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CONNECTION BETWEEN CARDIOMYOCYTE LYSOSOMES AND SOME CHARACTERISTICS OF FUNCTION, METABOLISM, AND ULTRASTRUCTURE OF THE INTACT RABBIT HEART

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KEY WORDS: heart; lysosomes; factor analysis.

Activation of the lysosomal apparatus of cells may reflect the degree of their injury or, on the other hand, may be associated with intensification of intracellular regeneration processes [4, 8, 12].

To study the role of lysosomes of cardiomyocytes in a multiparametric living object (intact cardiomyocytes, intact heart) we excluded some parameters which have been studied and replaced them by a smaller number of functions of them, in order to establish the most general relations between the various characteristics of function, metabolism, and ultrastructure of the test object. Since the investigation had to be carried out over a long period of time, seasonal influences on the object were excluded and an attempt was made to discover relations between parameters that are independent of season, for the writers previously found seasonal changes in certain physiological, biochemical, and morphological characteristics of the working intact heart [4, 6, 7]. Factor analysis, one of the methods of multidimensional statistical analysis, was used for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on 180 intact male chinchilla rabbits weighing 3.5 ± 0.5 kg. In the course of the year, at the middle of the month, during winter, spring, summer, and fall 28 physiological, biochemical, and morphological characteristics of cardiac activity were investigated (from 3 to 10 measurements during each season). The peak systolic pressure under real conditions (BP_p) and during occlusion of the ascending aorta or pulmonary artery for 5 sec, i.e., under conditions when the heart developed its maximal contractile power (BP_m), was measured electromanometrically in the left ventricle (LV) and right ventricle (RV) of the animals. The duration of the diastolic pause (T_d) was calculated from the BP_pLV curve. Activity of lipoprotein lipase (LPL), triacylglycerolipase (TGL), and monoacylglycerolipase (MGL) in pre- and postheparin blood plasma, myocardium, and adipose tissue was determined by the writers' own method [1]: The concentration of free fatty acids (FFA) in the blood was determined [3]. Heart sections were studied histochemically by Burstone's reaction for acid phosphatase, followed by counting the number of formazan granules in the section. For electron-microscopic investigation the heart was perfused through the aorta (after preliminary washing with physiological saline) with a 2.5% solution of glutaraldehyde, followed by postfixation of areas of papillary muscles in a 1% solution of OsO_4 at pH 7.2-7.4 . The material was then embedded in Araldite and sections were cut on a Reichert-Jung Ultracut ultramicrotome and stained with lead hydroxide and uranyl acetate, after which they were examined in the Tesla BS 540 electron microscope under a magnification of 22,000×. For quantitative analysis of the electron micrographs (EM) modified methods suggested by Paukov and Frolov [2, 4] were used. The mean number of mitochondria in one standard EM (M), the mean area of one mitochondrion (AM), the mean total area of the mitochondria in one EM (AM_{em}), the mean number of cristae per mitochondrion (CM), the mean total number of cristae per EM (CM_{em}), the coeffi-

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cient of energy efficiency of a single mitochondrion (CEE_{mc}) and of all mitochondria in an EM as a whole ($CEEM_{em}$), the coefficient of intensity of division of mitochondria (CIDM), and the number of lysosomes per EM (L) were calculated. Parameters of contractile activity of the heart and of quantitative analysis of EM were calculated separately for the left and right ventricles.

EXPERIMENTAL RESULTS

Among the 28 parameters studied some were found which correlated strongly with one another and, consequently, from the point of view of factor analysis, they differed from one another only a little as regards information about the phenomenon chosen for study. A symmetrical correlation matrix was constructed, the elements of which were coefficients of correlations for all possible pairs of the 28 characteristics studied (random values). The characteristics were numbered as follows:

1) BP_pLV; 2) BP_pRV; 3) BP_mLV; 4) BP_mRV; 5) T_d; 6) FFA; 7) LPL; 8) TGL; 9) MGL; 10) M LV; 11) M RV; 12) AM LV; 13) AM RV; 14) CM LV; 15) CM RV; 16) AM_{em}LV; 17) AM_{em}RV; 18) CM_{em}LV; 19) CM_{em}RV; 20) $CEEM_{mc}$LV; 21) $CEEM_{mc}$RV; 22) $CEEM_{em}$LV; 23) $CEEM_{em}$RV; 24) CIDM LV; 25) CIDM RV; 26) L LV; 27) L RV; 28) CF.

Groups with strong internal correlation, i.e., of those populations of random values in which correlation between all possible pairs of variables belonging to it was equal to or exceeded a certain arbitrarily assigned level, were isolated from the general correlation matrix by the method of branching connections [10] and the method of correlation profiles [13]. In the present study two random values were regarded as connected if the corresponding value of the coefficient of correlation was higher than or equal to 0.65. The first step in the procedure of isolation of groups by the branching connections method was organization of the characteristics, for which purpose the order of random values in the matrix given above was chosen. Next, from the order thus established, each random value was chosen in order from the 28th random values, together with all values with which the given characteristic was linked with a significant degree of correlation. Starting wtih the first characteristic, the sequence was constructed as follows. The first random value was chosen, after which all the features were examined and those which showed strong correlation with the first random value were selected. Each new random value chosen must correlate significantly with all random values of this sequence. The procedure was continued until all characteristics were exhausted. The procedure as described above was repeated for all 28 random values. As a result, characteristics of cardiac activity chosen in this manner formed groups. Analysis of the chosen groups showed that random values 1, 2, 3, 4, 7, 8, 24, 27, and also 6, 10, 12, 27 form closed groups which are factors, since all the values forming them correlate strongly with one another. The correctness of formation of these factors was confirmed by a study of relations between the 28 measured parameters by the correlation profiles method [13]. The advantage of this method is abolition of overlapping of groups or at least, reduction of this overlapping. The procedure of the method was as follows. Values of modules of correlation coefficients for a given random value with all the remaining 27 characteristics were plotted along the ordinate and the serial number of the random values in the same order as in the matrix on the abscissa. The set of points joined together on the graphs forms a correlation profile. During the investigation correlation profiles of all 28 random values and their agreement with each other were studied. The same two groups (Figs. 1 and 2) were isolated by the correlation profiles method and by the branching connections method.

Among characteristics of cardiac activity which were studied, a series of parameters can thus be distinguished which form two closed groups, and which can therefore be considered as factors and regarded as one of the general principles governing the phenomenon under observation [9]. Hence, from the set of characteristics pertaining to the object chosen for study, the two most important factors were distinguished by methods of factor analysis, and each of them includes the random value of the lysosome.

When these principles are interpreted, the following connection of cause and effect can be postulated. The contractile function of the intact heart largely depends on the intensity of lipolysis in the body. Meanwhile the intensity of lipolysis is connected to a large degree with activity of lysosomes. As the investigation shows, lysosomes of the right ventricle affect functional parameters of activity and lipid metabolism of the heart. However, internal correlation between the number of lysosomes in the left ventricle, the blood FFA level, and the number of mitochondria in the left ventricle indicates a very important role for ly-

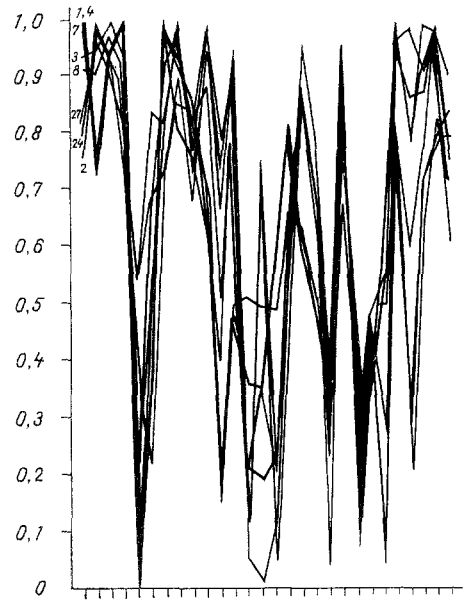

Fig. 1

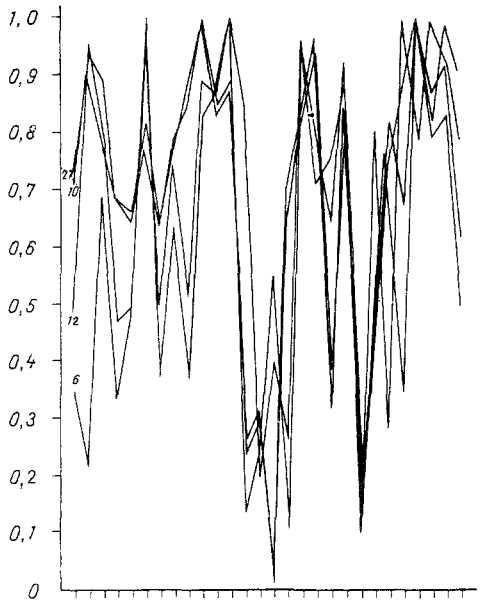

Fig. 2

Fig. 1. Correlation analysis of first factor. 1) BP_pLV; 2) BP_pRV; 3) BP_mLV; 4) BP_mRV; 7) LPL; 8) TGL; 24) CIDM LV; 27) L RV.

Fig. 2. Correlation profile of second factor. 6) Blood FFA level; 10) M LV; 12) AM LV; 27) L RV.

sosomes in the regulation of mechanisms maintaining the working capacity of a heart muscle. At the same time, fatty acids are known to affect the rate of release of lysosomal enzymes in the myocardium [11]. On the basis of connections established between parameters of cardiac activity the following chain of events can be submitted. A change in the number of lysosomes in the right ventricle affects activity of lipolytic processes in the heart as a whole, and through release of FFA and their utilization by the myocardium, this enables the energy metabolism of the heart to be maintained at the necessary level. The FFA concentration, in turn, affects the outflow of lysosomal enzymes in the myocardium, thereby regulating the state of the mitochondrial apparatus on the cardiomyocytes and changing the contractile function of the heart.

It can accordingly be postulated that the liposomal apparatus of the cardiomyocytes is a regulator of important biochemical reactions determining the functional state of the intact heart cell.

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IMPORTANCE OF FORCED EXPIRATORY CURVE DEVIATION FROM THE EXPONENTIAL TYPE

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KEY WORDS: forced expiration curves; obstruction of the respiratory passages; restriction of the lungs; small respiratory passages; large respiratory passages; time constant.

The forced expiration (FE) curve for the healthy subject is close to the exponential type. It is suggested in this paper that deviation of the FE curve from exponential be used to determine the time course of changes in patency of the respiratory passages during FE, to determine whether an obstruction is in the small or the large respiratory passages, and to establish the site of action of broncholytic agents.

Preliminary communications on the suggested method were published previously in thesis form [4, 5].

EXPERIMENTAL METHOD

The original source of information on the state of the human bronchopulmonary system is the ordinary FE spirogram. For healthy subjects, as was shown previously [3, 4], the FE curve represents volume as an exponential function of time. The volume V_t which the subject can still breathe out at time t depends on time in the following manner: $V_t = V_0 e^{-t/\tau}$, where V_0 is the initial volume, namely the forced vital capacity (FVC), e is the base of natural logarithms, and τ the time constant, characterizing the velocity of the exponential process. It can be considered that τ is the product of the resistance of the respiratory passages and the compliance of the lungs, at least for healthy persons [3-6]. However, during FE the conditions under which this is applicable are disturbed, i.e., the flow in many parts of the tracheobronchial tree becomes turbulent, and FE itself cannot be considered to be free emptying of the lungs. That is why τ is not the product of compliance of the lungs and resistance of the respiratory passages [7], although for healthy persons it is perhaps close to it in magnitude and essence [6]. Nevertheless, because FE is exponential in character, τ can be considered to characterize the ability of the lungs to empty quickly. The higher its value, the greater the obstructive changes in the respiratory passages.

In the present investigation informative changes in the FE curve were sought in its deviation from an exponential curve. In the course of FE the time course of the parameter τ was monitored, by splitting up the FE curve into separate segments (Fig. 1), where it was approximated by the corresponding exponential functon, similar to that given above. For the i-th segment, for instance, this relationship assumed the following form:

$$V_{t_i} = V_{t_{i-1}} e^{-\Delta t_i/\tau_i},\ \Delta t_i = t_i - t_{i-1},\ i = 1, \ldots, m.$$

Hence: $\tau_i = \Delta t_i / (\ln Vt_{i-1} - \ln Vt_i)$.

Here V_t is the volume to be expired at time t_i, Δt_i is the time interval defining the i-th segment of the subdivided curve, τ_i the time constant characterizing the velocity of the exponential process in the i-th segment, and m the number of segments of the subdivided curve. Dependence of τ on volume in the course of FE was determined and plotted graphically by computer, using the equations given above. The corresponding program was effected at the Information-Computer Center of the Fourth Main Board, Ministry of Health of the RSFSR, and also at

Moscow Tuberculosis Research Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 10, pp. 22-24, October, 1983. Original article submitted February 18, 1983.

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