MORPHOLOGICAL CHARACTERISTICS OF TISSUE COMPONENTS OF DIFFERENT LAYERS OF THE RAT MYOCARDIUM

V. A. Fedoseev and T. V. Pistsova

UDC 611.127-018-019

KEY WORDS: layers of the myocardium; morphometric analysis.

Studies of the principles governing cardiac function have revealed differences in values of metabolic parameters in different layers of the myocardium [10, 14, 15]. Investigations have shown that anaerobic pathways of energy formation predominate in the subendocardium and oxidative pathways in the subepicardial layer [15]. Differences in resistance of the layers of the myocardium to the action of experimental factors have been discovered in a few investigations using physiological methods [11, 12, 13].

On the basis of the above facts it can be postulated that the different layers of the myocardium play different roles in cardiac activity, but there is virtually no information on specific differences in their organization. Most studies of myocardial structure and, in particular, those using morphometric methods, give averaged values of structural parameters for the whole thickness of the heart muscle [2, 4, 7]. Comparatively few investigations have been devoted to the determination of morphological parameters of the different layers of the myocardium [1, 6]. However, in view of difficulties in the morphological identification of separate layers, different authors have distinguished the layers and defined their boundaries differently [6, 8, 9].

This paper describes a morphological study of the left ventrifuclar myocardium of rats aimed at discovering differences in the organization of the subepicardial, subendocardial, and intermediate layers of the myocardium. The anterior wall of the left ventricle was chosen as the test object because of the considerable difference in the direction of the muscle fibers in this chamber of the heart, a factor which can be used as the criterion by which to distinguish the layers [1].

EXPERIMENTAL METHOD

Experiments were carried out on five male Wistar rats weighing 180-200 g. Under pentobarbital anesthesia (0.05 mg/g body weight) the chest was opened. The heart was perfused with 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 10 min and then removed. Slabs 0.5-1.0 mm thick were cut from the middle third in a plane perpendicular to the axis of the left ventricle. The slabs were prefixed with 1% osmium tetroxide solution in phosphate buffer, dehydrated, and embedded in Epon. Blocks were sawn from the slabs, and semithin sections obtained from them with transverse orientation of the cardiomyocytes. The sections were stained with toluidine blue and measured under the MBI-3 microscope with magnification of 1350, using a multiple purpose universal ocular morphometric grid and an ocular micrometer.

The following parameters were determined: the relative volume of the myocytes (V_V^m), the relative volume of connective tissue (V_V^{ct}), the relative volume of the capillaries (V_V^c), the surface area of the myocytes (S_V^m), the surface area of the capillaries (S_V^c), the surface area of the cardiomyocytes expressed per unit volume of a cardiomyocyte (S_V^m/V_V^m), the surface area of a cardiomyocyte expressed per unit of surface area of the capillary (S_V^m/S_V^c), the volume of the cardiomyocytes expressed per volume of capillaries (V_V^m/V_V^c), and the volume of the capillaries as a fraction of the volume of connective tissue (V_V^c/V_V^{ct}). In addition, the mean values of the diameters of the cardiomyocytes (D) were calculated and the distribution of the cells by diameter also was estimated. The MIR-2 computer was used for the calculations and for statistical analysis of the data by the t test, allowing for primary and secondary objects [3].

Department of Morphology and Cytology, Medico-Biological Faculty, N.I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kupruyanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 9, pp. 349-351, September, 1985. Original article submitted October 8, 1984.

0007-4888/85/0009-1263$09.50

TABLE 1. Morphometric Parameters of Muscle and Connective Tissue Structures in Different Layers of Left Ventricular Myocardium ($\bar{X} \pm \sigma_{\bar{X}}$)

Parameter studied	Layer of myocardium			
	subendocardial	inner intramural	outer intramural	subepicardial
V_v^m	0,820±0,007	0,713±0,010*	0,72±0,008*	0,699±0,009
S_v^m	48,56±0,85*	48,68±0,87*	46,96±0,67	58,62±1,18
V_v^{st}	0,173±0,008	0,287±0,010*	0,273±0,008*	0,302±0,010
V_v^c	0,063±0,004	0,162±0,006*	0,102±0,007	0,169±0,007*
S_v^c	9,84±0,28	22,76±0,33	16,70±0,30	27,30±0,50
$\bar{D}$	12,07±0,02	10,49±0,03	13,27±0,01	9,28±0,02
S_v^m/V_v^m	54,90±0,67	63,00±0,35*	62,16±0,43*	79,80±0,29
S_v^m/S_v^c	4,93±0,05	2,15±0,08*	2,81±0,04	2,15±0,10*
V_v^m/V_v^c	12,95±0,50	4,40±0,32*	7,11±0,42	4,13±0,25*
V_v^c/V_v^{st}	0,408±0,025*	0,636±0,077	0,382±0,025	0,570±0,031*

Legend. *$P < 0.05$; in all other cases $P < 0.01$.

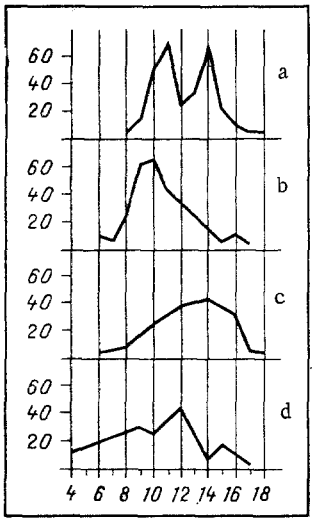

Fig. 1. Distribution of diameters of cardiomyocytes in different layers of left ventricular myocardium of rats. Abscissa, diameter of cells (in μ); ordinate, fraction of cells. a) Subendocardial layer, b) inner intramural, c) outer intramural, d) subepicardial layer. Number of cells for each layer was 30.

EXPERIMENTAL RESULTS

Differences in the direction of the muscle fibers were used as the criterion for distinction of layers in the myocardium. Depending on the direction of the fibers it was possible to distinguish four layers: outer — subepicardial, two layers in the intermediate zone — outer and inner, bounded respectively by the subepicardial and subendocardial zones, and the subendocardial layer. In the zone studied (the middle third of the wall of the left ventricle) these layers could be identified comparatively easily.

The results of the measurements and calculations for the different layers are given in Table 1 and Fig. 1. They show that the contribution of the volume of the cardiomyocytes to the total volume differs very little in the different layers. The relative surface area of the cells was maximal in the subepicardial layer. This is responsible for the significant differences between the surface areas of the cardiomyocytes when divided by their volume, in different layers, a parameter with which some authorities associate the degree of myocardial contractility [8]. The bulk density and surface area of the capillaries were minimal in the subendocardial layer, and differed significantly from those in the central and subepicardial layers, so that perceptible differences were found between the surface area and volume of the

capillaries, respectively. The value of this parameter, according to data in the literature, is linked to some degree with the level of available metabolism in the cardiomyocytes [1, 8].

The character of distribution of the cardiomyocytes by diameter in the different layers of the myocardium is interesting. The incidence of muscle cells of different sizes varied widely in all the layers. In the subendocardial layer highest frequencies were observed for myocytes measuring 11 and 14 μ. The character of distribution suggests the presence of mainly two types of muscle cells. However, it must be pointed out that the degree of variation of the diameter of the muscle cells is less in the central part of the myocardium. The subepicardial layer was characterized by considerable fluctuations of diameters over a wide range.

The study of the morphological characteristics of the tissue components in different layers of the left ventricular myocardium thus revealed differences in the morphometric parameters of the muscle and vascular elements of the heart. These variations in the structure of the different layers may perhaps be associated with their definite specialization in the course of cyclic activity of the heart.

LITERATURE CITED

1. G. G. Avtandilov and T. A. Gevondyan, Arkh. Anat., No. 7, 33 (1980).
2. M. S. Gnatyuk, Arkh. Anat., No. 5, 33 (1983).
3. G. S. Katinas and Yu. Z. Polonskii, Tsitologiya, No. 3, 339 (1970).
4. L. V. Kolesnikova and L. M. Nepomnyashchikh, Arkh. Anat., No. 4, 28 (1978).
5. C. Tasche, Introduction to Quantitative Cyto-Histological Morphology [in Russian], Bucharest (1980).
6. P. Anversa, A. V. Loud, F. Giacomelli, et al., Lab. Invest., 38, 597 (1978).
7. P. Anversa, G. Olivetti, M. Melissari, et al., Lab. Invest., 40, 341 (1979).
8. M. Aomine, M. Arita, S. Imanshi, et al., Jpn. J. Physiol., 32, 895 (1982).
9. A. F. Grimm, Hun-Lin Lin, and B. R. Grimm, Am. J. Physiol., 239, No. 1, H101 (1980).
10. M. A. Goldstein and D. A. Murphy, J. Mol. Cell. Cardiol., 15, 325 (1983).
11. M. Higuchi, J. Mol. Cell. Cardiol., 13, Suppl. 2, 41 (1981).
12. J. I. E. Hoffman, Clin. Sci., 61, 657 (1981).
13. S. Koyanagi, et al., Circ. Res., 50, 55 (1982).
14. W. M. Merrill, S. L. Alexander, and D. M. Conkle, J. Thorac. Cardiovasc. Surg., 82, 365 (1981).
15. E. Page and L. P. McCallister, Am. J. Cardiol., 31, 172 (1973).
16. J. L. Swain et al., Proc. Natl. Acad. Sci. USA, 79, 655 (1982).
17. I. Taira, H. Kanaide, and M. Nakamura, J. Mol. Cell. Cardiol., 13, Suppl. 2, 38 (1981).