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AMP deaminase, 5′-nucleotidase and adenosine deaminase in rat myocardial tissue in myocardial infarction and hypothermia

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Summary. AMP deaminase, 5′-nucleotidase and adenosine deaminase have been estimated in skeletal muscle and myocardial tissue in normal rats and in rats subjected to experimental myocardial infarction or hypothermia. A difference in the enzyme distribution was found between the right and left ventricles in the normal rat. A decrease in the activity of 5′-nucleotidase and an increase in the activity of adenosine deaminase were observed in infarcted myocardial tissue. The activity of all 3 enzymes was found to be depressed in the myocardium in rats subjected to hypothermia. These results are discussed in relation to adenosine production and its beneficial effects.

Within the last few years a considerable amount of evidence has accumulated for a hormonal role for adenosine[2]. Adenosine has been implicated in a number of biological processes such as inhibition of cell proliferation[3], immuno suppression[4] and hormone liberation[5]. Adenosine has been shown to produce local vasodilatation[6]. This particular function has been exploited in the treatment of ischaemic heart disease by using drugs which maintain the adenosine levels in the tissues[7]. The immediate precursor of adenosine in the tissues is adenylic acid which is most quickly derived from the breakdown of ATP, though it is also synthesized de novo from simple metabolites. The production of adenosine from AMP in the tissues depends upon the relative activities of AMP deaminase and 5′-nucleotidase. While the former enzyme makes AMP unavailable for the production of adenosine, and AMP is diverted into the purine nucleotide cycle[8], the latter enzyme is specifically concerned with the production of adenosine. The preservation of the adenosine so liberated depends upon a number of factors, one of which is the activity of adenosine deaminase. In this paper we describe the activities of AMP deaminase, 5′-nucleotidase and adenosine deaminase in rat myocardium under 2 experimental conditions, namely myocardial infarction and hypothermia; the former requires more adenosine to maintain blood circulation in the tissue deprived of a blood supply, and the latter requires less adenosine due to depressed metabolic function and consequent low blood circulation. These enzymes have also been estimated in normal rat skeletal muscle tissue for purposes of comparison.

Materials and methods. Albino rats of the local strain were used for the experiments. Rats of both sexes were randomly used; they were killed by decapitation and exsanguinated, and the heart and a piece of skeletal muscle (gastrocnemius) were quickly removed and placed in ice-cold saline; they were then washed in the same solution to remove all traces of blood and a 10% homogenate of each tissue was prepared in 0.25 M sucrose using a Potter-Elvehjem type of homogenizer.

AMP deaminase activity was estimated by the method of Ogasawara et al.[9]. The assay mixture comprised 0.5 ml of 20 mM phosphate buffer, pH 7.0, 0.1 ml of 150 mM sodium chloride solution, 0.1 ml of 0.05% bovine serum albumin, 0.1 ml of 15 mM AMP solution and 0.1 ml of 10% tissue homogenate. Incubation was at 37 °C for 10 min. The ammonia liberated was estimated by Berthelot's method. The intensity of the indophenol-blue color developed was measured in a Spectronic-20 spectrophotometer at 640 nm against water. The activity of the enzyme was expressed as μmoles of ammonia liberated per h per g wet weight of tissue using ammonia standards processed in a like manner.

5′-Nucleotidase activity was determined by the method of Buniatian as described by Sadasivudu et al.[10]. The assay mixture consisted of 0.2 ml of 0.2 M Tris-HCl buffer, pH 7.5, 0.1 ml of 0.12 M magnesium sulphate solution, 0.5 ml of 0.01 M AMP solution, 0.2 ml of 10% tissue homogenate and 0.5 ml of distilled water. This was incubated at 37 °C for 2 h. The reaction was terminated by the addition of 1.5 ml of 10% TCA solution and inorganic phosphate liberated was determined in a 1 ml aliquot of the supernatant. The activity of the enzyme was calculated using phosphate standards and expressed as μmoles of Pi liberated per h per g wet weight of the tissue.

Adenosine deaminase activity was estimated by the method of Martinek[11]. The substrate solution comprised 0.5 ml of 0.675 mM adenosine in 0.2 M phosphate buffer pH 7.05. To this 0.05 ml of 5% tissue homogenate was added, and incubated at 37 °C for 1 h. A control tube was simultaneously prepared for each sample by adding phenol color reagent to the substrate prior to the addition of the tissue homogenate. At the end of 1 h the reaction was stopped by adding 2.5 ml of phenol color reagent (liquefied phenol

AMP deaminase, 5′-nucleotidase and adenosine deaminase activity in rat myocardium

	Normal			Experimental	
	Skeletal muscle	Right ventricle	Left ventricle	Myocardial infarction	Hypothermia
AMP deaminase*	845.55 ± 117.78 (6)	21.11 ± 13.13 (6)	23.69 ± 13.44 (6)	22.59 ± 6.56 (6) NS	13.04 ± 6.47 (6) NS
5′-Nucleotidase**	71.35 ± 4.40 (10)	340.86 ± 13.88 (10)	301.44 ± 14.13 (10)	211.74 ± 19.77 (6) $p < 0.001$	159.07 ± 15.81 (10) $p < 0.001$
Adenosine deaminase*	2.76 ± 2.38 (10)	10.21 ± 5.58 (10)	11.71 ± 3.41 (10)	22.00 ± 6.72 (6) $p < 0.001$	3.61 ± 3.35 (6) $p < 0.02$

Results are Mean ± SD, figures in parenthesis represent number of observations. NS, Statistically not significant. *μmoles of ammonia liberated/h/g wet wt of tissue. **μmoles of inorganic phosphate liberated/h/g wet wt of tissue.

5.4 ml and sodium nitroprusside 25 mg made up to 500 ml with distilled water), and 2.5 ml of alkaline hypochlorite solution (62.5 ml of 1 N sodium hydroxide and 4.2 ml of 5% sodium hypochlorite solution made up to 500 ml with distilled water). The tubes were incubated at 37 °C for a further 15 min to complete color development. The blue color developed was measured at 640 nm against water and the activity of the enzyme expressed as μmoles of ammonia liberated per h per g wet weight of the tissue.

Myocardial infarction was produced experimentally by ligation of the left coronary artery, keeping the rat on artificial respiration by means of a respiration pump for a period of 90 min after which the heart was excised and the infarcted tissue was cut out on the basis of change in color of the area devoid of blood supply. The tissue was then homogenized and used for enzyme assays.

Hypothermia was produced by placing crushed ice over the entire body, except the head, of a Nembutal (4 mg/100 g) anesthetized rat. Rectal temperature was recorded from time to time. The temperature was usually maintained between 20 and 25 °C.

Results and discussion. Though the ventricles of the heart are concerned with the pumping of blood, the functional capacities of the left ventricle are greater in this respect than those of the right ventricle. Consequently there will be a difference in the metabolic capabilities of the 2 ventricles. Earlier studies indicated that in rats the left ventricle contained more aspartate aminotransferase and glutamine synthetase[12]. Though the level of activity of adenylate deaminase was almost the same in both ventricles, the activity of 5′-nucleotidase was found to be significantly higher in the right ventricle. The far higher activity of adenylic acid deaminase in skeletal muscle may be explained in terms of the presence of an appreciable activity of the purine nucleotide cycle in that tissue, while the low activity of this enzyme in the myocardium may point to the insignificance of this cycle in myocardium, if it exists in it at all. In contrast to this enzyme the high activity of 5′-nucleotidase in myocardium in comparison to the skeletal muscle might indicate the need for the production of adenosine in conditions of need by the myocardium.

As a result of ischaemia ATP is rapidly degraded to AMP. Though the activity of AMP deaminase did not show a change in the infarcted myocardial tissue its full activity in vivo may not be expressed in the infarcted tissue owing to depletion of ATP, which is a known activator of this enzyme[13]. At the same time the activity of 5′-nucleotidase in infarcted tissue, though found to be decreased, will occur at an uninhibited rate, because of the depletion of ATP, a known inhibitor of this enzyme[14]. As a result, a greater amount of adenosine would be formed in the infarcted myocardial tissue and may leak out. However, in contrast to 5′-nucleotidase the adenosine deaminase activity was found to be increased in the infarcted tissue. This would lead to greater production of inosine which again may leak out of the damaged tissue. Earlier studies revealed that the activity of adenosine deaminase in the serum of patients suffering from myocardial infarction was found to be very low. It was also observed that the low activity of this enzyme in serum was due to the presence of more inosine, an inhibitor of this enzyme[15] in the serum. Paradoxically the serum of these patients with myocardial infarction showed a much greater activity than normal when the serum was diluted showing that the infarcted myocardial tissue may be releasing more of this enzyme which, however, remained inhibited until dilution. The higher amounts of the enzyme reported in a similar study by Khalfen and Denisova may be explained as being due to the different method used in the estimation of adenosine deaminase[16].

The above changes pertaining to enzymes involved in the production and disposal of adenosine occuring in myocardial tissue could be regarded as compensatory in nature, directed to preserving the tissue and its function by improving local blood supply.

The process of hypothermia is often adopted in cardiac surgery. In hypothermia the cardiac metabolism could be suppressed to a great extent. Because of this, the needs for oxygen and nutrient supplies are minimized[17]. The decrease in the activities of adenylate deaminase and 5′-nucleotidase in myocardial tissue in rats subjected to hypothermia could be regarded as part of a generalized depression in tissue metabolism. The factors responsible for the decrease of activities of these enzymes in hypothermia are not properly understood. It can, however, be mentioned that in hypothermia the degradation of ATP is low, and the need for adenosine production will also be low.

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