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PROTEIN-SYNTHESIZING FUNCTION OF THE LIVER IN RABBITS WITH EXPERIMENTAL MYOCARDIAL INFARCTION

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UDC 577.217.3:616.12.005.8

KEY WORDS: experimental myocardial infarction; cell-free protein-synthesizing system of the liver.

Myocardial infarction gives rise to profound functional and metabolic changes both in heart muscle and in other tissues [4]. Histochemical and biochemical investigations have shown that the most marked changes in the liver during myocardial infarction are observed in the first hours and days of the disease [2]. Disturbance of the protein-synthesizing function in this period is particularly interesting, because the liver plays an important role in the supply of necessary proteins to other organs and tissues and, in particular, to the blood serum. Meanwhile, the molecular mechanisms of the changes in protein biosynthesis observed in the liver in myocardial infarction have virtually not been studied.

The aim of the present investigation was to study the function of the cell-free protein-synthesizing system obtained from the liver of intact rabbits (control) and 6, 12, and 24 h after production of experimental myocardial infarction (EMI).

EXPERIMENTAL METHOD

Rabbits weighing 2.5-3.0 kg were used. The model of EMI consisted of occlusion of the left descending coronary artery [10]. Thoractomy was performed under thiopental anesthesia (40 mg/kg), using sterile instruments. The degree of ischemia was monitored electrocardiographically [10]. The serum albumin level was determined by polyacrylamide gel (PAG) disc electrophoresis [8]. The total serum protein level was determined by the method in [7]. To prepare the cell-free protein-synthesizing system the liver was homogenized in 2.5 volumes of 30 mM HEPES (pH 7.5), containing 0.25 M sucrose, 70 mM KCl, 5 mM magnesium acetate, 0.25 mM EDTA, and 2 mM dithiothreitol. The homogenate was centrifuged for 15 min at 30,000g (unpurified S-30 fraction). Part of this fraction was filtered through Sephadex G-25 gel to remove as much as possible of the endogenous low-molecular-weight compounds (purified S-30 fraction). The standard incubation mixture, in a volume of 100 µl, contained 30 mM HEPES, 0.5 mM ATP, 0.02 mM GTP, 10 mM creatine phosphate (CP), and 2 µg creatine phosphokinase (CPK), each of the unlabeled amino acids (except leucine) in a concentration of 0.02 mM, 0.02 mM [^{14}C]leucine, 5 mM magnesium acetate, 120 mM KCl, 2 mM dithiothreitol, and 1 optical unit (A_{260}-A_{320}) of the purified S-30 fraction. In the case of unpurified S-30 the incubation mixture contained the same components except ATP, GTP, CP, CPK, and unlabeled amino acids.

The kinetic tests showed that to obtain the maximal level of translation, the essential incubation time is 30 min at 37°C. In the case of determination of the velocity of this pro-

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 0007-4888/85/9901-0070$09.50 © 1985 Plenum Publishing Corporation

TABLE 1. Serum Albumin Concentration of Rabbits with EMI (M ± m; n = 9-10)

Parameter	Control	Duration of EMI, h				
		6	12	24	48	72
Albumin concn., mg/ml	37,1±2,6	31,1±3,3*	26,0±1,9	23,6±2,5	23,3±2,6	27,2±3,1*

Legend. Here and in Table 2 * indicates changes that are not statistically significant.

TABLE 2. Incorportion of [^{14}C]Leucine into TCA-Insoluble Translation Product of Endogenous mRNA of Cell-Free Protein-Synthesizing Systems (μmoles/optical unit of S-30) of Rabbit Liver (M ± m; n = 11-14)

Type of protein-synthesizing system	Incubation time, min	Control	Duration of EMI, h		
			6	12	24
Unpurified S-30 fraction	30	24,6±2,9	16,6±2,7	15,6±1,8	21,0±3,2*
Unpurified S-30 fraction with addition of 60 mM creatine phosphate	30	48,3±6,7	64,7±6,1	73,1±9,5	60,8±7,1
Purified S-30 fraction	2	13,6±1,6	18,2±1,8	21,6±2,2	19,0±2,1
	30	87,8±6,1	105,2±8,0	132,6±11,1	111,6±7,9

TABLE 3. Ratio of Products Sedimented by Immunoprecipitation with Antialbumin Serum and TCA (M ± m; n = 6-7)

Incubation time, min	Control	Duration of EMI, h		
		6	12	24
2	0,38±0,5	0,28±0,02	0,26±0,02	0,21±0,02
30	0,31±0,04	0,27±0,01	0,21±0,02	0,16±0,04

cess, the incubation time was 2 min. The reaction was stopped by the addition of 0.5 ml of 0.1 N KOH, and after 20 min of incubation at 37°C, the samples were treated with 0.5 ml of 10% TCA and kept on an ice bath for 1 h. The precipitates were transferred to millipore (Synpor, Czechoslovakia) filters and washed with 5% TCA. Radioactivity was determined in toluene scintillator on SL-40 (Intertechnique, France) and Delta 300 (The Netherlands) counters. Efficiency of translation was estimated as incorporation of [^{14}C]leucine into the TCA-insoluble product. The level of albumin synthesis in this system was determined by immunoprecipitation. Albumin was isolated from the rabbits' blood serum by preparative PAG electrophoresis [1]. Serum containing antibodies against albumin was obtained by immunizing rats in accordance with the scheme in [6]. The antibody titer in the serum was determined by Ouchterlony's test [9]. The equivalent precipitation point was determined as described previously [5]. Immunoprecipitation of albumin synthesized in the cell-free system was carried out as follows: 10 μl of albumin (0.25 mg/ml), 20 μl of 0.14 M NaCl, 20 μl of buffer containing 30 mM HEPES, 5 mM magnesium acetate, 120 mM KCl, 0.25 mM EDTA, and 50 μl of antiserum with a titer of 1:4 were added to a sample with a volume of 100 μl. The mixture was incubated for 1 h at 37°C and allowed to stand over night at 4°C. The immunoprecipitates were collected on Synpor millipore filters and washed thoroughly with 20 mM potassium-phosphate buffer, pH 7.5, containing 0.14 M NaCl and 0.1 mM leucine. Samples containing serum of unimmunized animals served as the control. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

During the first 3 days of EMI, starting from 6 h the serum albumin level fell, with a tendency to recover 72 h after the operation (Table 1).

Since albumin is a secretory protein and since it is mainly synthesized in the liver, there is reason to suppose that the hypoalbuminemia in EMI is due to a decrease in albumin biosynthesis by the liver cells. To test this hypothesis the velocity and level of incorporation of [^{14}C]leucine into the translation product of endogenous mRNA *in vitro* were studied. The cell-free protein-synthesizing system containing unpurified S-30 fraction reflects most closely the level of protein biosynthesis in the cytoplasm *in vivo*, for it utilizes its own

resources for protein biosynthesis. As Table 2 shows, the level of incorporation of [^{14}C]-leucine into the TCA-insoluble translation product in such a system fell considerable 6-12 h after the operation.

Meanwhile, after removal of the low-molecular-weight compounds from the S-30 fraction and addition of optimal concentrations of components of the energy-yielding mixture and amino acids, the protein-synthesizing capacity of the S-30 fraction isolated after 6 h of EMI was higher than in the control. The maximal increase was observed 12 h after occlusion of the coronary artery. Consequently, the protein-synthesizing apparatus of the liver cells preserves its activity on the whole during the first day of EMI. Since the intensity of energy metabolism both in heart muscle and in the liver is disturbed in EMI, it was necessary to test the hypothesis that the energy supply of the unpurified S-30 fraction of the liver in EMI was inadequate for optimal functioning of the protein-synthesizing system. Accordingly, the kinetics of incorporation of [^{14}C]leucine into the total translation product was studied in the presence of different concentrations of ATP, GTP, and CP. Only a sixfold increase in the CP concentration in the system was found to lead to a considerable increase in the level of synthesis of the TCA-insoluble product in the cell-free system of protein biosynthesis from the liver (Table 2). The total level of protein biosynthesis *in vitro* in systems from the liver of the control and experimental animals was thus determined more by the energy supply than by any decrease in activity of the protein-synthesizing apparatus.

Consequently, it was particularly interesting to discover how the level of albumin synthesis changed in EMI relative to the total level of translation of endogenous mRNA. Comparative determination of radioactivity of the translation product sedimented by immunoprecipitation with antialbumin serum and TCA showed that in EMI the velocity and level of synthesis of albumin in the purified S-30 fraction of the liver relative to total protein synthesized in the cell-free system declined (Table 3).

Analysis of the results shows that, despite the high level of total protein biosynthesis in a cell-free system containing purified S-30 fraction of the liver from rabbits with EMI, the fraction of albumin synthesized in it decreased. This phenomenon can be explained by the reduction in the fraction of membrane-bound ribosomes observed by the writers previously in the liver of animals after occlusion of the coronary artery [3]. On the whole, therefore, activity of the protein-synthesizing apparatus of the liver does not decline in EMI, but there in a redistribution of the levels of synthesis of individual proteins. In EMI, however, the protein-synthesizing capacity of the liver is evidently not fully realized because of a reduction in the energy resources of the cell.

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