Heterogeneity of Left Ventricular Cardiomyocytes from Rat Heart

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Contractile cardiomyocytes in various parts of the heart differ in shape, size, ploidy, and other parameters. However, it is not known whether their population is heterogeneous within each heart chamber. In this paper, dry weight and ploidy of cardiomyocytes were estimated in different parts of rat left ventricle. It was found that the dry weight of cardiomyocytes in medial part of left ventricular anterior wall is higher than in other parts of the ventricle. Cardiomyocyte ploidy is the same in different regions of the left ventricle.

Key Words: *cardiomyocytes; heterogeneity; ploidy*

Proliferative activity of cardiomyocytes (CMC) decreases rapidly after birth. In adult animal heart, the amount of DNA-synthesizing myocytes is practically zero [8]. Postnatal increase in animal heart weight is mainly due to cell hypertrophy [9]. According to many researchers, the higher is the functional load on the heart compartment, the more pronounced CMC hypertrophy and ploidy are observed in it [4,11]. Under pathological conditions, these indicators may undergo significant changes, which may be relevant for diagnostics and prognostic purposes [1]. The data on the degree of CMC polyploidy and hypertrophy in different regions of the heart are almost lacking. Here we studied the degree of CMC hypertrophy and polyploidy in different of the left ventricle (LV) of rat heart.

MATERIALS AND METHODS

We used LV samples from healthy adult Wistar rats (n=6, body weight 250-300 g). LV wall was divided along the vertical axis into three parts: apical (closer to the apex of the heart), medial, and basal (closer to the atria). LV tissue was dorsoventrally divided into the anterior and posterior walls (Fig. 1). Pieces of tissue of each part were fixed in 10% neutral formalin. To prepare the smears of isolated CMC, alkaline dissociation of the tissue was used [2].

Dry weight of rat CMC was measured on fixed unstained smears embedded in glycerol, with MBIN-4 interference microscope (LOMO, Russia) in monochromatic light using an interference filter λ_{max}=550 nm and objective 10×0.30.

Dry weight of CMC was calculated by the formula:

$$M=\frac{\delta S}{100\alpha},$$

where M is cell dry weight (g), δ is optical path difference (cm), S is cell area (cm^2), and α is specific refractive index increment (cm^3/g) [3].

Optical path difference was determined by adjusting for darkness by the formula:

$$\delta=\frac{(\varphi_1-\varphi_2)}{K}\times\lambda,$$

where δ is path difference (cm), φ_1 and φ_2 are Senarmont compensator data (degrees), λ is wavelength (cm), and K=180°.

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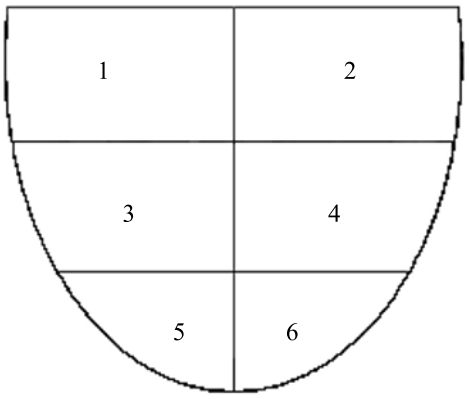

Fig. 1. Scheme of LV wall division into parts. *1*, *2*, basal part; *3*, *4*, medial part; *5*, *6*, apical part. Parts *1*, *3*, and *5*, posterior wall; *2*, *4*, and *6*, anterior wall.

To measure the cell area, image analyzer was used. The specific refractive index increment of proteins in glycerol is 0.0095 cm^3/g [12].

To determine CMC ploidy, the smears were stained after Feulgen [6]. DNA content in CMC nuclei was determined using fluorescence cytophotometry VideoTesT-Morphology image analysis system (Ista VideoTesT), which includes ES-Bimam microscope, CPT-8360 digital CCD-camera, and IBM PC [10]. The measurements were carried out in monochromatic light (interference light filter, λ_{max}=545 nm) using 40×0.65 objective. In each region of the ventricles 200 cells was analyzed. The mean (X) and error of the mean (S_x) were calculated for each parameter. When summing up the results from different animals, weighted means were calculated [7]. The significance of differences was evaluated by Student's t test at $p<0.05$.

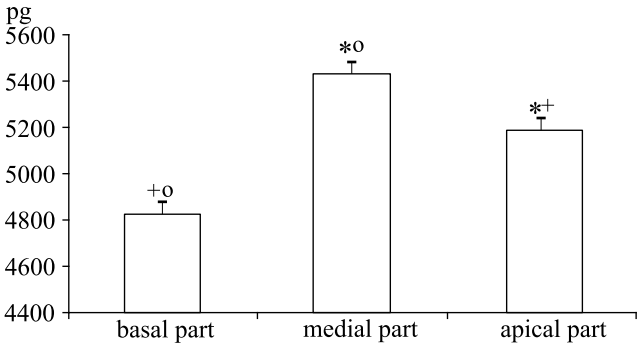

Fig. 2. Dry weight of CMC from basal, medial, and apical LV parts. $p<0.05$ relative to *basal part; +medial part; °apical part.

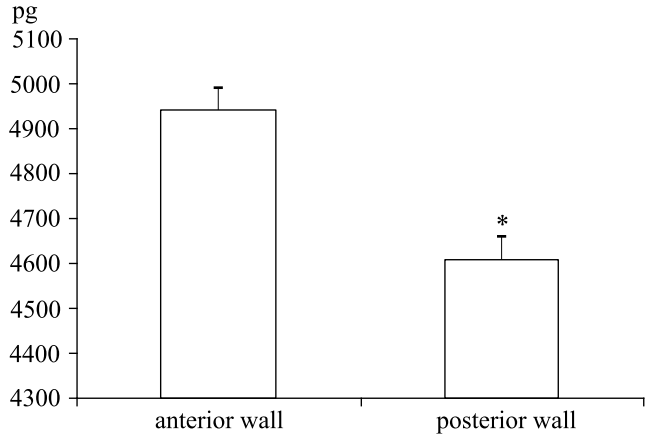

Fig. 3. Dry weight of CMC from anterior and posterior LV walls. *$p<0.05$ relative to anterior wall.

RESULTS

The results of CMC dry weight measuring in different regions of rat LV attest to heterogeneity of CMC population by this parameter. CMC dry weight in the medial region LV (4827±55 pg) is higher by 11% than that in the basal region (5429±54 pg), and 4.4% more than of those in apical region (5190±113 pg; Fig. 2). We have also shown that the dry weight of CMC in the anterior LV wall is higher than in the posterior wall by 6.7% (4940±51 *vs*. 4608±49 pg; Fig. 3). Since most of the sarcoplasm in working cardiomyocytes is occupied by contractile elements, these structures determine the dry weight of these cells. Thus, we can assume that cells of the medial part of LV anterior wall are subjected to maximum loads.

The size of CMC also depended on their ploidy [11]. It is known that CMC polyploidy occurs in postnatal animals [8]. CMC ploidy in LV of different animal species significantly exceeds the level of CMC ploidy in other heart departments [4]. This is related to the fact that LV pumps greater blood volume than the right ventricle and atria. However, the heterogeneous CMC ploidy pattern in various LV regions has not been investigated previously. Our study shows that the modal ploidy class of CMC is (2c×2) cells in all regions (Table 1).

Mononuclear diploid (2c) CMC are less numerous. Other ploidy classes (4c, 4c×2, *etc*.) are very rare in rats; their combined percentage is about 1-5% (Table 1). There were no significant differences between apical, medial, and basal LV parts in distribution of CMC by ploidy classes (Table 1). We can assume the trend towards reducing the proportion of 2c cells and increase in the proportion of 2c×2 cells in a direction extending from the base to the apex of the heart. The mean CMC ploidy did not differ in these regions.

There were no differences between the anterior and posterior LV walls in distribution of ploidy classes and average CMC ploidy (Table. 2).

Thus, the DNA ploidy pattern in CMC population of rat heart is homogenous, but the level of hypertrophy differs significantly both in dorsoventral and basal-apical directions.

TABLE 1. Percentage of CMC of Different Ploidy Classes in the Basal, Medial, and Apical LV Parts ($X \pm S_x$)

LV region	CMC of different ploidy classes, %				Mean ploidy
	2c	2c×2	4c	4c×2	
Basal	13.5±4.8	83.5±4.6	1.5±0.3	1.5±0.6	3.79±0.10
Medial	10.5±2.1	87.2±2.1	1.2±0.1	1.1±0.4	3.83±0.05
Apical	8.9±1.9	88.7±2.3	1.4±0.2	0.9±0.4	3.86±0.03

TABLE 2. Percentage of CMC of Different Ploidy Classes in the Anterior and Posterior LV Walls ($X \pm S_x$)

LV region	CMC of different ploidy classes, %				Mean ploidy
	2c	2c×2	4c	4c×2	
Anterior wall	15.7±5.7	81.8±4.5	1.2±0.5	1.3±0.7	3.74±0.14
Posterior wall	16.8±5.8	79.3±5.7	1.7±0.1	2.2±0.2	3.75±0.12

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