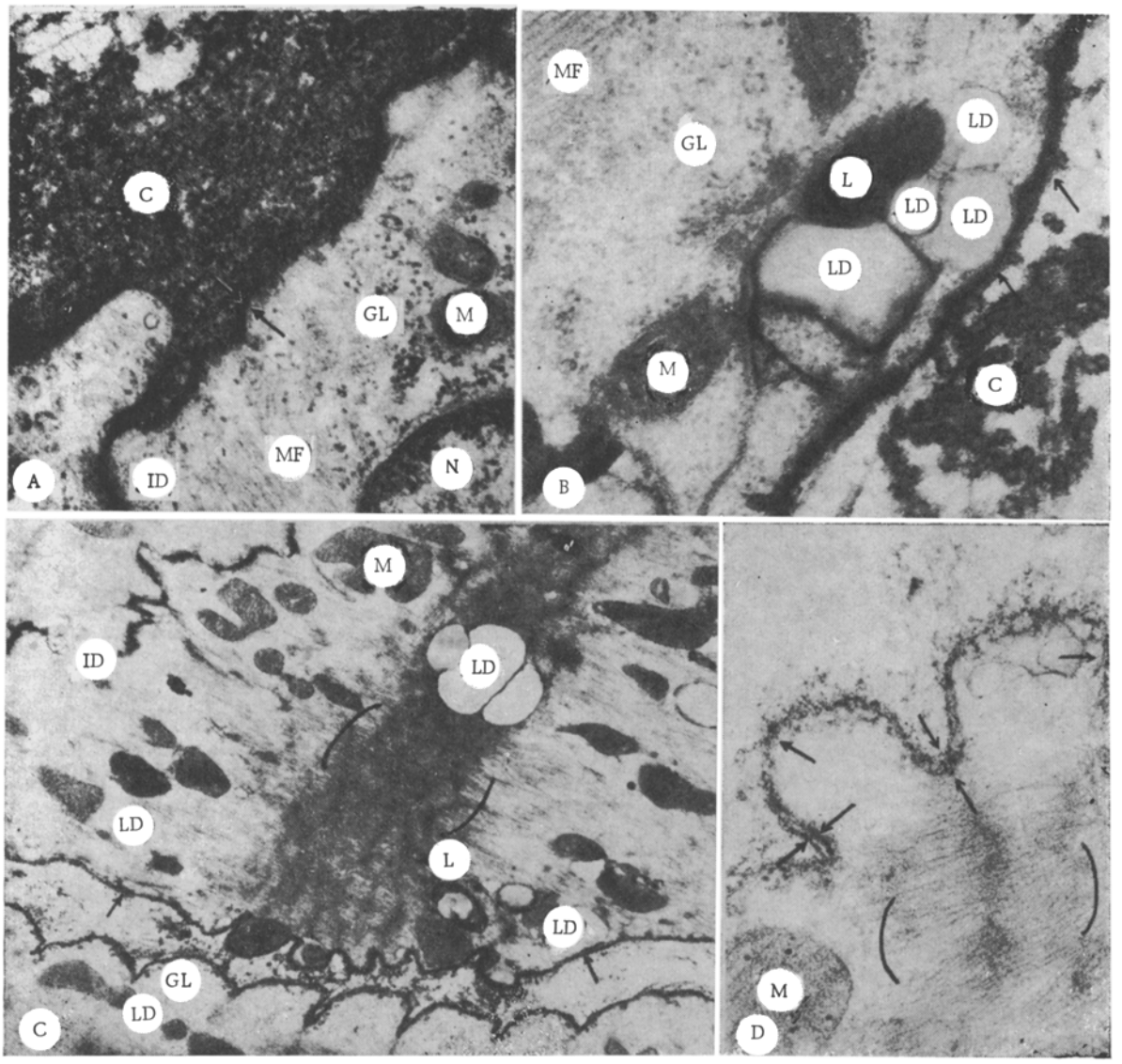

Fig. 1. Subendocardial Purkinje cell in left ventricle of dog's heart 24 h after ligation of left descending coronary artery. Sarcolemma stained with ruthenium red. C) Collagen; MF) myofibrils; M) mitochondria; ID) intercalated disks; N) nucleus; L) lysosome; LD) lipid drop; GL) glycogen; arrows indicate glycocalyx; foci of overcontraction of myofibrils shown between parentheses; section stained with uranyl acetate and lead citrate. A) Purkinje cell from nonischemized region of left ventricle; ruthenium red stained the thick outer layer of the sarcolemma (layer IV of collagen), in close contact with the glycocalyx, 50 nm thick; 60,000 ×; B) Purkinje cell from focus of infarct, differing only by its increased content of lipid drops and lysosomes; outer collagen layer of sarcolemma preserved only as fragments, glycocalyx unchanged; 60,000 ×; C) overcontracted Purkinje cell from infarct zone, sarcolemma practically without its outer layer, 15,000 ×; D) translucency and reduced thickness of glycocalyx of overcontracted Purkinje cell located in zone of infarct; double arrows indicate site of translucency of glycocalyx, single arrows denote where its thickness is reduced to 30 nm, 80,000 ×.

bloodless part of the myocardium of the infarct zone; tissue from the nonischemized myocardium of the same ventricle was used as the control. With a sharp razor blade pieces of tissue were cut out so that each sample included endocardium and the subjacent layer of myocardium about 1 mm thick.

Ruthenium red (from Merck, West Germany), as the best knowns of the mucopolysaccharide stains, was used for the histochemical detection of the carcolemma. Fixation with

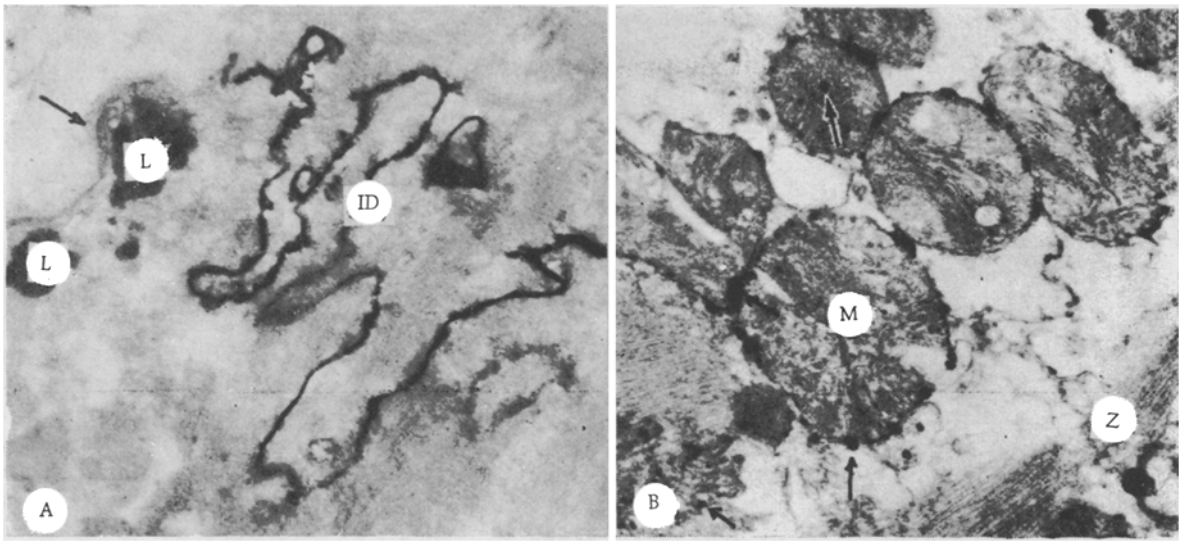

Fig. 2. Localization of colloidal lanthanum in subendocardial cells of infarct zone. ID) Intercalated disks; M) mitochondrion; L) lysosome; Z) Z-disk. A) Colloidal lanthanum does not penetrate into sarcoplasm of ischemized Purkinje cells but stains lumen of intercalated disks; arrows indicate glycocalyx free from third layer of sarcolemma; unstained preparation, 20,000 ×; B) contractile cardiomyocyte containing particles of colloidal lanthanum in its sarcolemma and the matrix of some mitochondria (arrow), 40,000 ×.

ruthenium red was carried out by the method in [10]; The samples were fixed for 1 h in glutaraldehyde and then postfixed for 3 h in osmic acid. Ruthenium red was added to the fixing fluid before use; the solution of ruthenium red was made up in deionized water.

The permeability of the sarcolemma was assessed by means of colloidal lanthanum, made up by the method in [13] at pH 7.8; for final postfixation the concentration of $La(OH)_3$ in the fixative was 1.3%. Glutaric fixation with colloidal lanthanum was carried out at room temperature for 12 h with constant agitation, after which the pieces of tissue were postfixed in osmic acid with the addition of the colloid for 1 h. After rapid dehydration in alcohols of increasing concentration the tissue was taken through two changes of propylene oxide and embedded in a mixture of Epon and Araldite. Capsules containing the myocardium were so oriented that each section could pass simultaneously through the endocardium and the subjacent cells. Ultrathin sections were cut on a Reichert $OM-U_3$ ultramicrotome and examined with the JEM-100B electron microscope.

EXPERIMENTAL RESULTS

The term "sarcolemma" is taken from light microscopy of heart cells and it means the boundary of the muscle fiber. Later, as a result of the use of the electron microscope, it was found that the sarcolemma, i.e., the cell boundary, consists of three layers. The first inner layer is the true plasma membrane, the plasmalemma, 9 nm thick. The second, middle layer, consisting of glycoproteins, is about 50 nm thick; in muscle cells it is usually called the glycocalyx. The third, outer component of the sarcolemma consists of type 4 collagen fibers; it separates the sarcolemma from the interstitial substance [3, 8, 11].

The experiments showed that the sarcolemma of the Purkinje cells, which were arranged in two layers and were immediately beneath the endocardium, in the control myocardium was constantly covered by a continuous third layer. On staining with ruthenium red the outer layer of the sarcolemma appeared to consist of a coarsely granular, loose, electron-dense material, varying in thickness from 50 to 500 nm, in close contact with a more electron-dense homogeneous layer of the glycocalyx, about 50 nm thick (Fig. 1A).

In the zone of ischemia only the Purkinje cells appeared viable. In those Purkinje cells which differed from the control only in their larger number of lipid drops and lysosomes, complete or almost complete disappearance of the third layer of the sarcolemma was observed (Fig. 1B). The layer of the glycocalyx showed no visible changes in this case. Among the

Purkinje cells were some in a state of more or less well-marked overcontraction. The sarcolemma of these cells was completely without its third layer, and the glycocalyx was thinner or it showed central translucency (Fig. 1C, D). Colloidal lanthanum did not penetrate into the Purkinje cells of the ischemized zone (Fig. 2A). Colloidal lanthanum was constantly observed in the irreversibly changed contractile cells of this zone as electron-dense granules on the surface of the mitochondria or in their matrix (Fig. 2B).

The morphological data provide an additional argument in support of the hypothesis put forward on the basis of electrophysiological observations, that the cells in the zone of ischemia have increased sensitivity to antiarrhythmic agents [4]. Fresh confirmation of this hypothesis was obtained in experiments using tetrodotoxin (TTX), which specifically blocks fast sodium channels. Intravenous injection of TTX into dogs 24 h after occlusion of the coronary artery led to abolition of the disturbances of rhythm [1]. The antiarrhythmic action of TTX was preserved on a heart isolated in the late stage of experimental myocardial infarction [2]. Restoration of the normal sinus rhythm through the action of TTX indicates that the conduction process in the myocardium outside the boundaries of the zone of ischemia was not substantially changed. Moreover the antiarrhythmic action of TTX was achieved in concentrations 100-1000 times less than the concentrations of TTX necessary to block conduction in the normal myocardium [2]. Hence, 24 h after occlusion of the coronary artery the subendocardial Purkinje fibers, in which arrhythmia developed [5, 9], exhibit extremely high sensitivity to TTX. This high sensitivity of the Purkinje fibers may be connected with the state of the glycocalyx and the layer of collagen fibers, if these structures are regarded as a diffusion barrier on the part of cardiotropic substances for the ionic channels located in the plasmalemma. Under normal conditions the presence of a diffusion barrier creates conditions under which cardioactive substances must cross the barrier formed by the intricately organized structures adjacent to the plasmalemma. Disappearance of these structures in Purkinje fibers as the result of ischemia makes the ionic channels and, in particular, the sodium channels more accessible for antiarrhythmic agents. The separation into layers of the glycocalyx, directly adjacent to the basement membrane, and the defects in its structure discovered in this investigation must also help to increase the sensitivity of the ionic channels.

Despite the essential changes in structure adjacent to the plasmalemma, that structure itself evidently remains relatively intact. This is confirmed by data showing that lanthanum does not penetrate into the Purkinje fibers of the ischemic zone (Fig. 2A). Meanwhile ionic transport in the ischemized Purkinje fibers is modified: It is shown by electrophysiological data according to which the Purkinje fibers isolated from the zone of ischemia have a low resting potential and increased ability for automatic activity as a result of an increase in the steepness of diastolic depolarization [5, 9]. Data on overcontraction of the Purkinje fibers in the zone of ischemia are also in support of changes in ionic transport (Fig. 1C, D). The absence of permeability for lanthanum in the cells of the conducting system thus indicate that the plasmalemma of these cells has no serious defect.

In fibers of the conducting system in the zone of infarction no great ultrastructural changes could be found compared with the control. However, these cells do experience oxygen insufficiency, as is shown by the accumulation of lipid drops in them (Fig. 1B, C). Accumulation of lipid drops in the subendocardial Purkinje fibers in the zone of ischemia was demonstrated previously [6].

In conclusion, it will be noted that the role of structures on the outer surface of the plasmalemma in the formation of ionic transport during the action of various substances has so far received little study. Nevertheless, the results of the present investigation suggest that the glycocalyx and the collagen layer may affect accessibility of ionic channels to various cardiotropic drugs, including antiarrhythmic agents.

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CHANGES IN THE GASTRIC MUCOSA IN THE EARLY STAGES OF CHRONIC RENAL FAILURE IN RATS

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KEY WORDS: renal failure; gastric mucosa; rats.

The nature and mechanism of formation of pathology of the gastric mucosa during chronic renal failure (CRF) (uremic gastritis) have not yet been explained [3, 6], so that a study of this problem from the experimental aspect is essential.

To determine the precise mechanisms of action of CRF in the early stages of its formation on the gastric mucosa, in the investigation described below a series of chronic experiments were carried out on rats. Special attention was paid to changes in the stomach during initial disturbances of kidney function, which have hardly been studied at all under clinical conditions.

EXPERIMENTAL METHOD

Altogether 30 mature rats of both sexes were used; under ether anesthesia a two-stage subtotal nephrectomy was performed through an extraperitoneal approach. Material for examination, consisting of pieces of the mucosa from the fundal and antral portions of the stomach, was taken 1, 4, and 6 months after the operation. Sections 5 μ thick, stained with hematoxylin and eosin, and by the Dominici–Kedrovsky method in Samsonov's modification [4], were used for histological investigation. For histochemical investigation pieces of stomach tissue frozen in liquid nitrogen were used. The following oxidoreductases — succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G6PDH), and monoamine oxidase (MAO), and the hydrolases acid phosphatase (AP) and ATPase — were studied in frozen sections 10 μ thick. To determine the distribution of neutral mucopolysaccharides the PAS reaction was used. Quantitative assessment of activity of these enzymes was carried out with the MUF-5 cytospectrophotometer. Activity of the enzymes was calculated from mean values obtained by the study of 100-150 cells. The results were subjected to statistical analysis. The degree of development of CRF was estimated by the increase in the blood serum urea level from 4.4 ± 0.2 mM in the control to 7.0 ± 0.5 mM after 1 month, to 10.0 ± 0.5 mM after 4 months, and to 8.5 ± 0.4 mM 6 months after the operation.

EXPERIMENTAL RESULTS

In all nephrectomized animals hyperplasia of the mucosa of the fundal portion of the stomach developed by the sixth month on account of an increase in the number of parietal and chief cells (in the normal gastric gland there are 42.0 ± 4.0 chief and 19.5 ± 3.0 parietal cells, but by the end of the experiment there were 80.5 ± 10.0 and 34.5 ± 4.0, respectively). This phenomenon is accompanied, as the data showed indirectly, by an increase in gastric secretion, as shown by an increase in SDH activity and a decrease in LDH activity (parietal

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