CHANGES IN ADENYLATE KINASE ISOZYME ACTIVITY IN THE ISCHEMIC RABBIT HEART

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Three isozymes of adenylate kinase (AK), differing in their kinetic, electrophoretic, and immunological properties, are known to exist in the myocardial cell [8]. These are the cytoplasmic isozyme AK_1, located in the cytoplasm [9], mitochondrial isozyme AK_2, located in the intermembranous space of the mitochondria [10], and the isozyme AK_3, located in the mitochondrial matrix [13]. The role of AK is connected with its involvement in the regulation of the energy charge of the adenylates, the phosphate potential, activation of fatty acids, synthesis of macromolecules, cell growth, muscular contraction, and creatine phosphate synthesis [3]. There is also evidence that isozymes AK_1 and AK_2 are involved in the transmission of energy stored in phosphate bonds of ATP from the mitochondria to the sites of its utilization [3].

The effect of ischemia on activity of AK isozymes has so far received only little study [1, 5]. After ischemia for 30 min, AK_2 activity in isolated mitochondria of the rabbit heart is depressed by 20% [5]. It is not clear whether this takes place through a decrease in activity of the isozyme itself or due to its release from the mitochondria into the external medium. It is likewise unknown how ischemia can affect activity of the other AK isozymes in the myocardium itself. The investigation described below was carried out to study these problems.

EXPERIMENTAL METHOD

A small piece of rabbit heart (about 0.2 g) was divided into two parts: one part (control) was immediately immersed in ice-cold 0.9% KCl solution, the other (experimental) was immersed in the same solution at 37°C, and thoroughly washed to remove blood. Myocardial ischemia was produced by autolysis [6] for 1 h. To prepare the extract each piece of tissue, after preliminary mincing, was homogenized with a glass homogenizer at the rate of 9 ml of medium (180 mM KCl, 10 mM Tris-HCl, 0.5 mM EDTA; 0°C, pH 7.7) and 1 ml of 10% Triton X-100 to 1 g tissue. The resulting homogenate was centrifuged for 3 min on an "Eppendorf-5414" bench-top ultracentrifuge at maximal speed. The supernatant, as tissue extract, was used for the measurements. Protein was determined by the biuret method [2].

Total AK activity was measured spectrophotometrically as the rate of ADP formation [3]. AK_3 activity was measured under the same conditions but in the presence of 100 μM diadenosinepentaphosphate(Ap_5A) since Ap_5A inhibits activity of isozymes AK_2 and AK_3 was measured in the same way after preliminary incubation of the tissue extract for 15 min with 2.0 mM dithio-(*bis*)-nitrobenzoic acid (DTNB) (final concentration in the cuvette 5 μM). DTNB inhibits only the cytoplasmic isozyme AK_1 [8]. AK_1 activity was calculated as the difference between total activity and activity of AK_2 + AK_3, and AK_2 activity was determined as the difference between AK_2 + AK_3 activity, and AK_3 activity.

EXPERIMENTAL RESULTS

The investigations showed that activity of AK isozymes in tissue extract from the control myocardium was distributed as follows: AK_1 46%, AK_2 29%, and AK_3 25%. After ischemia for 1 h the values were as follows: AK_1 46%, AK_2 43%, and AK_3 7%. Values of activity of the individual AK isozymes are given in Table 1. The ratio of activities of AK_1 and AK_2 isozymes was 1.57:1. This is in agreement with data obtained by other workers [12], for bovine heart muscle, for which the ratio is 3:3.

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TABLE 1. Distribution of Activity of AK Isozymes in Rabbit Myocardium under Normal Conditions and after Ischemia for 1 h ($M \pm m$, $n = 7$)

Exptl. conditions	Enzyme activity, nmoles ADP/min/mg protein			
	total	AK_1	AK_2	AK_3
Control	214±18	94±18	60±3	52±8
Ischemia for 1 h	194±21	98±14	84±9*	12±2*

Legend. *$p < 0.05$. Significant changes compared with control.

When the changes discovered in AK isozyme activity in myocardial tissue extract after ischemia are examined, the first feature of note is the 40% rise in activity of the AK_2 isozyme. Activity of the AK_3 isozyme decreased by 77%. No significant changes were found in either total activity or activity of the AK_1 isozyme.

The decrease in the activity of the AK_2 isozyme discovered previously [5] after ischemia for 30 min in isolated mitochondria apparently contradicts our results. It must be pointed out that during ischemia [4] and during isolation, the integrity of both the outer and the inner mitochondrial membranes is disturbed. This may lead to the release of the isozyme located in the intermembranous space into the external medium. This could account for the decrease in the activity of the AK_2 isozyme in isolated mitochondria. The reasons for the increase in AK_2 activity in the tissue extract are not yet clear. Perhaps there is a change in the transfer of AK_2 from the mitochondria into a different structural and functional medium and a change in the physicochemical properties in the cytoplasm in the ischemic cell.

On the one hand, release of the AK_2 isozyme from the mitochondria may adversely affect functioning of the energy transport system [3] in the postischemic period, and on the other hand, an increase in AK_2 activity ought to lead to an increase in power of the ATP-regenerating system, and ought thus to play a beneficial role in ischemia.

The cause and the purpose of the reduction of isozyme AK_2 activity are not yet clear. Since this isozyme plays an important role in substrate phosphorylation [7], the considerable decrease in its activity observed after ischemia may adversely affect the energy state and the stability of the mitochondria themselves. On the other hand, GTP formed during substrate phosphorylation can be utilized in the protein-synthesizing system and can promote the course of repair processes.

Thus the changes observed in AK isozyme activity cannot be unequivocally interpreted. The data described above add significantly to the picture of ischemic changes found in the energy-providing system of the myocardial cell.

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