Endogenous Phosphorylation of Lysosomal Proteins of Rat Heart and Liver in the Early Postreanimation Period

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The lysosomal fraction isolated in a Percoll gradient from rat liver and heart after 30 min of the postreanimation period following a 5-min heart arrest stimulated incorporation of ^{32}P in total lysosomal protein. Addition of cAMP and protein inhibitor of cAMP-dependent protein kinase changed the rate of phosphorylation. Endogenous phosphorylation of total lysosomal protein in the postreanimation period was reduced in the heart and increased in the liver. This may be due to a change in the intracellular level of cAMP, which fell in the heart and rose in the liver.

Key Words: *lysosomes; cAMP-dependent phosphorylation; postreanimation period; heart; liver*

The lysosomal enzymes play an important role in the development of destructive changes in the heart and liver tissues during both hypoxia and ischemia, as well as during postischemic rehabilitation [8]. The permeability of lysosomal membranes and the activity of acid hydrolases in the cytosol of hepatic and myocardial cells have been shown to be increased in the early postischemic and postreanimation period [2,3]. The detected relationship between the intracellular cAMP concentration and the state of lysosomal membranes during postischemic recovery permits one to hypothesize a cAMP-mediated mechanism regulating lysosomal activity [6,10].

In this research we investigated the relationship between the rate of endogenous phosphorylation of proteins in the lysosomal fraction of rat liver and heart and the level of cAMP during the early postreanimation period.

MATERIALS AND METHODS

Male Wistar rats were used in the study. Circulation was arrested by clamping the cardiac vascular bundle after short-term ether narcosis [4,9]. Reanimation was carried out 5 min after heart arrest. The reanimation complex included indirect massage of the heart and artificial ventilation of the lungs with room air through an intubator inserted in the trachea. The efficacy of reanimation was assessed by the time needed for the recovery of heart work, spontaneous respiration, and corneal reflexes [10,12]. The control group consisted of animals decapitated synchronously with experimental groups after light ether narcosis. The lysosomal fraction was isolated from the heart and liver tissue in a Percoll gradient [13]. The resultant fraction contained about 70% total proteolytic lysosomal activity. Endogenous phosphorylation was measured by incorporation of ^{32}P-ATP (Radiopreparaty, Russia) in the total fraction of lysosomal protein (TLP) or in histone II-A (Sigma). The reaction mixture contained 5 to 10 μg lysosomal protein, 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 50 μg/ml pepstatin, and 0.1 mM ^{32}P-ATP (1 μCi) in a final volume of 130 μl. The reaction was stopped by adding 1 ml of 10% trichloroacetic acid containing 100 μg/ml bovine serum albumin. The samples were transferred to Whatman 3MM filters for radioactivity scintillation [7]. Protein concentration was measured as described previously [11].

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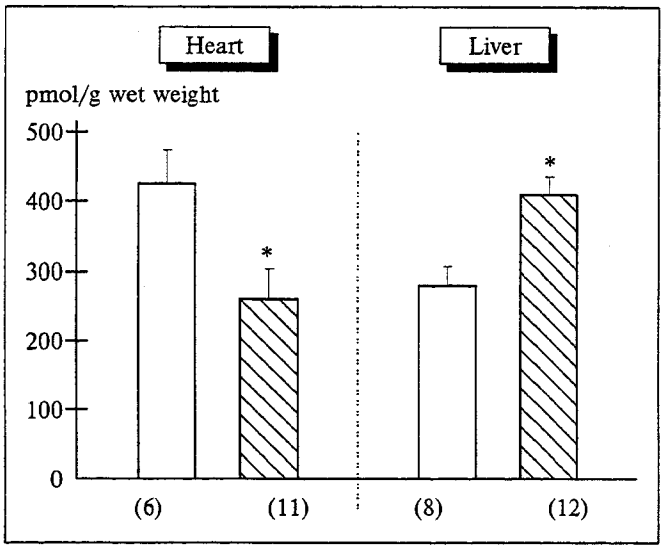

Fig. 1. Content of cAMP in rat heart and liver tissue. White bars: control; shaded bars: 5 min heart arrest+30 min postreanimation. Asterisk shows $p<0.05$ in comparison with the control. Number of experiments shown in parentheses.

The concentration of cAMP was measured by radioimmunoassay using Amersham kits after rapid freezing of tissue samples in liquid nitrogen.

RESULTS

The lysosomal fraction isolated from cardiac and hepatic tissue contained protein kinase (PK) activity, which was evident from ^{32}P incorporation in both TLP and histone II-A added as exogenous substrate (Table 1). The rate of ^{32}P incorporation in TLP and histone II-A decreased in the lysosomal fraction of the heart and increased in the lysosomal fraction of the liver 30 min after reanimation in comparison with the control group. Addition of protein inhibitor of cAMP-dependent PK (Sigma) suppressed the phosphorylation of histone II-A and TLP in the lysosomal fraction isolated from the heart of control rats and did not influence the endogenous phosphorylation in the postreanimation period. In the liver, the suppressive action of cAMP-dependent PK inhibitor on phosphorylation of histone II-A and TLP manifested itself both under conditions of postreanimation recovery and in the controls (Table 1). Addition of 5 μM cAMP to the lysosomal fraction of the heart of control rats did not affect ^{32}P incorporation in TLP but normalized the rate of protein phosphorylation in the lysosomal fraction isolated from rat heart under conditions of the postreanimation period.

The results indicate a different capacity of the lysosomal fraction of the heart and liver to endogenous phosphorylation. The extent of endogenous phosphorylation of lysosomal proteins may be related to the difference in the concentrations of intracellular cAMP in cardiac and hepatic tissue after reanimation. Measurement of the cAMP level in the myocardium and liver 30 min after reanimation showed that the intracellular cAMP content was changing in the same direction as the rate of endogenous phosphorylation (Fig. 1). Figure 1 shows that the cAMP level in the postreanimation period is reliably reduced in heart tissue and increased in the liver. It is possible that the initial rate of endogenous phosphorylation of lysosomal proteins is different in the heart and liver and depends on the intracellular cAMP concentration.

Moreover, other factors may influence the rate of endogenous phosphorylation of lysosomal proteins in the liver and heart of both control animals and rats subjected to heart arrest followed by reanimation. The different magnitude of endogenous phosphorylation of lysosomal proteins may

TABLE 1. Phosphorylating Activity of Lysosomal Fraction Isolated from Rat Heart and Liver ($M\pm m$)

Source of lysosomes	Lysosomal fraction	Lysosomal fraction with additives			
		cAMP	cAMP+PK inhibitor	histone II-A	histone II-A +PK inhibitor
Heart					
Control	132.83±24.95 (8)	75.26±13.85 (8)	97.28±14.17 (8)	139.45±3.86 (3)	12.84±5.28 (3)
5−min heart arrest+ 30 min postreanimation	94.29±8.22* (8)	128.80±12.10* (8)	110.43±20.71 (8)	27.02±2.86* (3)	45.75±5.00* (3)
Liver					
Control	101.97±9.79 (8)	86.00±9.58 (8)	74.86±12.89 (8)	13.10±1.74 (3)	9.52±2.49 (3)
5−min heart arrest+ 30 min postreanimation	148.70±27.40 (8)	97.76±4.67 (8)	50.69±14.45* (8)	147.03±15.47* (3)	18.19±4.29* (3)

Note. Phosphorylating activity was measured by ^{32}P incorporation in total lysosomal protein fraction and expressed in pmol of ^{32}P incorporated in min/mg protein. The number of experiments is shown in parentheses. cAMP was added in a concentration of 5 μM, inhibitor of cAMP−dependent PK at 15 μg per sample, and histone II−A at 2 mg/ml. Asterisk shows $p<0.05$ *vs.* the control.

be due to the presence of multiple forms of PK in the lysosomal membranes [7]. For example, the development of ischemia is known to be related to changed sensitivity of cAMP-dependent PK to cAMP, which may determine the rate of endogenous protein phosphorylation [1]. The absence of a stimulating effect of cAMP on endogenous phosphorylation in the lysosomal fraction isolated from the liver may likewise be caused by an endogenous thermolabile inhibitor of cAMP-dependent PK which suppresses cAMP binding to the enzyme [5].

The results attest to cAMP-dependent phosphorylation of lysosomal proteins in the lysosomal fraction isolated from the liver and heart of rats. The rate of endogenous phosphorylation of TLP depends on the levels of cAMP and inhibitor of cAMP-dependent PK and is achieved in different ways in the heart and liver under conditions of postreanimation recovery.

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REFERENCES

1. A. E. Antipenko and S. N. Lyzlova, *Biokhimiya*, **50**, 12-16 (1985).
2. T. N. Ivanova, E. D. Polyakova, and A. I. Ivanov, *Vopr. Med. Khim.*, **36**, № 6, 28-32 (1990).
3. S. E. Kolpakova, E. V. Vinogradova, L. V. Molchanova, *et al., Ibid.*, **33**, № 5, 89-92 (1987).
4. V. G. Korpachev, S. P. Lysenkov, and L. Z. Tel', *Pat. Fiziol.*, № 3, 78-80 (1982).
5. I. N. Nagibneva, O. M. Sidorkina, and T. M. Morozova, *Biokhimiya*, **54**, 1379-1385 (1989).
6. S. E. Nikulina, O. Yu. Krylova, T. M. Goncharenko, *et al., Vopr. Med. Khim.*, **35**, № 5, 103-108 (1989).
7. C. A. Collins and W. W. Wells, *J. Biol. Chem.*, **257**, 827-831 (1982).
8. B. F. Dickens, I. T. Mak, and W. B. Weglicki, *Mol. Cell. Biochem.*, **82**, 119-124 (1988).
9. K. Kawai, N. Saito, S. Xu, *et al., Maturation Phenomenon in Cerebral Ischemia*, Tokyo (1990), pp. 75-86.
10. L. V. Molchanova, S. E. Nikulina, T. N. Ivanova, *et al., Resuscitation*, **22**, 261-274 (1991).
11. T. Spector, *Analyt. Biochem.*, **86**, 142-146 (1978).
12. J. Waskiewicz, L. V. Molchanova, E. Walajtys-Rode, and U. Rafalowska, *Resuscitation*, **16**, 287-293 (1988).
13. M. Yoshinary, A. Taurog, and P. P. Krupp, *Endocrinology*, **117**, 580-590 (1985).