ATPase ACTIVITY OF SMOOTH AND ROUGH MICROSOMAL SUBUNITS OF THE RABBIT HEART AND LIVER AFTER BRIEF DISTURBANCE OF THE MICROCIRCULATION

N. M. Dmitrieva, G. V. Chernysheva, and M. D. Vakar

UDC 616.16-008.1-092.9-07:[616.12+616-008.36].931-074

A brief disturbance of the microcirculation in rabbits differed in its effects on ATPase activity of the smooth and rough microsomes of the liver and heart. In the liver, Mg-ATPase activity was lowered in both fractions of microsomes. In the heart, the activity of the enzyme was raised only in the smooth (light) microsomes.

KEY WORDS: ATPase activity; fractions of microsomes of heart and liver; microcirculation.

Research into the metabolism of organs and tissues in the early stages of disturbance of the microcirculation is essential to the study of the pathogenesis and treatment of several diseases, notably myocardial infarction. By the use of an experimental model reproducing a disturbance of the microcirculation by intravenous injection of high-molecular-weight dextran, causing aggregation of red blood cells, and of vasopressin, which constricts the lumen of the coronary vessels, a series of changes in the energy metabolism and contractile function of the heart was discovered in the early and late stages of disturbance of the microcirculation [3, 4].

Since an important role in musuclar contraction is played by the sarcoplasmic reticulum (SPR), in the investigation described below ATPase activity of the microsomal subfractions of the heart, which consist mainly of SPR proteins, was studied during acute and brief disturbance of the microcirculation. The results were compared with the enzyme activity of the smooth and rough subfractions of liver microsomes, which perform different functions from the microsomes of the heart.

EXPERIMENTAL METHOD

Male rabbits weighing 2-3 kg were used. To remove glycogen from the liver the animals were deprived of food for 20-24 h before the beginning of the experiments. An acute disturbance of the coronary circulation was produced by intravenous injection of high-molecular-weight dextran (0.5-1 g/kg body weight) and vasopressin (5 pressor units/kg body weight). The experiments were carried out under urethane anesthesia. The ECG and respiration of all animals were recorded before and after injection of substances. During the period of maximal change in the ECG, 5 min after injection of vasopressin, the heart and liver were removed and the tissue washed with 0.14 M KCl to remove blood. The liver microsomes were fractionated in a stepwise sucrose density gradient in the presence of $MgCl_2$ [5]. The liver tissue was homogenized in 0.25 M sucrose containing 20 mM Tris-HCl, pH 7.4, and 10 mM $MgCl_2$, after which the 10% homogenate was centrifuged to remove the nuclei (600g, 10 min) and mitochondria (10,000g, 10 min). The postmitochondrial supernatant was carefully layered above 1.3 M sucrose containing 20 mM Tris-HCl, pH 7.4, and 10 mM $MgCl_2$. As a result of centrifugation in the horizontal rotor (100,000g, 1 h) a light fluffy layer (the subfraction of smooth microsomal vesicles) formed at the boundary between the sucrose gradients, and the residue at the bottom of the tube consisted of the subfraction of rough vesicles of liver microsomes. The subfraction of smooth microsomes was carefully collected and centrifuged at 105,000g (1 h) to obtain a solid residue. The residues of the smooth and rough membranes from the liver were suspended in 0.25 M sucrose, made up in Tris-HCl, pH 7.4, and the protein concentration was determined by Lowry's method [7]. The residues were kept in the frozen state at −10°C.

Laboratory of Molecular Pathology and Biochemistry, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 1, pp. 26-28, January, 1978. Original article submitted April 7, 1977.

0007-4888/78/8501-0029 $07.50

TABLE 1. ATPase Activity of Microsomal Subfractions of Heart and Liver of Rabbits under Normal Conditions and after Combined Administration of Vasopressin and High-Molecular-Weight Dextran (M ± m)

Experimental conditions	Organ	ATPase activity, μmoles Pi/mg protein/h					
		total microsomal fraction		fraction of smooth membranes		fraction of rough membranes	
		Mg-ATPase	Ca-ATPase	Mg-ATPase	Ca-ATPase	Mg-ATPase	Ca-ATPase
Normal (13 experiments)	Heart	23,52±1,11	22,63±1,45	22,8±1,30	20,1±1,49	17,0±2,14	12,7±1,96
	Liver	9,09±0,64	6,54±0,62	11,3±0,98	7,4±0,98	7,1±0,87	4,5±0,68
Vasopressin+ dextran (11 experiments)	Heart	—	—	27,3±0,91*	23,2±1,34	15,6±0,84	13,7±0,66
	Liver	8,3±1,29	5,2±1,04	9,6±0,75	5,2±0,63*	6,4±0,98	3,3±0,45

*P < 0.05 compared with corresponding control (normal).

Legend. Hydrolysis of ATP in presence of Mg^{2+} or Ca^{2+} only taken as activity of Mg- and Ca-ATPase respectively.

TABLE 2. Glucose-6-Phosphatase Activity of Microsomal Subfractions of Liver under Normal Conditions and after Combined Administration of Vasopressin and High-Molecular-Weight Dextran (M ± m)

Experimental conditions	Glucose-6-phosphatase activity, μmoles Pi/mg protein/h		
	total microsomal fraction	fraction of smooth membranes	fraction of rough membranes
Normal (11 experiments)	6,5±0,80	3,91±0,40	7,33±0,53
Vasopressin+ dextran (11 experiments)	6,9±0,66	3,65±0,37	6,97±0,45

The microsomes of the heart were fractionated in a stepwise sucrose density gradient [6]. The residue of the coarse microsomal fraction of the heart [2] was suspended in 0.25 M sucrose containing Tris-HCl, pH 7.4, and layered above a previously prepared stepwise gradient consisting of equal volumes of 35 and 20% sucrose, containing 20 mM Tris-HCl, pH 7.4. After centrifugation in the horizontal rotor (100,000g, 1.5 h) a light layer (smooth membranes) was formed at the boundary between the 35 and 20% sucrose layers, and a residue (rough membranes) collected at the bottom of the tube. The fraction of smooth membranes was collected by centrifugation at 105,000g for 1h. To remove the contractile proteins the subfractions of the heart membranes were treated with 0.6 M KCl in histidine buffer, pH 7.2 [1]. After removal of the contractile proteins the protein content was determined in the subfractions of the heart membranes and they were kept at −10°C.

ATPase activity was determined [2] in the fractions of smooth and rough membranes from the heart and liver, and glucose-6-phosphatase activity was determined in the subfractions from the liver [14].

EXPERIMENTAL RESULTS

The comparative study of enzyme activity of the smooth and rough microsomal membranes of the heart and liver of intact rabbits revealed differences (Table 1). First, the smooth membranes of both organs, compared with the rough membranes, possessed higher ATPase activity. Second, activity of the ATPases of the microsomes of the heart was higher than in the microsomes of the liver. Third, just as in investigations by other workers [11-13], in these experiments no glucose-6-phosphatase activity was discovered in the microsomes of the heart. It was concentrated chiefly in the membranes of the rough endoplasmic reticulum of the liver (Table 2).

Injection of vasopressin, superposed upon the aggregation effect of the high-molecular-weight dextran, led to enlargement of the T wave and its conversion to negative at the fourth to fifth minute. Marked bradycardia and extrasystoles thereupon developed and the heart beat and respiration rate were slowed. Changes in ATPase activity in different directions were observed in the microsomal subfractions isolated from the heart

and liver 5 min after injection of vasopressin. In the heart, a change in its activity was observed only in the smooth membranes, when Mg-ATPase activity increased by 19% and Ca-ATPase activity by 15% (Table 1). In the liver, despite fluctuation in ATPase activity under normal conditions and during disturbance of the microcirculation, the mean data showed that ATPase, activated by Mg^{2+} and Ca^{2+} ions, fell equally in fractions of both smooth and rough membranes (Table 1). Under these conditions the glucose-6-phosphatase activity remained unchanged (Table 2).

The results showed that the microsomal fraction of the heart consisted of smooth (light) and rough (heavy) subfractions of membranes. Both subfractions possessed ATPase activity. The subfraction of light vesicles is known [10] to consist to the extent of 90% of proteins of Ca-activated, Mg-dependent transport ATPase [8-10, 15], whereas the subfraction of heavy vesicles contains Ca-binding protein and only a small quantity of protein with transport Ca-ATPase. The tendency observed for hydrolysis of ATP to increase in the presence of Ca^{2+} in the light microsomes can be interpreted as a possible increase in the work of the Ca pump of the SPR in response to acute brief disturbance of the microcirculation.

LITERATURE CITED

1. N. G. Gimmel'reikh, Biokhimiya, No. 5, 925 (1972).
2. N. M. Dmitrieva, G. V. Chernysheva, and M. D. Vakar, Byull. Éksp. Biol. Med., No. 12, 1433 (1976).
3. A. M. Chernukh, M. D. Vakar, P. N. Aleksandrov, et al., Kardiologiya, No. 11, 10 (1971).
4. G. V. Chernysheva, M. D. Vakar, L. V. Stoida, et al., Vopr. Med. Khim., No. 1, 14 (1973).
5. G. Dallner, Acta Pathol. Microbiol. Scand., Suppl., 166 (1963).
6. A. M. Katz and D. J. Repke, Circulat. Res., 21, 153 (1967).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
8. G. H. MacLennam, J. Biol. Chem., 246, 2762 (1971).
9. A. Martonosi and G. Feretos, J. Biol. Chem., 239, 659 (1964).
10. G. Meissner, Biochim. Biophys. Acta, 389, 51 (1975).
11. H. Remmer and H. J. Merker, Klin. Wschr., 41, 276 (1963).
12. H. U. Schulze and H. J. Standinger, Hoppe-Seyler's Z. Physiol. Chem., 352, 1659 (1971).
13. M. K. Stetten and S. B. Ghosh, Biochim. Biophys. Acta, 233, 167 (1971).
14. M. A. Swanson, Methods Enzymol., 2, 541 (1955).
15. A. Weber, R. Herz, and Z. Reiss, Biochem. Z., 345, 329 (1966).