EFFECT OF ATP-SPLITTING CARDIAC ECTOENZYMES DURING POSTISCHEMIC MYOCARDIAL REPERFUSION

V. V. Alabovskii, O. D. Oleinikov, and V. V. Ryabova

UDC 616.127-005.4-092.9-003.9-07: 616.12-008.931

Key Words: ectoenzymes; ATP; ischemia; reperfusion

Ectoenzymes were discovered in 1974. They are enzymes located on the outer side of plasma membranes and carry out catalytic conversions of substrates in the interstitial space. Among the ectoenzymes of the heart, that with the highest activity is ecto-ATPase, which hydrolyzes ATP. Its activity is judged by the rate of hydrolysis of ATP, which is passed together with the perfusion solution through the isolated heart [3]. An essential condition for the identification of this enzyme is that the cells must be intact. Damage to the cell membrane can distort the true value of enzyme activity on account of enzymes "leaking" from the cytoplasm, and catalyzing the same reaction. Meanwhile it is tempting to study activity of ectoenzymes in various pathological states. Since they are plasma membrane proteins their activity may depend directly on the state and integrity of the sarcolemma. The only alternative solution to this problem is evidently to record ecto-ATPase activity at those times of the pathological process when the plasma membrane becomes impermeable once more for intracellular enzymes.

The aim of this investigation was to study activity of ATP-splitting ectoenzymes in the heart with ischemic damage.

EXPERIMENTAL METHOD

Experiments were carried out on isolated hearts of albino rats (n = 24) weighing 200-250 g. The spontaneously contracting hearts, removed from animals anesthetized with ether, were perfused through the aorta with Ringer–Locke solution, saturated with oxygen at pH 7.4 and 37°C. The rate of perfusion was established by means of a peristaltic pump at 10 ml/min/g. The hearts contracted under auxovolumic conditions. The perfusion pressure in the aortic system was recorded by means of an electromanometer, and changes in the volume of a small balloon, introduced into the left ventricle, were recorded by means of a photodetector. The index of contractile function (ICF) of the heart was calculated as the product of the change in volume of the balloon (from systole to diastole) and the number of contractions per minute. Reperfusion-induced injury to cardiomyocytes after prolonged myocardial ischemia followed by restoration of coronary perfusion, served as the experimental model. ATP was added to the perfusion fluid in a concentration of 50, 100, or 150 μM after 15-20 min – the time required for contractile activity of the heart to stabilize – and the heart was perfused with this solution for 1 min. Total ischemia for 30 min was created by occluding the aortic cannula. Reperfusion, which lasted 30 min, was undertaken with perfusion solutions containing and not containing ATP. The time of administration of ATP was 1 min. Using the ATP concentration difference in perfusion fluid flowing into and out of the heart, activity of ecto-ATPases was calculated. ATP concentrations were determined by an enzymic method. The myoglobin concentration in perfusion fluid leaving the heart was measured on an SF-26 spectrophotometer at a wavelength of 420 nm [2]. The significance of differences in the case of tied samples was estimated by Wilcoxon's matched pairs test, and in the case of independent samples, by the Wilcoxon–Mann–Whitney test [1].

EXPERIMENTAL RESULTS

During perfusion of the isolated heart with solution containing ATP in a concentration of 50 μM, all of it underwent hydrolysis. In this case, it was impossible to measure ectoenzyme activity on the basis of the amount of reaction substrate. If the ATP concentration was increased to 100 μM, its concentration in perfusion fluid leaving the heart was 7.5 $\pm$ 3.8 μM. The use of

Department of Biochemistry, N. N. Burdenko State Medical Institute, Voronezh. (Presented by Academician of the Academy of Medical Sciences of the USSR T. T. Berezov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 5, pp. 436-438, May, 1990. Original article submitted December 31, 1988.

 0007-4888/90/0005-0574$12.50

TABLE 1. Rate of Hydrolysis of ATP (100 μM) during Incubation (10 min) in Perfusion Fluid Leaving the Heart, and Myoglobin Concentration in Perfusion Fluid Collected before Ischemia and during Reperfusion of Myocardium

Parameter	Initial value (before ischemia)	Riperfusion, min			
		1—4		10	20
Rate of hydrolysis, μmoles/min·mg	0	0,045 (0,032—0,058)	0,030 (0,02—0,051)	0,008 (0,005—0,013)	0
p		<0,001	<0,001	<0,001	
Myoglobin concentration, nM	4,0 (0,6—6,2)	22,3 (6,4—37,0)	9,5 (2,4—11,7)	7,0 (2,7—9,8)	3,5 (0,7—5,5)
p		<0,001	<0,05	<0,05	>0,05

Legend. Arithmetic mean values and limits of variations: p) significance of differences from initial values.

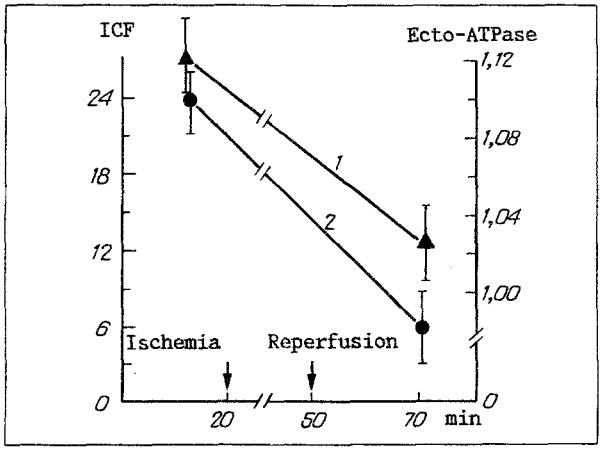

Fig. 1. Activity of cardiac ecto-ATPase and index of contractile function of the heart (ICF) before ischemia and after 20 min of myocardial reperfusion. 1) Rate of hydrolysis of ATP (150 μM, μmoles/min · g) by cardiac ecto-ATPase; 2) index of contractile function of heart (μl/min).

ATP in a concentration of 150 μM was accompanied by its partial hydrolysis in the heart: 37.4 ± 9.5 μM or 25 ± 6% of ATP (of its initial concentration) remained in the outflowing solution.

Addition of 100 μM ATP to the solution passed through the heart, and incubation in it for 10 min did not lead to ATP hydrolysis. Consequently, exogenous ATP is hydrolyzed in the heart itself and not in the outflowing perfusion fluid, which could contain ATPases originating from the cytoplasm of the cardiomyocytes.

Reperfusion of the isolated heart immediately after total ischemia for 30 min, with solution containing 150 μM ATP, was accompanied by hydrolysis: at the 1st, 2nd, 3rd, and 4th minutes of reperfusion the ATP concentration in the solution leaving the heart was 9.0 ± 2.1, 8.0 ± 1.9, 8.0 ± 2.2, and 7.0 ± 1.9% of the initial concentration respectively.

Incubation of 100 μM ATP in perfusion fluid collected during the first 4 min of reperfusion led to hydrolysis of ATP (Table 1). Consequently, besides enzymes, ATP was hydrolyzed by those ATPases and enzymes involved in ATP conversion (myo-, hexo-, and pyruvate-kinases, etc.) which escaped from the cells during ischemic and reperfusion damage. This explains the hydrolysis of ATP observed during the first 4 min of reperfusion of the heart by solution containing ATP.

Incubation of 100 μM ATP in solutions collected 5, 10, and 15 min after the beginning of reperfusion of the heart was accompanied by a gradual decrease in the rate of ATP hydrolysis from the 5th to the 15th minute (Table 1). Perfusion fluid obtained at the 20th minute of reperfusion was unable to hydrolyze ATP. Consequently, at the 20th minute of reperfusion, escape of ATPases from the cells had completely ceased, thus enabling activity of the ectoenzymes themselves to be determined.

The intensity of hydrolysis of ATP by the heart during its passage through the heart, at the 20th minute of reperfusion, was lower than before ischemia. Besides a decrease in the rate of ATP hydrolysis, there was a simultaneous fall in the value of ICF of the myocardium (Fig. 1). Release of myoglobin into the perfusion fluid at this time fell to its initial value (Table 1). De-

pression of the contractile function of the heart thus did not correlate with the period of recovery of membrane permeability of the cardiomyocytes for intracellular proteins, but was somehow connected with recovery of ectoenzyme activity. Plasma membranes evidently succeeded in creating a barrier, during the short period of reperfusion, which prevented high-molecular- weight substances from leaving the cells. However, the decrease in membrane permeability did not signify simultaneous restoration of its former structural and functional organization. Reduced activity of ecto-ATPase, a cell membrane protein, at this time confirms this conclusion. This depression may be caused by partial loss of the cardiac ectoenzymes during the first few minutes of reperfusion, or by disturbance of the structure of the enzyme or of its lipid environment. Whatever the case, the level of ecto-ATPase activity can serve as a marker of injury of the heart cell membranes and as a criterion for assessing the depth of reperfusion-induced myocardial damage.

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