DEAMINATION OF AMP AND ADENOSINE AND DEPHOSPHORYLATION OF AMP IN SUBCELLULAR STRUCTURES OF THE MYOCARDIUM IN EXPERIMENTAL AORTIC STENOSIS

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On the 8th-10th day after constriction of the abdominal aorta in rabbits the weight of the left ventricle increased and the velocity of breakdown of AMP and adenosine in the microsomes extracted from the myocardium at 75,000 g fell: the velocity of deamination of AMP by 71%, the velocity of dephosphorylation of AMP by 41%, and the velocity of deamination of adenosine by 74%.

One of the special properties of heart muscle is its ability to produce ammonia from adenylic acid (AMP) by conversion of the acid into adenosine and subsequent deamination of the adenosine by a specific deaminase [12,13,15]. At the same time, direct splitting of ammonia from AMP can also take place in the myocardium [9].

The connection between ammonia formation and functional activity of muscles [10,17], and the participation of AMP in oxidative phosphorylation [16] makes the study of enzymes splitting AMP in heart muscle under conditions of normal and disturbed activity of particular interest.

In previous investigations the action of AMP deaminase, 5^1-nucleotidase, and adenosine deaminase in the subcellular structures of heart muscle was studied in intact animals [7,8].

The object of the present investigation was to study the activity of the same enzymes in the subcellular structures of the myocardium during aortic stenosis.

EXPERIMENTAL METHOD

Experiments were carried out on rabbits weighing 2-3 kg. The diameter of the proximal part of the abdominal aorta was reduced to 2 mm by means of a ligature.

The rabbits were taken for investigation on the 8th-10th day after operation. The ratio between the weight of the left ventricle and that of the right averaged 5 in the animals, whereas in intact rabbits it was about 4.

The animals were decapitated, and the heart was quickly removed and immersed for a few minutes in cold 0.25 M sucrose solution.

The left ventricle was cut up finely with scissors and then homogenized for 1-2 min with 4.5 volumes of 0.25 M sucrose solution. Cell fractions were isolated from the homogenate by differential centrifugation: the myofibrillary-nuclear fraction at 600 g (the upper layer of the residue was removed), mitochondria at 3500 g, and microsomes at 75,000 g. The first two fractions were washed twice with 0.25 M sucrose solution.

The enzyme activity of the fractions was determined 1-3 h after they had been obtained. The test mixtures contained 40 mmoles tris-buffer, pH 7.0, and 4 mmoles substrate. The fractions and homogenate were taken in the experimental mixture in sufficient volume to ensure that splitting of the substrate did not exceed 30%. The mixtures were incubated for 30 min at 37°.

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TABLE 1. Splitting of AMP and Adenosine by Cell Fractions of Myocardium of Left Ventricle (in μmoles/mg protein; M ± m)

Animals	Deamination of AMP				Dephosphorylation of AMP				Deamination of adenosine			
	homogenate	myofibrils of nucleus	mitochondria	microsomes	homogenate	myofibrils of nucleus	mitochondria	microsomes	supernatant	homogenate	myofibrils of nucleus	mitochondria	microsomes
1. Intact	0,172± 0,023 (8)	0,152± 0,037 (8)	0,433± 0,110 (9)	2,291± 0,640 (9)	0,071± 0,006 (8)	0,057± 0,013 (6)	0,133± 0,034 (10)	0,751± 0,095 (10)	0,302± 0,057 (8)	0,048± 0,003 (7)	0,028± 0,006 (5)	0,090± 0,024 (7)	0,833± 0,040 (7)
2. With aortic stenosis	0,166± 0,035 (7)	0,116± 0,025 (7)	0,152± 0,045 (7)	0,662± 0,102 (4)	0,063± 0,008 (8)	0,034± 0,006 (8)	0,088± 0,016 (8)	0,395± 0,069 (8)	0,184± 0,045 (7)	0,042± 0,004 (6)	0,014± 0,005 (6)	0,075± 0,016 (7)	0,214± 0,028 (5)
Significance of difference (1 – 2)	$P>0,5$	$0,2<P<0,5$	$0,02<P<0,05$	$0,02<P<0,05$	$0,2<P<0,5$	$P>0,5$	$0,2<P<0,5$	$0,001<P<0,01$	$0,1<P<0,2$	$0,2<P<0,5$	$0,1<P<0,2$	$P>0,5$	$P<0,001$

Note. Number of animals given in parentheses.

The degree of deamination was assessed quantitatively by Kalckar's spectrophotometric method [14]; phosphorus split from AMP was assessed from the increase in inorganic phosphorus, using the Fiske–Subbarow method.

The experimental results were expressed as μmoles deaminated substrate or phosphorus formed per milligram protein of homogenate or fraction.

The protein content in the homogenate and fractions was estimated as nitrogen after mineralization of the TCA residues after washing and freeing from phospholipids.

EXPERIMENTAL RESULTS AND DISCUSSION

The results are given in Table 1. Of all fractions investigated, the microsomes possessed the greatest ability to deaminate AMP and adenosine, whether from healthy or experimental animals. In both groups of animals, the microsomes also had the highest 5^1-nucleotidase activity. These results are in agreement with earlier findings [7,8,9].

Comparison of the control and experimental rabbits shows that the specific deaminase and dephosphorylating activity of the microsomes was much lower in the animals with aortic stenosis than in intact animals. In the controls, for instance, for each milligram microsomal protein 2.29 μmoles AMP was deaminated, compared with only 0.66 μmole for the experimental animals, i.e., a reduction of 71%.

In the animals with aortic stenosis, the adenosine deaminase activity was reduced by 74% and the 5^1-nucleotidase activity of the microsomes by 41%. A decrease in activity of the investigated enzymes was also found in the mitochondria and supernatant, the activity of which was presumably due to contamination by microsomes. However, these changes cannot be regarded as significant. The decrease in enzyme activities in the homogenate from animals with aortic stenosis likewise was not significant. This fact may be explained by the small content of microsomes in the homogenate and inability to detect a decrease in their activity in the presence of large quantities of myofibrils, which are inert in this respect.

On the basis of the results described above and also of previous findings [7,8,9], it can be concluded that the reaction of ammonia formation from AMP mainly takes place in the microsomes of the myocardial cells. The decrease in the velocity of deamination of AMP and adenosine, and also in the velocity of dephosphorylation of AMP in the microsomes in these experiments may therefore indicate a decrease in ammonia formation in the myocardium in aortic stenosis.

The disturbance of cardiac activity in the initial stage of aortic stenosis is accompanied by a decrease in the intensity of aerobic oxidation and in the conjugated phosphorylation [3,11], and also by other changes in intermediate metabolism in the myocardium [1,2,4-6]. At the same time, as Vyalykh and Meerson [1] have shown, the content of ATP and AMP in the heart muscle is essentially unchanged in aortic stenosis. This fact, together with the decrease in the velocity of AMP deamination in the microsomes, may indicate that a certain level of equilibrium is reached between breakdown and restoration of adenine nucleotides in the myocardium under the modified conditions of its activity.

LITERATURE CITED

1. M. F. Vyalykh and F. Z. Meerson, Vopr. Med. Khimii, No. 1, 19 (1960).
2. M. F. Vyalykh and N. G. Lutsenko, Vopr. Med. Khimii, No. 2, 186 (1961).
3. M. F. Vyalykh, Vopr. Med. Khimii, No. 6, 625 (1961).
4. F. Z. Meerson, M. E. Egorova, and S. Ya. Guz, Vopr. Med. Khimii, No. 5, 336 (1955).
5. F. Z. Meerson and T. L. Zayats, Byull. Éksperim. Biol. i Med., No. 7, 33 (1960).
6. F. Z. Meerson, G. V. Chernyshova, and L. S. Rozanova, Vestn. Akad. Med. Nauk SSSR, No. 5, 32 (1961).
7. Z. Yu. Nechiporenko, E. G. Goncharenko, and O. P. Goloborod'ko, Ukr. Biokhim. Zh., No. 4, 398 (1966).
8. Z. Yu. Nechiporenko and E. G. Goncharenko, Ukr. Biokhim. Zh., No. 2, 192 (1967).
9. Z. Yu. Nechiporenko and O. P. Goloborod'ko, Ukr. Biokhim. Zh., No. 3, 322 (1967).
10. D. L. Ferdman, The Biochemistry of Diseases of Muscles [in Russian], Kiev (1953), p. 40.
11. M. F. Argus, J. C. Arcos, V. M. Sardesai, et al., Proc. Soc. Exp. Biol. (N.Y.), 117, 380 (1964).
12. E. J. Conway and R. Cooke, Biochem. J., 43, 479 (1939).
13. D. A. Goldthwait, J. Clin. Invest., 36, 1572 (1957).
14. H. M. Kalckar, J. Biol. Chem., 167, 445 (1947).
15. P. Ostern and T. Mann, Biochem. Z., 260, 326 (1933).
16. T. Ozawa, Arch. Biochem., 117, 201 (1966).
17. J. K. Parnas and P. Ostern, Biochem. Z., 234, 307 (1931).