ATPase ACTIVITY OF CARDIAC MICROSOMES, REGULATION OF CALCIUM TRANSPORT IN THEM, AND CALMODULIN LEVELS IN EXPERIMENTAL MYOCARDIAL INFARCTION

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It has recently been shown that the Ca transporting capacity of the sarcoplasmic reticulum (SR) of the heart is controlled by cyclic AMP-dependent and calmodulin- (CaM-) dependent phosphorylation [8]. The target protein for the corresponding protein kinases in this case is phospholamban, a low-molecular-weight component of SR membranes. One result of the firm bond between Ca, Mg-ATPase and phospholamban in SR membranes is a change in the velocity of active Ca^{++} transport through the membrane of SR vesicles coupled with phosphorylation [5], and in turn, this determines the normality of diastole and, consequently, of systole.

Previously [2] the writers showed that the process of cAMP-dependent phosphorylation of phospholamban is disturbed in experimental myocardial infarction, and that the vulnerability of this protein to proteinases due to this disturbance is greater in the affected myocardium than in the control.

The aim of this investigation was to continue the study of the integral system: phosphorylation of phospholamban — Ca-activated ATPase of SR — CaM — Ca^{++} transport in SR in experimental infarction.

EXPERIMENTAL METHOD

Experiments were carried out on mongrel dogs weighing 15-18 kg. Operations were performed on the animals under intravenous thiopental anesthesia with controlled respiration [1]. Myocardial infarction was induced by ligation of the descending branch of the left coronary artery. The animals were killed 24 h after ligation of the coronary artery by respiratory arrest due to injection of muscle relaxants. Necrotic tissue of the left ventricle was investigated, and the same regions of myocardium from healthy animals served as the control.

The microsomal fraction was isolated by the method in [8] and cAMP-dependent protein kinase was obtained as described previously [3]. CaM protein was isolated from bovine brain by chromatogrphy on DEAE-cellulose and Sephacryl S-200 [9]. The CaM level in the myocardium was determined as the degree of activation of phosphodiesterase with the aid of cAMP, using a calibration curve, and it was expressed in conventional units (CU): 1 CU corresponds to the quantity of CaM causing 50% activation of phosphodiesterase. cAMP- and CaM-stimulated Ca^{++} transport in the microsomes were induced by the writers' modification of the method in [10]. After treatment of 30 μg protein for 10 min in incubation medium in the presence or absence of 10^{-6} M cAMP, 0.2 mg/ml protein kinase, and 10 μg/ml CaM, the reaction was started by addition of 10 μM Ca^{++} containing 0.05 μCi of ^{45}Ca. Aliquots were filtered through Millipore (HA, 0.45 μM Ca^{++}) filters and the quantity of Ca^{++} accumulated in the microsomes was determined as the difference between the radioactivity of the original reaction mixture and the radioactivity of the filter. Ca-activated ATPase activity in the microsomes was estimated as the quantity of ^{33}P formed in the medium [7].

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TABLE 1. Ca-Activated ATPase Activity in Cardiac Microsomes in Experimental Infarction

Regions of myocardium	Number of animals	Ca-ATPase activity, μmoles ^{33}P/mg protein/min
Control	6	0,19±0,03
Ischemic	5	0,11±0,02*

Legend. *P < 0.05.

TABLE 2. CaM Levels (in CU) in Myocardium, in Cytosol and Microsomes, in Experimental Infarction (M ± m)

Regions of myocardium	Cytosol (A)	Microsomes (B)	A/B
Control	4151±125	2883±130	1,44
Ischemic	5482±286	1355±205	4,06

Legend. Data for 6-7 animals shown.

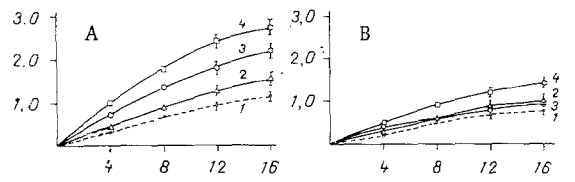

Fig. 1. Oxalate-dependent accumulation of ^{45}Ca by cardiac microsomes in experimental infarction. Abscissa, time (in min); ordinate, accumulation of ^{45}Ca (in μmoles/mg protein). A) Normal animals, B) myocardial infarction. 1) Basal level of accumulation, 2) level of accumulation in presence of CaM, 3) level of accumulation in presence of CaM and cAMP, 4) level of accumulation in presence of CaM, cAMP, and protein kinase.

EXPERIMENTAL RESULTS

Studies of oxalate-dependent Ca^{++}-transporting capacity of SR membranes showed that the basal (unstimulated) level of ^{45}Ca accumulating in preparations of intact microsomes after incubation for 16 min at 25°C averaged 1.10 ± 0.06 μmole/mg protein. CaM (10 μg/ml) added to the incubation medium increased the quantity of accumulated ^{45}Ca to 1.50 ± 0.17 μmole/mg protein. Addition of 10^{-6} M cAMP led to an increase of about 48% in the rate of CaM-stimulated accumulation of ^{45}Ca. Addition of exogenous protein kinase as well as CaM and cAMP to the incubation medium caused an increase of Ca^{++} accumulation in this case of 75% compared with medium not containing exogenous protein kinase and cAMP (Fig. 1A).

A different picture was observed in myocardial infarction (Fig. 1B). For instance, the basal level of accumulation after incubation for 16 min was 0.80 ± 0.09 μmoles ^{45}Ca/mg protein, and addition of 10 μg/ml CaM to the incubation medium had no stimulating effect on calcium transport. In experimental infarction, addition of 10^{-6} M cAMP to the incubation medium caused no increase in ^{45}Ca accumulation compared with the corresponding CaM-stimulated level. Addition of 0.2 mg/ml of protein kinase caused an increase in CaM-stimulated accumulation of ^{45}Ca by only 35%.

Comparison of levels of Ca^{++} accumulation in microsomes from intact and ischemic regions of myocardium revealed a fall in the basal level of accumulation of this ion in experimental infarction. A marked decrease in Ca-activated ATPase activity of SR was observed under these circumstances (Table 1) in the ischemic muscle compared with the intact myocardium.

Ca-ATPase activity was calculated as the difference between activities of Ca,Mg-ATPase and Mg-ATPase (the latter was determined in the presence of EGTA). It can be concluded from these results that correlation exists between the work of the Ca-pump and unstimulated Ca^{++} transport in SR (changes follow the same trend) in myocardial infarction.

In healthy muscle CaM-dependent, like cAMP-dependent, enhancement of the Ca-transporting capacity of SR in healthy muscle is evidently a function of phosphorylation of membrane-bound target proteins by CaM- and cAMP-dependent protein kinases respectively. One such target protein for both protein kinases is phospholamban (phosphorylation takes place in this case at different residues of the serine substrate), whereas for the cAMP-activated enzyme, CaM-dependent protein kinase is evidently another [5]. In the latter case the mechanism of activation may be of the cascade type, as, for example, during activation of the kinase of phospholipase b or the kinase of myosin light chains (CaM-sensitive enzymes) with the aid of cAMP-dependent phosphorylation.

Reduction of the effect of cAMP-dependent exogenous (in the presence of 0.2 mg/ml of protein kinase) activation of Ca^{++} transport observed in infarction compared with the control may be due, in particular, to changed ability of molecules of the target proteins (phospholamban and, perhaps, CaM-activated protein kinase, bound with the SR membrane, to undergo phosphate modification. In this case, for instance, exogenous phosphorylation of phosphorylation of phospholamban amounted to 71% of the control [2]. The absence of stimulation of Ca^{++} transport on addition of 10^{-6} M cAMP correlates with the fall (26% of the control) in the level of endogenous (in the presence of 10^{-6} M cAMP) phosporylation of phospholamban [2] and it may be due to disturbance of the ability of the holoenzyme of protein kinase in infarction to undergo dissocation under the influence of endogenously produced cAMP, as the writers demonstrated previously [1] in infarction. Besides cAMP, the content of which fell appreciably in the zone of infarction [1], determination of the level of the other coenzyme in protein kinase reactions (CaM) in the affected myocardium also is interesting. The results of measurement of the CaM levels in myocardial infarction in fractions of cytosol (100,000 g) and microsomes are given in Table 2.

It will be clear from Table 2 that in myocardial infarction the total content of CaM (A + B) in the two fractions was in fact virtually unchanged compared with the control, possibly on account of the very conservative properties of this protein — its thermostability, its high resistance to proteolysis [4, 6], and its marked increase in the zone of myocardial infarction studied [2]. However, the CaM content increased under these circumstances in the cytosol fraction (by 32%) and decreased in the microsomal fraction (by 53%) compared with the corresponding control. Changes in compartmentalization of CaM during ischemia may be due to labilization of the cell membranes and associated weakening of affinity of CaM for the membranes. In turn, the increase in CaM content in the cytosol of an affected cardiomyocyte may lead to additional stimulation of phosphodiesterase with the aid of cAMP, and thus to a fall in the level of this nucleotide.

To conclude, it will be noted that the disturbance of cAMP- and CaM-dependent regulation of Ca^{++} transport in SR discovered in myocardial infarction may be one of the decisive factors in the development of irreversible damage to cardiomyocytes. For instance, the excess accumulation of Ca^{++} ions in the cytosol of the affected cell (which is a critical factor in the development of profound changes in the heart cell) arising while ischemia is still in the reversible stage, may be due to a disturbance of regulation of Ca^{++} transport in SR effected by different protein kinases.

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