in the synaptosomes only a passive transport system has been found [4, 11]. If the supply of glutamine to the synaptosomes is in fact not controlled by the Na^+ concentration in the external medium, the importance of other factors in the control of transport may be increased. In particular, some action of the combined addition of Ca^{++} and GABA to the medium on the glutamine supply is possible.

LITERATURE CITED

1. R. N. Glebov and G. N. Kryzhanovskii, Usp. Fiziol. Nauk, 6, 3 (1975).
2. S. V. Levin, Structural Changes in Cell Membranes [in Russian], Leningrad (1976).
3. A. I. Silakova, G. P. Trush, and A. Yavilyakova, Vopr. Med. Khimii, 8, 538 (1962).
4. R. J. Baldessarini and C. Yorke, J. Neurochem., 23, 839 (1974).
5. A. M. Benjamin and J. H. Quastel, J. Neurochem., 25, 197 (1975).
6. J. P. Bennett and S. H. Snyder, J. Neurochem., 21, 1533 (1973).
7. P. Gazzotti, Biochem. Biophys. Res. Commun., 67, 634 (1975).
8. F. Hajos, Brain Res., 93, 485 (1975).
9. W. J. Logan and S. H. Snyder, Brain Res., 42, 413 (1972).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
11. P. J. Roberts and P. Keen, Brain Res., 67, 352 (1974).
12. A. Vinogradov, A. Scarpa, and B. Chance, Arch. Biochem., 152, 646 (1972).

ACTION OF ACUTE CARDIAC ISCHEMIA ON ACTIVITY OF THE PROTEIN-SYNTHESIZING SYSTEM OF THE INNER MITOCHONDRIAL MEMBRANES OF THE MYOCARDIUM

L. A. Kopteva, L. I. Zhevalyukova, and V. I. Biryuzova

UDC 616.12-005.4-036.11-07: 616, 127-008.939.6-074

Acute cardiac ischemia caused by ligation of the descending branch of the left coronary artery leads to a sharp increase in the activity of the protein-synthesizing system of the inner mitochondrial membranes of the myocardium throughout the period of organization of the experimental infarct, as reflected in an increase in the synthesis of protein and RNA in the mitochondria. During development of the infarct considerable changes are observed in the ultrastructure of the inner mitochondrial membranes of the myocardium, the degree of which is directly dependent on the stage of development of the pathological process.

KEY WORDS: mitochondria of the heart; ischemia; protein and RNA synthesis; ultrastructure.

The writers showed previously that necrotic lesions of the heart have a marked effect on the activity of protein and RNA synthesis in the inner membranes of the mitochondria of the undamaged parts of the myocardium [2-4]. However, the question arose whether these results reflect a true effect of ischemia or whether they may be in part the result of the effect of the sympathomimetic drug isoproterenol, which was used to create the necrotic foci in the myocardium.

In the present investigation classical ischemia of the myocardium caused by ligation of the descending branch of the left coronary artery was used as the experimental model.

EXPERIMENTAL METHOD

Male Wistar rats weighing 250-280 g were used. Since the development of the experimental myocardial infarct in the animals continued for about three weeks, and since the typical picture of proliferation of con-

Institute of General Pathology and Pathophysiology, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. N. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 9, pp. 295-297, September, 1978. Original article submitted March 2, 1978.

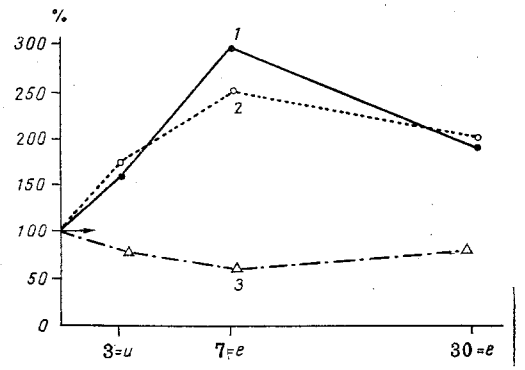

Fig. 1

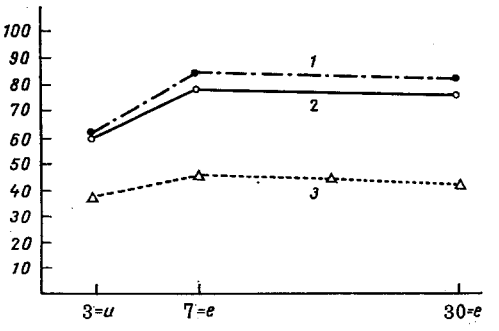

Fig. 2

Fig. 1. Protein synthesis in inert mitochondrial membranes at different stages of development of experimental infarct. Abscissa, time of investigation (in days); ordinate, changes, in %). 1) Inner membranes with matrix; 2) Inner membranes without matrix; 3) Number of destroyed mitochondria in tissue preparations (in %).

Fig. 2. RNA synthesis in inner mitochondrial membranes of myocardium. Abscissa, time of investigation (in days); ordinate, specific radioactivity, in cpm/mg RNA ($\times 10^3$).

nective tissue is found after the 30th day in the region of the organizing infarct [1, 6], the times chosen for the investigation (3rd, 7th, and 30th days) lay within the stage of organization of the infarct.

On the 3rd and 30th days electrocardiography was carried out on the animals. At the times of testing the animals were decapitated, the heart was removed, and the necrotic area not to be used in the experiments was excised. The mitochondria and their inner membranes were isolated as described previously [4, 6]. The purity of the preparations was verified electron-microscopically. The rate of uptake of radioactive label into proteins and RNA in the mitochondrial membrane was determined in vitro. Chlorella protein hydrolyzate-^{14}C with specific radioactivity of 1000 mCi/mmole was incorporated into the proteins of the mitochondrial membranes, and the final protein concentration in the sample was 4-6 mg.

RNA synthesis in the mitochondrial membranes was judged from the incorporation of UTP-^{3}H with a specific radioactivity of 55 mCi/mmole (5 μCi/mmole per sample). The final protein concentration in the sample was 2-4 mg. After incubation (30 min, 37°C) the reaction was stopped with 20% TCA. The residues were washed on membrane filters (1.5μ, Czechoslovakia). Radioactivity was counted in an Intertechnique L-30 scintillation counter (France). Protein was determined by the method of Jacobson and Lodish [7].

EXPERIMENTAL RESULTS

Clear changes typical of an ischemic heart lesion, expressed as the appearance of a pathological Q wave in lead I, elevation of the ST segment above the isoelectric line, and the appearance of a negative T wave, were observed on the ECG obtained three days after the appearance of the infarct. After 30 days, i.e., when the formation of the infarct was virtually complete, the Q wave was deepened, the ST segment remained above the isoelectric line, and the T wave was smoothed. Consequently, even though the 30th day is regarded as the period of final formation of the necrotic focus, areas of myocardium in the zone next to the infarct and areas lying a considerable distance away from the pathological focus continued to be in a state of acute ischemia.

The inner mitochondrial membranes were investigated both together with the matrix and without it [3, 4].

In acute ischemia a marked increase in protein synthesis was found in the inner mitochondrial membranes of the myocardium depending on the stage of development of the pathological process (Fig. 1). On the seventh day after the onset of acute ischemia the specific radioactivity (SR) of proteins of the inner membranes reached a maximum, an increase of almost 200%. By the 30th day SR had fallen again, but was still high compared with SR of the intact animals. The difference in protein-synthesizing activity of the mitochondrial membranes observed on the seventh day after the onset of infarction may perhaps have been due to the fact that the most critical situation arose in the mitochondria at that stage of development of the pathological process, and in order to overcome it, it was necessary to involve certain matrix proteins, activation of which increases the powers of compensation of the myocardial mitochondria, in the process of synthesis in the mitochondria.

A similar trend was found with RNA synthesis in the mitochondrial membranes. The maximal values of SR of RNA in the mitochondrial membranes occurred on the seventh day of formation of the experimental

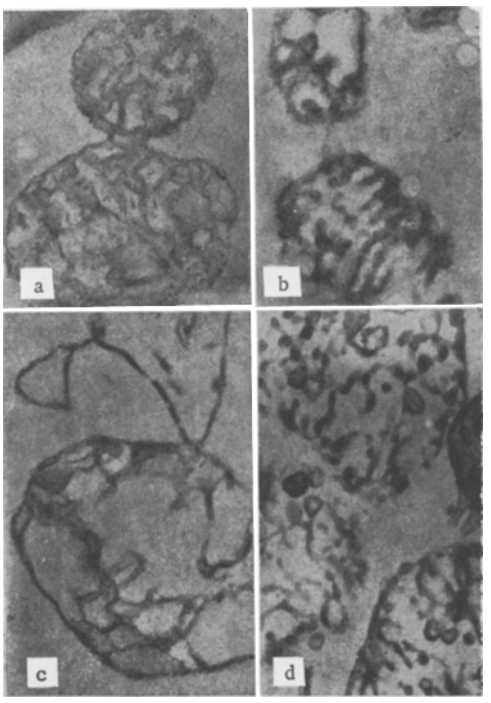

Fig. 3. Ultrastructure of inner membranes of myocardial mitochondria during formation of experimental infarct, 25,000×. a) In intact animals; b) after third day of ischemia; c) seventh day, d) 30th day of ischemia.

infarct (Fig. 2). The virtual absence of differences in the intensity of incorporation of UTP-^{3}H into the test fractions evidently indicated that RNA synthesis in the mitochondria takes place only on their inner membranes.

The number of damaged mitochondria in the tissue preparations at different stages of development of acute ischemia is shown in Fig. 3. All 100% of the mitochondria in the intact animals were of normal structure and were undamaged, on the 3rd day the number of damaged mitochondria was 19%, on the 7th day 34%, and by the 30th day the mitochondrial apparatus was practically fully restored and the number of damaged organelles was now only 9%.

The results indicate that acute ischemia leads to activation of the protein-synthesizing system of the myocardial cell; this activation develops along the lines of an initial increase in protein synthesis in the mitochondria, leading to restoration and reproduction of the organelles; in this way the energy deficiency is partly overcome and the powers of adaptation of the myocardial cell are increased. Electron-microscopic investigation showed that on the third day after the onset of experimental infarction fragmentation and partial lysis of the inner mitochondrial membranes were observed (the matrix was transparent and considerably swollen). By the seventh day the inner membranes were swollen even more and showed considerable maceration, their triplet structure was poorly defined. By the 30th day the swelling of the membranes was reduced, they were less macerated, some cristae were detached, and the fraction of inner membranes consisted chiefly of vesicles.

Restoration of the normal mitochondrial population in the course of adaptation to the disease evidently took place on account of an increase in the reproductive activity of mitochondria which still remained in the normal state [5]. The possibility cannot be ruled out that at the ultrastructural level in vivo also the cells are able to compensate for the loss of function through the activation of reserve organelles. An example of this is given by the present investigation, in which the energy deficiency arising in acute ischemia activated the protein-synthesizing system of the mitochondria, thereby leading to reproduction of the organelles and maintaining the energy balance of the cell.

LITERATURE CITED

1. A. V. Vinogradov, Pat. Fiziol., No. 1, 15 (1962).
2. L. A. Kopteva and V. I. Biryuzova, Byull. Éksp. Biol. Med., No. 7, 19 (1968).
3. L. A. Kopteva, L. I. Zhevalyukova, et al., Byull. Éksp. Biol. Med., No. 12, 90 (1975).
4. L. A. Kopteva and L. I. Zhevalyukova, Byull. Éksp. Biol. Med., No. 11, 19 (1977).
5. K. S. Mitin, Electron-Microscopic Analysis of Changes in the Heart in Infarction [in Russian], Moscow (1974).
6. C. Hoppel and C. Cooper, Arch. Biochem., 135, 17 (1969).
7. A. Jacobson and H. Lodish, Analyt. Biochem., 54, 513 (1973).

EFFECT OF NEUROGENIC INJURY TO THE HEART ON ITS GLUCOSE-6-PHOSPHATE DEHYDROGENASE CONTENT

N. A. Novikova

UDC 616.127-008.931-02:616.8-092.9

Neurogenic damage to the rat heart muscle by electrical stimulation of the arch of the aorta leads to the development of a deficiency of tissue noradrenalin (NA), the mediator of the sympathetic nervous system. This is accompanied by a marked increase in glucose-6-phosphate dehydrogenase (G6PD) activity. Preliminary administration of actinomycin D, an inhibitor of RNA synthesis, completely prevents the increase in G6PD activity in the heart muscle tissue following neurogenic injury. The results indicate the more rapid induction of this enzyme in the tissue of the myocardium after neurogenic injury, which correlate with changes in the tissue NA balance.

KEY WORDS: neurogenic injuries; heart muscle; noradrenalin; glucose-6-phosphate dehydrogenase; actinomycin D.

Previous investigations have shown that destruction of the tissue of the myocardium through its neurogenic injury is preceded by metabolic disturbances [1, 4, 5], based on changes in the velocity of enzyme reactions [2, 3, 7]. Simultaneously with disturbance of the tissue noradrenalin balance, on account of exhaustion of its reserves, a marked increase was found in the activity of hexokinase and lactate dehydrogenase, with a consequent increase in the rate of glycolysis, and also in glucose-6-phosphate dehydrogenase (G6PD) activity, limiting the pentose phosphate metabolic pathway [37]. However, the mechanisms of the changes in enzyme activity in the heart muscle associated with neurogenic lesions have not yet been explained. It was accordingly decided to study changes in G6PD activity in the soluble fraction of heart muscle after neurogenic injury with the aid of actinomycin D, an inhibitor of protein synthesis.

EXPERIMENTAL METHOD

Experiments were carried out on 36 male albino rats weighing 180-200 g. Neurogenic damage to the myocardium was caused by electrical stimulation of the arch of the aorta [1]. The series of investigations was undertaken on four groups of animals: 1) 10 intact rats (control); 2) 5 rats undergoing a mock operation, the operative control; 3) 10 rats subjected to electrical stimulation of the arch of the aorta for 3 h on the second day after the operation; 4) 11 rats receiving an intraperitoneal injection of actinomycin D in a dose of 100 μg per rat before electrical stimulation.

G6PD activity in the soluble fraction of heart muscle tissue (20,000 g, 40 min) was determined spectrophotometrically at 340 nm, 24 h after stimulation of the arch of the aorta, from the rate of reduction of added

Laboratory of Experimental Pharmacology, Department of Pharmacology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR S. V. Anishkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 9, pp. 297-298, September, 1978. Original article submitted November 15, 1977.

