EFFECT OF A POLYPEPTIDE PREPARATION ON THE STATE OF MYOCARDIAL CELL ENERGY METABOLISM DURING HYPOXIA AND ISCHEMIA

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UDC 616.127-005.4-07:616.127-018.1-008.9

KEY WORDS: ischemia; hypoxia; energy metabolism

The treatment of myocardial infarction (MI), which is aimed at restoring the blood flow, can prevent death of cells located in the zone of hypoxia and ischemia. However, the efficacy of thrombolytic treatment in relation to survival of cardiomyocytes is limited by the fact that the cells in the zone deprived of its blood flow can survive only for a short time. To lengthen the period of effective use of thrombolytic treatment, substances capable of delaying death of cells exposed to hypoxia and ischemia can be used in the early period of MI.

It was reported in previous publications that a polypeptide preparation obtained from the heart (cordialin) delays widening of the boundaries of the zone of necrosis in rats with coronary occlusion [2, 3].

The aim of the present investigation was to study the effect of cordialin on the state of cardiomyocytes kept under conditions of hypoxia and ischemia.

EXPERIMENTAL METHOD

Experiments were carried out on 100 noninbred albino rats weighing 160-180 g. A model of acute myocardial ischemia was created by ligation of the left coronary artery. The lactate concentration was determined by means of kits from "Boehringer" and the glucose concentration by "Bio-La-Test" kits in the blood 6 and 24 h after coronary occlusion. The glycogen concentration in the myocardium at these same times was determined by the anthrone method, and ATP by kits from "Boehringer." In a separate series of experiments, cardiomyocytes were isolated by the method in [10]. Oxygen consumption was recorded polarographically by means of a closed Clark's electrode with cell volume of 1 ml at 30°C. The protein concentration was determined by Lowry's method. Respiration of the cardiomyoctyes was recorded under both normoxic and hypoxic conditions, created in vitro. The protein content in the polarographic cell was 0.4-0.6 mg. The heart cells were oxygenated by saturation of the cardiomyocyte suspension with carbogen in a test tube. Cordialin was used in a concentration of 10^{-7} g/ml. The respiration rate of the cardiomyocytes was expressed in nanogram-atoms O_2/min/mg protein. Succinate was used in a concentration of 0.1 mM and NADH in a concentration of 0.5 mM.

EXPERIMENTAL RESULTS

The increased blood glucose level, a sharp increase in the lactate concentration, and a threefold decrease in the glycogen concentration in the myocardium of rats with experimental MI (Table 1) indicate that cells kept under conditions of hypoxia and ischemia change to ATP production by activation of glycolysis. Since the efficiency of glycolysis is low

Research Institute of Cardiology, Tomsk Scientific Center, Academy of Medical Sciences of the USSR. "Tsitomed" State Medico-Biological Research and Production Complex, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR R. S. Karpov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 7, pp. 24-27, July, 1991. Original article submitted April 26, 1990.

 0007-4888/91/0007-0926$12.50

TABLE 1. Biochemical Parameters in Rats with Myocardial Ischemia

Parameter	Intact rats	Solution of cordialin in physiological saline			
		6 h	24 h	6 h	24 h
Glucose, mmoles/liter	2,91±0,12	4,77±0,33*	4,04±0,15*	3,18±0,10**	3,06±0,13**
Lactate, mmoles/liter	1,44±0,7	4,03±0,26*	2,01±0,05*	1,31±0,14**	1,50±0,07**
Glycogen in heart, g/kg tissue	1,35±0,13	0,41±0,06*	0,46±0,07*	0,98±0,13***	1,14±0,17**

Legend. *$p < 0.05$ compared with intact rats, **$p < 0.05$ compared with rats with coronary occlusion and receiving physiological saline.

compared with aerobic energy production, utilization of ATP exceeds its production, as a result of which the ATP concentration in the tissue falls sharply [5]. For instance, whereas the ATP concentration in the myocardium of intact rats was 4.08 ± 0.18 μmoles/g tissue, 24 h after coronary occlusion it was 2.24 ± 0.30 μmoles.

Administration of cordialin to animals with MI led to normalization of their myocardial glycogen concentration and blood glucose and lactate levels (Table 1). However, inhibition of glycolysis did not lead to a fall in the ATP concentration in the myocardium. In animals not receiving cordialin the ATP concentration in the myocardium was 3.30 ± 0.20 μmoles/g tissue. It was reported previously that many glycogen granules can be seen in the sarcoplasm of cells of the peri-infarct zone in animals receiving cordialin, only single lipid drops are present, the mitochondria, although slightly swollen, are densely packed with several parallel rows of distinctly outlined cristae, and the mean number of mitochondria, the number of cristae per mitochondrion, and the efficiency of mitochondrial energy production are all appreciably higher than in animals not so treated [3].

Thus the ability of cordialin to inhibit glycolysis, to protect ATP reserves, and to prevent destruction of the mitochondria, by maintaining their energy efficiency, i.e., to normalize the bioenergetics of the cardiomyocytes under ischemic conditions, may be linked in some definite manner with the possible effect of cordialin on oxidative phosphorylation processes in the cardiomyocytes.

A characteristic feature of myocardial cell damage in hypoxia and ischemia is disturbance of the respiratory function of the mitochondria. We know that oxygen deficiency stimulates processes of reduction of pyridine-nucleotides, intensifies synthesis and oxidation of succinic acid, and leads to hyperactivation of succinate dehydrogenase (SDH) [8]. Under ordinary conditions succinate penetrates into the cell with difficulty and is oxidized slowly [1]. The coefficient of stimulation of respiraton (CSR) on this substrate does not exceed 1.2-1.5. On the addition of succinate to hypoxic cells, respiration is stimulated almost sixfold relative to the endogenous level of oxygen consumption (Fig. 1). This strong stimulation of respiration of the cardiomyocytes arises for two reasons. First, in hypoxia the permeability of the plasma and mitochondrial membranes evidently is increased, so that succinate can enter the cells, and second, SDH, which oxidizes succinate, is activated [8].

Addition of succinate and NADH to oxygenated cells does not cause any significant increase in CSR (Fig. 2). Like succinate, NADH penetrates with difficulty into the cell under normal conditions [9], but in hypoxia, even despite increased permeability of cell membranes, by contrast with succinate it virtually is not oxidized [1].

Hyperactive oxidation of succinic acid leads to exhaustion of substrates and to further uncoupling of oxidative phosphorylation [4, 7]. Inhibition of oxidation of succinic acid therefore becomes advantageous during the energy deficiency which develops in the tissue during profound ischemia. Normalization of succinate oxidation was observed when carbogen was blown through the cell suspension or cordialin was added. For instance, the addition of cordialin to a suspension of cardiomyocytes oxidizing succinate under hypoxic conditions led to reduction of CSR from 5.76 to 1.58, but blowing carbogen through the suspension reduced this parameter to 2.02. The presence of cordialin in a suspension of oxygenated succinate-oxidizing cells does not change the oxygen consumption relative to the level of endogenous respiration (Fig. 2). Consequently, cordialin, in the concentration tested, while not affecting endogenous respiration, inhibits respiration stimulated by succinate. On addition of a second amount of succinate, despite the presence of cordialin in the medium, activation of cardiomyocyte respiration is observed, although admittedly, not to the same degree as after the first addition (Fig. 1).

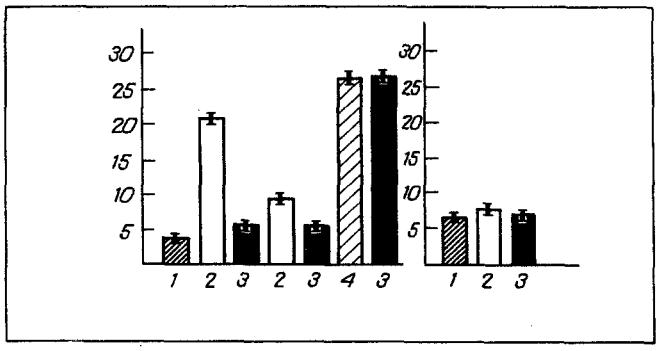

Fig. 1 Fig. 2

Fig. 1. Effect of cordialin on respiration of cardiomyocytes during hypoxia. Here and in Fig. 2: 1) endogenous respiration; 2) succinate (0.1 mM); 3) cordialin (10^{-7} g/ml); 4) NADH (0.5 mM). Ordinate, rate of O_2 consumption (in ng-atoms O_2/min/mg protein).

Fig. 2. Effect of oxygenation by carbogen on respiration of cardiomyocytes.

When analyzing these results relating to the effect of cordialin on respiration of a cardiomyocyte suspension, it can be tentatively suggested that the decrease in the intensity of oxidation of succinic acid is evidently not the result of a membrane-stabilizing effect, for the strongest suppressive action on respiration of cardiomyocytes by cordialin occurred after the hypoxic cells had already oxidized succinate actively. The point of application of cordialin is probably oxidation of succinic acid, proceeding inside the cell.

This view may be confirmed by data on the process of respiration of cardiomyocytes kept under hypoxic conditions, when NADH is used as the substrate (Fig. 1). Addition of this substrate against the background of cordialin caused considerable activation of cardiomyocyte respiration. Consequently cordialin, while not changing membrane permeability, activates oxidation of NADH, and subsequent addition of cordialin does not change CSR.

The results of investigations conducted on a model of a cardiomyocyte suspension provides a partial explanation of the mechanisms of the positive action of cordialin on models in vivo. Since reproduction of ischemia by ligation of the coronary artery causes uncoupling of oxidative phosphorylation in the mitochondria of cells kept under hypoxic conditions, inside the cells NADH accumulates (and is not oxidized) synthesis and oxidation of succinic acid are stimulated, and SDH is activated [1, 8]. Cordialin, which converts the respiration of cells exposed to hypoxia and ischemia from oxidation of FAD-dependent succinate to oxidation of the energetically more advantageous NAD-dependent substrates [9], thereby optimizes oxidative phosphorylation processes in the mitochondria. As a result, injury to the mitochondria and their inactivation are prevented. Injury to mitochondria and lowering of their energy-producing efficiency lead to inhibition of β-oxidation of fatty acids and of the tricarboxylic acid cycle which, in turn, leads to accumulation of lipid inclusions in the intracellular space. The absence of lipid drops in animals receiving cordialin and also high values of their myocardial ATP concentration are evidence of high activity of energy metabolism in these cells. Inhibition of glycolysis by cordialin may perhaps be the result of the fact that there is no longer any need to activate this process, and ultimately excessive accumulation of lactate and acidification of the medium are prevented in cells of the peri-infarct zone, the length of survival of the cells is increased, and their functional activity preserved.

Thus the results can provide a theoretical basis for the use of cordialin in the treatment of myocardial diseases accompanied by hypoxia and ischemic states. Cordialin can be used to treat the acute stage of MI and to prolong courses of thrombolytic treatment, and for the treatment of ischemic heart disease, angina, and other pathological conditions in which the oxygen supply to myocardial cells is disturbed.

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USE OF THE NEW MUSCARINIC CHOLINOLYTIC KG-62 TO CORRECT THE COURSE OF EXPERIMENTAL GASTRIC ULCER

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UDC 616.33-002.44-085.217.34-036.8-092.9-07

KEY WORDS: muscarinic cholinolytic KG-62; gastric ulcer in rats.

Muscarinic cholinolytics, which inhibit the acid-forming function of the stomach and restore its normal motor activity, are widely used in therapeutic schedules aimed at correcting gastric function in patients with peptic ulcer [5]. One widely used drug is metacin (oxyphenonium bromide), which, because of its chemical structure, has an exclusively peripheral muscarinolytic action, thereby ensuring absence of many side effects A series of iodomethylates of oxyalkylphosphinic acids with muscarinolytic action has been synthesized at the N. A Nesmeyanov Institute of Organoelementary Compounds, Academy of Sciences of the USSR [2]. One of the most active of these compounds has proved to be the substance conventionally named KG-62. Our investigations on animals showed that KG-62 in fact possesses a muscarinic cholinolytic action similar to that of metacin [3, 4].

The aim of this investigation was to assess the efficacy of KG-62 on the course of experimental gastric ulcer.

EXPERIMENTAL METHOD

Experiments were carried out on 46 noninbred rats weighing 160-200 g, anesthetized with hexobarbital (70 mg/kg). An experimental ulcer was produced with glacial acetic acid [11], for this model most completely reproduces the morpho-

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 7, pp. 27-30, July, 1991. Original article submitted July 5, 1990.