MORPHOLOGICAL AND BIOCHEMICAL CORRELATIONS IN MUSCLE TISSUE OF THE ATRIA AND VENTRICLES

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An electron-microscopic investigation of heart muscle tissue showed that the cardiomyocytes of the atria differ from those of the ventricles. Three types of cells differing in the character of relations between the organoids and the number of specific secretory granules in them, were found in the atrial muscle tissue. A study of the distribution of the activity of lactate dehydrogenase (LD) and its isozymes by disc electrophoresis and isoelectric focusing showed that LD isozyme spectra of the ventricles and atria differ considerably from each other. It is suggested that the sources of energy and the mechanism of energy production are not the same in the muscle cells of the atria and ventricles.

KEY WORDS: cardiomyocytes of the atria and ventricles, ultrastructure, lactate dehydrogenase isozyme spectrum.

The main structural unit of cardiac muscle tissue is a highly differentiated cell – the cardiomyocyte. Until recently it was considered that the typical cross-striated muscle fibers of the heart perform only a contractile function. These views were formed because mainly the myocardium of the ventricles has been studied and the results obtained in this way were automatically extrapolated to the heart as a whole.

Recent publications have shown not only the structural specificity, but also certain distinguishing features of the metabolism of the muscle tissue of the atria and ventricles in higher vertebrates [1, 2, 5, 6, 10, 11]. The results of physiological observations indicate that the positive inotropic effect per contraction is greater in the ventricle than in the atrium [4]. However, the value of the investigations so far undertaken is considerably reduced because their results have not hitherto been compared with respect to the same object.

It was accordingly decided to undertake a combined morphological and biochemical investigation of heart muscle tissue in rats in order to elucidate certain structural and metabolic characteristics of different regions of the heart.

EXPERIMENTAL METHOD

Experiments were carried out on 30 noninbred male albino rats weighing 150-200 g. The muscle tissue of the ventricles and atria were studied by the usual histological method and by electron microscopy. Material for electron-microscopic analysis was fixed in 3% glutaraldehyde (in phosphate buffer, pH 7.4) and embedded in Araldite. Ultrathin sections were cut on the LKB-1 ultratome and stained with uranyl acetate followed by lead citrate.

For biochemical investigation, hearts from 3 or 4 animals were used in each experiment. Homogenates of the tissues of the atria and ventricles were prepared in the cold by the usual method with the addition of EDTA and Triton X-100. The supernatant was used to determine total lactate dehydrogenase (LD) activity [9], for electrophoresis in flat blocks of polyacrylamide gel [15], for isoelectric focusing with ampholines in a thin layer of polyacrylamide gel [12], and for determination of the protein content [8]. At the end of electrophoresis and isoelectric focusing the localization of the LD isozymes was studied by the phenazine metasulfate–tetrazolium reaction [13]. For quantitative electrophoretic analysis the ERI-65m automatic integrating densitometer was used. Statistical analysis of the results was carried out by the method of indirect differences [3].

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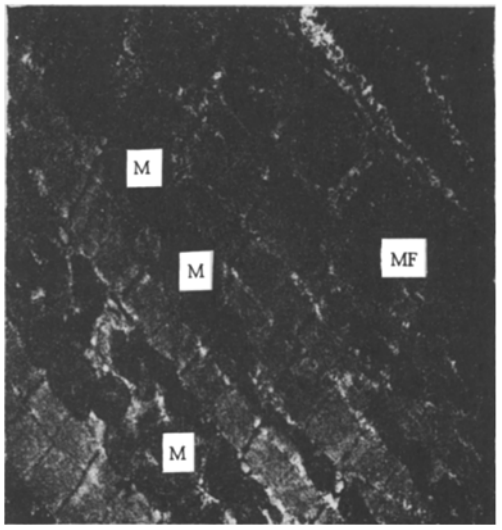

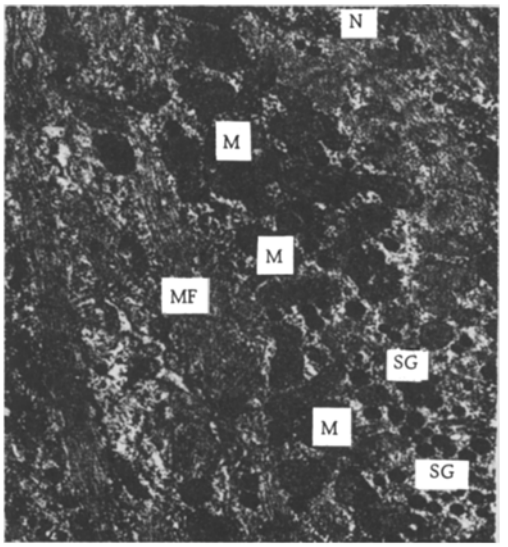

Fig. 1 Fig. 2

Fig. 1. Ventricular cardiomyocyte (4000×). M) Mitochondria; MF) myofibrils.

Fig. 2. Atrial (musculo-secretory) cardiomyocyte (4200×). N) Nucleus; MF) myofibrils; M) mitochondria; SG) secretory granules.

TABLE 1. Activity of LD and Its Isozymes (in μmoles NADH/mg protein/min) in Atrial and Ventricular Myocardium of Rats (M ± m)

Test object	Total LD	LD isozymes				
		LD_1	LD_2	LD_3	LD_4	LD_5
Atrium (5)	0,63±0,07	0,34±0,02	0,11±0,005	0,07±0,01	0,09±0,01	0,03±0,01
Ventricle (5)	1,90±0,30	0,77±0,01	0,29±0,02	0,45±0,02	0,34±0,01	0,05±0,005

Legend. Number of experiments in parentheses.

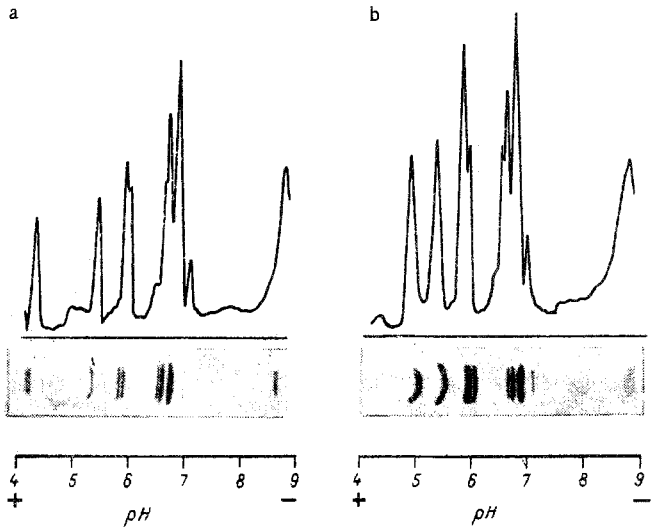

Fig. 3. Data of isoelectric focusing and densitograms of isozymes of soluble LD from atria (a) and ventricles (b) of intact rats. pH gradient 3.5-9.5. Duration of isoelectric focusing 2 h 20 min. Final voltage 1050 V. Temperature 5°C.

EXPERIMENTAL RESULTS

The electron-microscopic investigation showed that muscle fibers of the myocardium of the rat ventricles consist of highly differentiated cardiomyocytes with a well-developed myofibrillary apparatus, which occupies nearly all of the cytoplasm (Fig. 1). Tubules of the smooth reticulum were found near the nucleus between the myofibrils. The mitochondria were near the cytolemma, compactly arranged between the myofibrils. Intercalary disks in the fibers of the ventricular myocardium consisted of folded membranes with many desmosomes.

The course of the muscle fibers in the atria was more tortuous and the places between the fibers wider than in the ventricles. The cell composition of the muscle fibers of the atrial myocardium differs significantly from the ventricular not only in the degree of development of the subcellular structures characteristic of contractile cells, but also in the presence of special elements – secretory granules (Fig. 2). This fact also was noted previously [10].

As regards the degree of development of the myofibrils, the character of relations between the organoids, and the number of specific granules all the muscle cells of the atria can be divided into three types. The first type includes cardiomyocytes with a well-developed myofibrillary apparatus and many mitochondria. Such cells resemble the ventricular cardiomyocytes. The second type includes cells with a poorly developed myofibrillary apparatus and with freely lying mitochondria (Fig. 2). The rough reticulum and Golgi complex are well developed in their cytoplasm and they contain numerous secretory granules surrounded by a single whole membrane. The morphological pictures, according to the results of electron microscopy, suggest that the substance of the granules is eliminated through the cytolemma. The granules studied differ from lysosomes and lipid inclusions. The whole description could indicate that the granules found in the heart muscle cells are secretory granules. Considering the morphological features of the cells of this type, they may be called musculo-secretory. The third type consists of cells with well-developed myofibrils and a few secretory granules.

As Table 1 shows, the LD isozyme spectrum of the myocardium is highly specific and characterized by a high content of LD_1 and LD_2, which determine the total activity of the enzyme. It must be emphasized that the total LD activity in the muscle tissue of the ventricles is 3 times greater than in the muscle tissue of the atria. Meanwhile the relative predominance of the anodal fractions of the enzyme (LD_1 and LD_2) was more marked in the atria than in the ventricles (70.3% and 55.7% of the total LD activity respectively). Differences in the LD isozyme spectra of these two parts of the hearts were seen most clearly when the distribution of activity of the cathodal forms of the enzyme, LD_3, LD_4, and LD_5, was analyzed. The activity of each of these isozymes was considerably higher in the ventricles than in the atria. Furthermore, the relative content of the cathodal fractions of LD was considerably higher in the ventricles than in the atria (44.3 and 29.7% of the total LD activity, respectively).

Analysis of the LD composition in the different parts of the heart thus indicates that the LD isozyme spectrum of the ventricles differs from that of the atria in the predominance, both absolute and relative, of cathodal forms of the enzyme: LD_3, LD_4, and LD_5.

Isoelectric focusing of LD from the ventricular and atrial myocardium of rats (Fig. 3) revealed many zones of specific activity mainly in the weakly acid regions of the gradient at pH 4.9-7.2. The isoelectric spectra of LD from the ventricle and atria differed considerably from each other in both the number and the activity of individual subunits of the enzymes. The difference between the isoelectric spectra of LD from different parts of the myocardium was seen most clearly on analysis of isozymes identified as LD_2 and LD_3 (isoelectric points 4.95, 5.35, 5.65, 5.85, and 5.90, respectively).

The polymorphism of LD isozymes discovered by differential analysis of the atria and ventricles, reflected in both qualitative and quantitative differences, thus correlates with the profound structural heterogeneity of the cardiomyocytes of these two regions of the heart. The reaction catalyzed by LD is the most suitable reaction for the investigation of the relationship between aerobic and anaerobic processes [7, 14]. The results now obtained suggest that the muscle cells of the atria and ventricles rely on different sources of energy and different mechanisms of energy production.

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EFFECT OF CLORPROMAZINE ON ULTRASTRUCTURE OF EPITHELIAL CELLS AND CELL CONTACTS IN THE FROG BLADDER

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Chlorpromazine causes swelling and vacuolation of cells of the mucous membrane of the frog urinary bladder and prevents their response to antidiuretic hormone (ADH) by increased permeability for water. The structure of the zone of intercellular contacts was undisturbed, and this could be the reason for the impermeability of the chlorpromazine-treated epithelium for water.

KEY WORDS: chlorpromazine; permeability for water; antidiuretic hormone; swelling of the cell; intercellular contacts.

For the last two decades the problem of the mechanism of the increased water transport under the influence of antidiuretic hormone (ADH) has been a subject of intense discussion. According to one hypothesis, ADH increases the permeability of the apical plasma membrane of the cell for water [6, 7], whereas according to another hypothesis it is the permeability of the ground substance that is increased [1, 4]. Chlorpromazine prevents ADH from increasing the absorption of water [3]. The object of the present investigation was to compare the effect of chlorpromazine on the ultrastructure of the cell and intercellular contacts during its inhibition of the ADH effect.

EXPERIMENTAL METHOD

The bladder of a frog was filled with Ringer's solution diluted with water 1:10, with the mucous membrane on the inner side, and immersed in aerated Ringer's solution. The degree of permeability for water was estimated from the volume of water absorbed from the bladder along the osmotic gradient [5]. Chlorpromazine and and ADH were added to the Ringer's solution on the side of the mucous membrane. For the electron microscopic investigation, bladders in different functional states were transferred for 3-5 min into a 2.5% solution of glutaraldehyde made up in Ringer's solution with cacodylate buffer (pH 7.4). The bladder wall was then incised,

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