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CREATINE PHOSPHOKINASE ACTIVITY IN MEMBRANES OF THE MYOCARDIAL SARCOPLASMIC RETICULUM IN EXPERIMENTAL CORONARY INSUFFICIENCY

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UDC 616.132.2-008.64-092.9-07: 616.127-008.931

KEY WORDS: myocardium; metabolism; creatine phosphokinase; membranes; ischemia.

Recently developed views on the creatine kinase mechanism of energy transport in myocardial cells [6, 11, 14] have compelled investigators not only to continue the search for new ways of solving complex and largely unexplained problems of energy supply for the contractile function of the heart, but also to reassess results already obtained. According to a current hypothesis [6, 8, 10], the creatine kinase reaction is reversible within the limits of one cardiomyocyte. For instance, in the intermembranous space of the mitochondria the direct reaction of transfer of phosphate from ATP to creatine is effected by means of the mitochondrial isozyme creatine phosphokinase (CPK), whereas in myofibrils the reverse reaction of phosphorylation of ADP with the formation of ATP takes place with the participation of the MM isozyme. CPK isozymes have now been found in all subcellular structures producing or utilizing energy: in the mitochondrial membrane, myofibrils, membrane of the sarcoplasmic reticulum (SPR), plasma cell membrane, etc. [3, 14].

According to one view [15], CPK isozymes, which are in close structural connection with cell membranes and provide the cellular apparatus with energy, are in close functional association with other enzymes responsible for the transfer of materials and ions through the membrane. For instance, CPK in mitochondria functions in close association with ATP-ADP translocase, and in myofibrils, the SPR membrane, and the sarcolemma, it is associated with Mg-dependent, Ca-dependent, and Na,K-dependent ATPase.

The study of the role of membrane formations in energy transport and utilization by the myocardial cells assumes particular importance in myocardial ischemia, when significant changes are observed in the structure and properties of the protein–lipid layer of the membranes [7, 12], and, in particular, in their phospholipid composition. This is a particularly important matter in connection with information in the literature that certain CPK isozymes are most probably bound with the membrane by means of phospholipids [13].

Changes in activity of the mitochondrial CPK isozyme during measured limitation of the coronary blood flow were found previously by the present writers [1]. Meanwhile the study of yet another unsolved problem is extremely important – that is energy supply for Ca^{++} transport through the SPR membrane of myocardial cells and the link between this process and certain other parameters determining the functional state of the membrane apparatus of the SPR.

Laboratory of Biochemistry, N. D. Strazhesko Ukrainian Research Institute of Cardiology, Kiev. Laboratory of Membrane-Active Compounds of β-Diketones, Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR, Riga. (Presented by Academician of the Academy of Medical Sciences of the USSR D. F. Chebotarev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 5, pp. 53-55, May, 1983. Original article submitted July 15, 1982.

 0007-4888/83/9505-0628$07.50

TABLE 1. SPK Activity, Concentrations of Phospholipids and Diene Conjugates (DC), and Accumulation of Malonic Dialdehyde (MDA) in SPR Membranes of Rabbit Myocardium in Experimental Ischemia (M ± m)

Experimental conditions	CPK, μmoles creatine/mg protein/h	Phospholipids, μmoles/P/mg protein	MDA, μmoles/mg protein	DC, μmoles/mg protein
Control	11,90±0,36	0,37±0,01	9,8±1,9	53,4±13,0
Vasopressin	8,22±0,10*	0,22±0,01*	17,4±1,6*	92,0±7,6*
Vasopressin after antioxidant	10,28±0,70†	0,42±0,01†	8,4±2,1†	78,0±6,5

Legend. *P < 0.01 compared with control, †P < 0.05 compared with experiments with vasopressin.

The importance of a solution to these problems under conditions of myocardial ischemia is determined by the extreme urgency of this problem in modern cardiology, and the investigation described below was undertaken for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on rabbits with acute limitation of the coronary blood flow (intravenous injection of vasopressin, 0.5 unit/kg), and after preliminary injection (1 h before vasopressin) of the antioxidant preparation AV-156Na into the animals. CPK activity [2], some parameters of lipid peroxidation (LPO) [4, 5], and the phospholipid concentration [7] were determined in SPR membranes isolated by the method in [9].

EXPERIMENTAL RESULTS

The results (Table 1) show a marked effect of experimental ischemia on all stages of myocardial metabolism studied. For instance, activity of the CPK isozyme bound with the SPR membrane was reduced by more than 25% in vasopressin-induced coronary insufficiency.

Under the experimental conditions used, definite activation of LPO processes was detected in the SPR membranes. This was shown by an increase in the content of principal LPO products in the various structures: an increase in the MDA concentration by 77% and an almost twofold increase in the DC concentration. This investigation also showed that changes found in CPK activity and LPO processes are closely linked with structural changes in the SPR membranes and, in particular, their lipid composition. For instance, under conditions of coronary insufficiency some decrease was observed in the concentration of membrane-bound cholesterol, as well as a sharp fall in the levels of phospholipids (by 40%), mainly on account of phosphatidylcholine, possibly as a result of their hydrolysis.

Similar determinations also were carried out on experimental animals treated with the antioxidant AV-156Na. As Table 1 shows, all the parameters studied in these experiments showed a consistent tendency toward normalization. This clear effect of the antioxidant can evidently be regarded as evidence that the level of peroxidation reactions in the myocardium largely determines the character of changes in individual metabolic processes on the membranes.

The question of the membranes and reactions which can be regarded as primary in the whole complex of metabolic changes in the myocardium during ischemia has not yet been settled. At the same time, there is no doubt about the exceptional importance of an adequate supply of energy for the contractile function of the heart, especially under conditions of oxygen deficiency. The decrease in CPK activity of the SPR membranes found in the present investigation under conditions of experimental ischemia may indicate a definite disturbance of the normal energy supply for transport Ca-ATPase of the SPR membranes. Under these circumstances the change in CPK activity took place against the background of a much lower phospholipid level in the SPR membranes of the heart of the experimental rabbits. The CPK isozyme bound with the SPR membrane may perhaps be a lipid-dependent enzyme, and preservation of the normal lipid structure of the membrane may be an important or essential condition for the manifestation of its complete activity.

The considerable activation of LPO processes under conditions of coronary insufficiency, observed in the present investigation, must also be taken into account. This may lead to

changes in the structural stability of the membranes, an increase in their fluid properties, and an increase in ionic permeability. Moreover, considering that a compound with marked antioxidant properties, if administered before production of ischemia, substantially protected the SPR membranes against the development of all the disturbances described above, including lowering of CPK activity, it can evidently be considered that a change in the lipid composition of the membranes and activation of LPO processes in them are among the possible causes of appearance and development of the combination of metabolic and functional disturbances observed in the ischemic myocardium.

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