ULTRASTRUCTURAL AND MORPHOMETRIC STUDY OF RAT HEART MUSCLE CELLS IN CULTURE

R. Meyer, H. David,
G. Vasilev, and H. Guski

UDC 612.172-085.23

An ultrastructural and morphometric investigation was made of cells from the right and left ventricles of young rats 4 days old during culture for 7 days, in order to study the morphological and stereological parameters of the mitochondria and myofibrils. The results of the morphometric measurements revealed the periodic character of structural changes in the mitochondria and myofibrils. The changes discovered must be regarded not as structural differentiation of the cells but rather as adaptation to the conditions of culture.

KEY WORDS: morphometry; rat cardiomyocytes; culture of cardiomyocytes.

Rat heart muscle cells in culture are used for various types of experimental, physiological, and biochemical research, for the conditions of the medium can be relatively constant and the rate of pulsation can be regarded as a parameter of their functional capacity [4-6].

It has not yet been established what morphological changes take place in heart muscle cells in virto during culture and how they can be harmonized with certain contradictory biochemical data [7-9].

Against this background it was decided that existing qualitative morphological data [10] should be augmented by the results of an ultrastructural and morphological investigation of heart muscle cells in culture in order to detect quantitative changes in the structure of the mitochondria and myofibrils.

EXPERIMENTAL METHOD

The method of obtaining heart muscle cells and the conditions of the medium were described previously [6-11]. Muscle cells from the ventricle of 4-day-old Wistar rats (original material) were cultured on coverslips. The cell density in each flask (Leighton's tube) was 2×10^6 cells in 2 ml of 5M 20_1 nutrient medium [4, 12] with the addition of 10% inactivated calf serum. The cells were cultured for 7 days and material for electron-microscopic investigation was taken each day. After fixation in glutaraldehyde the cells were carefully separated from the substratum with a razor blade, then fixed in OsO_4 and embedded in Micropal. Sections were cut on an LKB Ultrotome and studied in the IA (Simens) electron microscope. On each day of tissue culture, 50 electron micrographs with secondary magnification of between 20,000 and 40,000 were subjected to morphometric examination by the quantitative stereological dot-counting method [1, 3, 13], and the random cutting method [2]. A square grid with intervals (distance between two neighboring points) of 0.5 μm was used for the calculations. By means of special computer programs, the following morphometric parameters were obtained from the primary data:

the bulk density of the mitochondria (V_{vm}) in cm^3/cm^3 and of the myofibrils (V_{vmy_0}) in cm^3/cm^3;

the surface density (relative surface area) of the outer membrane of the mitochondria (S_{vm_0}) in m^2/cm^3;

the relative surface density of the outer membrane of the mitochondria (the surface area of the outer membrane per unit volume of mitochondrion) $(S_{vm_0})/V_{vm})$ in $\mu m^2/\mu m^3$;

the number of sections through the mitochondria per unit area of myocytes (N_{0m}) in cm^{-2};

the numerical density (relative number of mitochondria) (N_{vm}) in cm^{-3};

the mean area of cross section of the mitochondria in a section (A_m) in μm^2;

Institute of Pathology, Humboldt University, Charite, Berlin. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 10, pp. 492-494, October, 1979. Original article submitted July 7, 1978.

 0007-4888/79/8810-1222 $07.50

the mean volume of a mitochondrion (V_m) in μm^3;

the mean diameter of a mitochondrion (D_m) in μm;

the mean length of a mitochondrion (L_m) in μm;

the mitochondrial-myofibrillary ratio (P_m/P_{my_0}).

The following indices also were calculated:

the number of sections of mitochondria in 100 μm^2 area of cytoplasm;

the relative proportion of micromitochondria (with a volume of below 0.02 μm^3), in%;

the proportion of degenerating mitochondria, in %;

the number of myofilaments per myofibril.

EXPERIMENTAL RESULTS

The results of the morphometric investigations are given in Table 1. They show that the bulk density of the myofibrils was considerably reduced by the 3rd day of culture, by which time only 34% of the original material still remained in culture. By the 4th day a considerable increase in this index was observed and it lasted until the 7th day. However, by that time the bulk density of the myofibrils had not yet regained its initial value (the bulk density of myofibrils of myocardiocytes from 4-day-old rats).

On the 1st day of culture, the number of myofilaments per sarcomere fell sharply, after which a gradual increase was observed until the 4th day. From the 4th through the 7th days a gradual decrease in their number again was observed. The bulk density of the mitochondria, like that of the myofibrils, fluctuated in a similar manner, and its values were always lower than initially.

The decrease in the bulk density of the mitochondria was mainly the result of a decrease in their size (mean area of cross section of the mitochondrion and its mean volume). During 7 days in culture the number of sections through the mitochondria per unit area of myocyte and their numerical density were higher than in the original material. The number of micromitochondria rose slightly from the 1st through the 4th day of culture, then fell and came close to the initial value.

It should be emphasized that the mitochondrial-myofibrillary ratio was higher than in the original material from the 1st through the 4th days of culture. This indicates a clear decrease in the relative volume of the myofibrils compared with the volume of the mitochondria. Throughout the period of culture and, in particular, on the 3rd and 5th days, an increase in the number of degenerating mitochondria was found. In this case the number of degenerating mitochondria was evidently inversely proportional to their total number.

The results of the morphometric investigation showed that heart muscle cells of 4-day-old rats can repair the structural changes arising as a result of enzymic degradation. This is evidence of their ability to

TABLE 1. Morphometric Indices of Myocardial Cells from 4-day-old Rats in Culture (M ± m)

Index	4-day old rats	Age of culture in days						
		1·	2	3	4	5	6-	7
V_{vm}	0,17±0,01	0,13±0,01	0,10±0,02	0,15±0,01	0,13±0,01	0,11±0,01	0,12±0,01	0,14±0,01
$V_{v\ my_0}$	0,56±0,02	0,30±0,03	0,20±0,02	0,20±0,02	0,46±0,03	0,46±0,02	0,43±0,03	0,41±0,02
S_{vmo}, m^2/m^3	2,20±0,16	1,83±0,20	1,44±0,17	1,61±0,16	1,94±0,18	1,36±0,11	1,74±0,15	2,02±0,16
S_{vmo}/V_{vm}, $\mu m^2/\mu m^3$	14,55±1,02	13,84±1,35	12,55±1,89	10,60±0,93	15,50±1,22	13,76±3,32	20,83±4,54	15,33±1,05
A_m, cm^{-2}	1,09±0,09	1,44±0,19	1,21±0,16	1,13±0,13	1,44±0,13	0,96±0,10	1,23±0,12	1,28±0,11
N_{vmo}, cm^{-3}	1,15±0,14	2,88±0,63	2,04±0,37	2,83±0,63	2,78±0,32	2,29±0,37	2,13±0,33	1,96±0,29
NAM	0,70±0,05	0,49±0,05	0,44±0,07	0,84±0,18	0,54±0,09	0,70±0,11	0,47±0,04	0,56±0,06
Volume, μm^3	0,25±0,04	0,12±0,04	0,10±0,02	0,33±0,25	0,12±0,03	0,23±0,09	0,13±0,02	0,17±0,03
length, μm	2,46±0,22	1,35±0,16	1,24±0,15	1,93±0,27	1,30±0,14	1,70±0,28	1,75±0,19	1,95±0,22
Diameter, μm	0,38±0,02	0,37±0,03	0,40±0,05	0,44±0,04	0,39±0,03	0,41±0,03	0,36±0,03	0,36±0,03
P_m/P_{my_0}	0,35±0,04	0,67±0,13	0,48±0,16	2,93±0,19	0,42±0,08	0,31±0,05	0,34±0,05	0,46±0,07
Number of mitochondria per 100 μm^2 cytoplasm	89,5	146,4	102,8	101,6	119,3	81,6	102,8	110,4
Number of mitochondria, %	10,0	14,9	46,7	42,9	50,5	68,8	19,9	13,7
No. of degenerating mitochondria	4,1	4,1	8,6	12,0	7,1	11,4	6,8	5,0
Number of myofilaments per myofibril	29,1±3,01	11,2±1,06	11,7±0,89	13,7±1,47	18,3±1,70	14,1±2,21	12,8±0,56	11,9±0,89

adapt themselves to the conditions of culture [10]. On the basis of the results of the morphometric investigation three periods of morphological changes in the myocardiocytes in vitro can be distinguished. In the first period, ending on the 2nd day in culture, destructive processes predominated in the mitochondria and myofibrils (a decrease in the bulk density, relative volume, number of myofilaments per sarcomere, and dimensions of the mitochondria).

In the second period (from the 3rd through the 5th day of culture) synthetic processes predominated, evidently in connection with adaptation of the myocardiocytes to conditions in vitro. In the third period the culture medium was inadequate to maintain the changes found in the structures during the second period. An increase in the number of cells in culture and a decrease in the dimensions of the mitochondria, their mean volume, the mean area of cross-section of the mitochondria in the tissue section, and the mitochondrial-myofibrillary ratio were observed.

The results of this morphometric study confirm the view that myofibrils can be synthesized in vitro in rat myocardiocytes [8] and they are in good agreement with the results of morphometry of myocardial muscle cells kept under hypoxic conditions [14]. Meanwhile the results do not agree with the view that disturbance of structural differentiation of myocardial cells takes place during culture [7].

LITERATURE CITED

1. G. G. Avtandilov, Morphometry in Pathology [in Russian], Moscow (1973).
2. S. A. Saltykov, Stereometric Metallography [in Russian], Moscow (1970).
3. G. Vasilev, H. Guski, E. F. Lushnikov, et al., Arkh. Patol., No. 9, 80 (1977).
4. W. Halle and K. D. Jentzsch, Biol. Rundschau, 10, 217 (1972).
5. W. Halle and K. D. Jentzsch, Pharmazie, 29, 433 (1974).
6. R. Meyer and K. Wenzelides, Dtsch. Gesundh.-Wes., 32, 1990 (1977).
7. I. Harary, A. Fugimoto, and H. Kuramitsu, Nat. Cancer Inst. Monogr., 13, 257 (1964).
8. A. Kössler, Acta Biol. Med. Germ., 29, 119 (1972).
9. G. L. Wallucat, H. Will, W. Schulze, et al., Acta Biol. Med. Germ., 33, 57 (1974).
10. H. Masson-Pévet, H. J. Jongsma, and J. de Bruijne, J. Mol. Cell. Cardiol., 8, 747 (1976).
11. R. Meyer, C. Schildhaus, J. Schmejkal, et al., Exp. Pathol. (Jena), 5, 53 (1971).
12. W. Halle and A. Wollenberger, Am. J. Cardiol., 25, 292 (1970).
13. E. R. Weibel and H. Elias (editors), Symposium on Quantitative Methods in Morphology, Berlin (1967).
14. H. Hermersdörfer, in: Gegenbaurs morphol. Jahrbuch (Leipzig), 122, 466 (1976).