# CALCIUM-BINDING ACTIVITY OF CARDIAC MITOCHONDRIA IN AGING RATS AND ITS POSSIBLE ROLE IN MYOFIBRILLARY RELAXATION

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During aging definite disturbances are observed in cardiac activity [4, 7]. The duration of the myocardial contractile cycle increases in old age, due to slowing of myofibrillary relaxation [7, 9, 13], which is attributed to a decrease in the rate of $Ca^{++}$ uptake by the sarcoplasmic reticulum [7]. Some workers have suggested that besides the sarcoplasmic reticulum, a role in regulation of the myocardial contractile cycle may also be played by mitochondria [3, 6, 10], which have high $Ca^{++}$-accumulating capacity and binding rate. Investigation of the $Ca^{++}$-binding activity of the cardiac mitochondria from the age aspect has not hitherto been undertaken.

This paper describes a study of the rate of $Ca^{++}$ binding by mitochondria of the rat heart during aging.

## EXPERIMENTAL METHOD

Male Wistar rats aged 1, 3, 12, and 24 months were used. Mitochondria were isolated from the heart by the method described previously [2], except that the rinsing and suspending media did not contain albumin and EDTA. $Ca^{++}$ transport into the mitochondria was recorded at 30°C fluorometrically, with the aid of chlortetracycline [12]. The composition of the medium was: 310 mM sucrose, 20 mM Tris-HCl (pH 7.3), 1 μM rotenone, 10 μM chlortetracycline, 100 μM $CaCl_2$, 4 mM succinate-Tris, 1 mg mitochondrial protein. Protein was determined by Lowry's method with some modifications [11].

## EXPERIMENTAL RESULTS

The curve reflecting binding of $Ca^{++}$ by rat heart mitochondria is illustrated in Fig. 1. In response to addition of succinate to the medium the intensity of fluorescence of the probe was considerably increased, and this could be prevented by 50 μM ruthenium red. Addition of 1 μM trifluoromethoxy-carbonyl-cyanide-phenylhydrazone caused liberation of $Ca^{++}$ bound with the mitochondria. These findings are evidence that binding of $Ca^{++}$ cations by mitochondria is energy-dependent.

Values of $Ca^{++}$-binding activity of cardiac mitochondria from rats of different ages, expressed in relative units of change of intensity of fluorescence in unit time, are given in Table 1. They show that the $Ca^{++}$-binding activity of the mitochondria did not change significantly with age.

We know [5] that the content of mitochondrial protein in 1 g wet weight of myocardium in rats aged 24 months is 22.4% less than in rats aged 14 months. Together with the results of the present investigation, these facts may also help to explain the decrease in the rate of relaxation of the myocardium during aging [7], if it is accepted that, besides the reticulum, mitochondria also participate in binding the $Ca^{++}$ released into the sarcoplasm to initiate contraction [3, 6]. In models suggested previously, $Ca^{++}$ bound with mitochondria ought to be liberated into the sarcoplasm by the mitochondria in response to a signal from the T system. However, a rather different version of mitochondrial participation in regulation of contraction-relaxation of the myocardium may also be examined. The modified version which we now suggest stems from a comparison of the properties of the cardiac mito-

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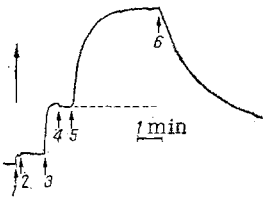


Fig. 1. Changes in intensity of chlortetracycline fluorescence during calcium binding by mitochondria. Additions of components of medium: 1) mitochondria, 2) rotenone, 3) chlortetracycline, 4) $CaCl_2$, 5) succinate, 6) addition of trifluoromethoxycarbonyl-cyanide-phenylhydrazone uncoupler. Broken line: 50 μM ruthenium red present in medium.

TABLE 1. $Ca^{++}$-binding Activity of Rat Heart Mitochondria, M ± m

| Age, months | Number of animals | $Ca^{++}$-binding activity, relative units |
|---|---|---|
| 1 | 5 | 71,4±4,1 |
| 3 | 7 | 70,8±3,9 |
| 12 | 4 | 67,6±5,5 |
| 24 | 5 | 67,8±2,3 |

chondria and sarcoplasmic reticulum, and is as follows. The $Ca^{++}$ concentration in the sarcoplasm on initiation of contraction is commensurate with the value of $K_m$ of the mitochondria for $Ca^{++}$ binding [3, 6, 8]. Consequently, $Ca^{++}$ ions ought to be transported into the mitochondria simultaneously with transport into the reticulum. The fraction of $Ca^{++}$ transported into the mitochondria may be considerable, for the content of mitochondrial protein in the myocardium is more than one order of magnitude higher than the content in the reticulum [6, 8]. With a decrease in free $Ca^{++}$ concentration in the sarcoplasm the contribution of transport into the reticulum will be increased because of the higher affinity of the latter for $Ca^{++}$. At a certain moment the value of the electrochemical $Ca^{++}$ gradient on the inner mitochondrial membrane is in equilibrium with the degree of energization of the membrane, and binding of $Ca^{++}$ by the mitochondria ceases. This moment may correspond to considerable relaxation of the myofibrils. A further decrease in the $Ca^{++}$ concentration in the sarcoplasm on account of its continued transport into the reticulum will give rise to more profound relaxation of myofibrils and to an increase in the elctrochemical $Ca^{++}$ ion gradient on the mitochondrial membrane, which now corresponds even less to the degree of energization of the inner membrane. The result is that $Ca^{++}$ starts to leave the mitochondria, and, notwithstanding its low concentration in the sarcoplasm, it is transferred into the reticulum, apparently unnoticed by the myofibrils. The diffusion potential of $Ca^{++}$ ions thus created on the inner mitochondrial membrane may participate in ATP synthesis [1]. At the stage of apparent rest, $Ca^{++}$ may therefore be transferred into the reticulum, so that the whole pool of ions which participates in the initiation of contraction could be released from the cisterns of the reticulum in response to a signal from the T system. Under these circumstances it is unnecessary to postulate the existence of a direct structural connection between the T system and the mitochondria [3] or another method of transmission of the signal from the T system to the mitochondria.

The important point is that, according to this model, participation of mitochondria in the regulation of the contractile cycle makes it possible to advance the time of the beginning of relaxation of the myofibrils and to regulate myocardial contractions by hormones and other physiologically active substances through their influence on the state of mitochondrial function. The results obtained in the present investigation, showing the Ca-binding activity of the mitochondria remains practically unchanged during aging in rats, together with the observed decrease in the content of mitochondria in the heart of old animals [5], within the context of the suggested model may provide a possible explanation of the decrease in the rate of relaxation of the myocardium during aging.

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# NONRECEPTOR INTERACTION OF LOW DENSITY LIPOPROTEINS WITH HUMAN FIBROBLASTS

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According to views on receptor-mediated endocytosis, low-density lipoproteins (LDL), on penetrating into the cell, are transported in "coated" vesicles into lysosomes, where degradation of the protein and hydrolysis of the cholesterol esters of the LDL take place [3]. If the LDL concentrations in the extracellular medium exceed the limits of receptor interaction, they may be taken up by the cell even without receptor participation. This method of LDL catabolism, independent of controlled receptors, may assume great importance in the development of hypercholesterolemia and of atherosclerotic changes in the arterial wall.

The object of the present investigation was to study catabolism of [$^{125}$I]-protein- and [$^{3}$H]-lipid-labeled LDL by human fibroblasts under conditions when the participation of specific receptors for LDL was suppressed.

## EXPERIMENTAL METHOD

Experiments were carried out on cultures of human embryonic lung fibroblasts at the 10th passage. The cells were cultured in Pavitskii flasks under conditions of restricted receptor-mediated LDL uptake by the cells. For this purpose fibroblasts were used in the experiment after monolayer formation in 10% bovine serum made up in Eagle's medium (without preincubation of the cells in medium not containing lipoproteins), and high concentrations of LDL (50-100 μg/ml as protein), suppressing receptor synthesis, were used. The duration of the experiments (24 h) also was much longer than the time required to inhibit synthesis of LDL receptors (with LDL present in the medium). To study the rate of intracellular LDL degradation under conditions of nonreceptor interaction, the "pulse labeling" method was used. In these experiments fibroblasts were incubated in medium containing [$^{125}$I]-LDL in a concentration of 100 μg/ml as protein for 24 h, after which the medium was poured off and the cell monolayer was washed with the solution five times [2], after which the cells were flooded with medium containing unlabeled LDL in the same concentration. In parallel experiments the cells were flooded with medium containing $^{125}$I-labeled LDL (100 μg/ml), and 24 h later extra-

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