EFFECT OF RIBOXINE ON MYOCARDIAL METABOLISM IN HIGH-ALTITUDE HYPOXIA

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A leading factor in the complex chain of adaptive reactions of the myocardium under hypoxic conditions is the development of metabolic acidosis and, consequently, of structural and functional transformation of the heart muscle. The search for possible ways of influencing metabolism in heart tissue by means of drugs, in order to increase its resistance to oxygen lack, is therefore very important. Considerable interest in this plane is being shown in the biosynthetic preparation riboxine (Inosine-F), a nucleoside which can potentiate oxidation–reduction processes and nucleic acid synthesis in heart muscle in certain diseases of the cardiovascular system and, in particular, in myocardial infarction [1-3, 5].

It was accordingly decided to study the effect of riboxine on some structural and metabolic processes in the myocardium during prolonged exposure of animals to high-altitude hypoxia, and the details are given below.

EXPERIMENTAL METHOD

Experiments were carried out on 72 noninbred rats weighing 150-200 g. The control group consisted of 18 rats kept under ordinary animal house conditions; 12 animals of group 1 were given riboxine in a dose of 0.03 mg/g body weight daily by gastric tube; 18 rats of group 2 were kept in a pressure chamber at an "altitude" of 6000 m for 6 h daily; the 24 rats of group 3 were given riboxine in the same dose as the animals of group 1, 30 min before each "ascent" in the pressure chamber. The rats were decapitated after 7, 15, and 30 days. Morphometric, histochemical and electron-microscopic methods of investigation were used. The body weight of the animals was determined and the heart weighed separately (by Müller's method in Il'in's modification); the area of cross section of the cardiomyocytes was studied by direct microplanimetry. Succinate dehydrogenase (SDH), cytochrome oxidase (CCO) by Nachlas'

TABLE 1. Enzyme Activity and Glycogen Concentration (in conventional units, data of cytophotometry) in Myocardium of Rats Treated with Riboxine (group 1), Exposed to Hypoxia in a Pressure Chamber (group 2), and Exposed to Both These Factors (group 3)

Time of investigation, days	Group of animals	SDH	CCO	NAD-diaphorase	ATPase	LDH	Glycogen
Control		63,5±1,52	65,6±1,31	70,5±1,43	55,1±1,91	31,9±1,46	42,3±1,45
7	1	47,3±1,44*	73,1±1,61*	73,6±1,54	57,4±1,43	69,9±1,73*	41,6±1,34
	2	41,7±1,96*	57,3±1,73*	43,9±1,73*	41,6±2,23*	56,5±1,70*	26,6±1,05*
	3	44,7±1,20	70,1±1,91**	72,0±1,60**	55,2±1,34**	67,2±1,57**	40,0±1,57**
15	1	42,0±1,32*	81,1±3,08*	68,0±0,89	66,0±0,13*	90,0±1,85*	46,0±3,15
	2	42,4±1,75*	53,7±1,20*	38,9±1,87*	52,9±1,77*	61,6±1,63*	21,2±1,68*
	3	56,3±1,63**	66,2±1,26**	68,5±1,51**	62,2±2,11**	60,3±1,85	43,8±1,57**
30	1	68,0±1,51*	61,0±1,55*	63,0±1,89*	66,0±2,15*	59,0±1,44*	33,0±0,81*
	2	50,6±1,31*	63,1±1,26	51,7±1,50*	42,0±1,50*	47,1±0,99*	34,9±3,81
	3	67,0±1,55**	91,3±2,28**	67,0±1,44**	65,8±1,70**	56,3±1,84**	36,0±2,82

Legend. *P < 0.02 compared with control; **P < 0.01 compared with group 2.

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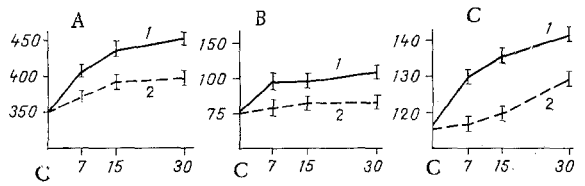

Fig. 1. Hypertrophy of the heart in rats kept in a pressure chamber at an "altitude" of 6000 m and receiving riboxine before exposure to hypoxia: a) weight of heart (in mg/100 g body weight); b) weight of myocardium of right ventricle (in mg/100 g body weight); c) area of cross section of cardiomyocytes (μm^2). C) Control. 1) "Altitude" 6000 m; 2) the same, administration of riboxine. Abscissa, time of investigation (days).

method, NAD-diaphorase, lactate dehydrogenase (LDH) by Gomori's method, and adenosine triphosphatase (ATPase) by Waschstein's method, were demonstrated histochemically in the myocardium. Glycogen was studied by Shabadash's method. Enzyme activity and glycogen were estimated quantitatively by cytophotometry, and the results were subjected to statistical analysis. Myocardial tissue for electron-microscopic study was fixed in a glutaraldehyde solution and postfixed in 1% OsO_4 solution, and embedded in a mixture of Epon and Araldite. Sections were examined in the JEM-100B microscope.

EXPERIMENTAL RESULTS

Changes in the ratios between the values obtained for enzyme activity and glycogen content were found in the myocardium of the rats of group 1 (Table 1). SDH activity on the 7th and 15th days of riboxine administration was reduced by 24.9 and 33.4%, respectively, but on the 30th day it rose to 2.7% above the initial level. CCO activity was increased on the 7th and 15th days by 11.3 and 23.5%, respectively, but on the 30th day it was reduced by 7.1% below the control value. Administration of riboxine caused no change in NAD-diaphorase activity during the first 7 days, but by the 15th and 30th days its activity was somewhat reduced (by 3.6 and 10.6%). Meanwhile there was a marked increase in LDH activity at all times of the experiment, to between 2 and 2.5 times the control level. Under the influence of riboxine, aerobic oxidation of lactic acid is evidently intensified in the rat myocardium, and, as a result of this, ATP resynthesis is increased. Other workers [5, 6], in the course of clinical-experimental studies in myocardial infarction, also found evidence of this. In the present experiments, it was confirmed by an increase in ATPase activity in the myocardium at all stages of the investigation (the increases on the 7th, 15th, and 30th days were by 3.2, 18.9, and 19.8%, respectively). The glycogen concentration in the myocardium of rats receiving riboxine for 15 days did not differ significantly from its initial level, but after 30 days it was reduced by 22.1%.

Keeping the animals under hypoxic conditions in the pressure chamber (group 2) was accompanied by considerable changes in metabolism in the heart muscle, especially in oxidative phosphorylation processes. This was shown by the fall in SDH, CCO, and NAD-diaphorase activity as early as on the 7th day of the experiment, by 34.4, 12.7, and 37.6%, respectively, i.e., activity of enzymes responsible for oxidation–reduction reactions in the Krebs' cycle and in the final electron transport chain was sharply reduced. As a compensatory reaction to maintain the energy balance of the heart during exposure to hypoxia, an increase was observed in the intensity of anaerobic glycolysis, reflected in an increase of 77.3% in LDH activity and a decrease of 37.2% in the glycogen concentration compared with its initial level. ATPase activity was reduced by 25.2% on the 7th day of the experiment. These relationships between the values of enzyme activity and glycogen concentration in the myocardium continued to be present with small fluctuations during a stay of 30 days by the rats at an "altitude" of 6000 m.

Preliminary administration of riboxine before the "ascent" of the rats in the pressure chamber (group 3) was accompanied by less-marked changes in SDH activity in their heart muscle. On the 7th and 15th days of the experiment its values were 7.2 and 32.7% higher than the corresponding levels in animals not receiving riboxine, but by the 30th day of the experiment they were the same as in the control. CCO and NAD-diaphorase activity in the myocardium of the rats of this group during the first two weeks of the experiment was almost indistinguishable from the control and was 23.2 and 76.1% higher than in the untreated animals. By the 30th day of the experiment differences in the levels of CCO and NAD-diaphorase activity in rats receiving (group 3) and not receiving (group 2) riboxine were 44.3 and 29.6%, respectively. LDH activity and the glycogen concentration in the myocardium of rats of this

group were higher at all times of the experiment than in untreated animals. This must be interpreted as the result of correction of the phenomena of acidosis in the myocardium by riboxine and improvement of the energy balance of the cell under its influence [2, 7]. This conclusion is confirmed by the better state of preservation of myocardial ATPase activity in rats receiving riboxine before exposure to hypoxia.

Electron-microscopic investigation of the myocardium of rats exposed to hypoxia in the pressure chamber (group 2) revealed considerable changes in the intracellular organelles of the cardiomyocytes and, in particular, in the energy-forming structures — mitochondria (swelling, vacuolation, translucence of the matrix, partial or total destruction of cristae). Separation, contractures, and lysis of myofibrils and a reduction in the number of cytogranules also were observed.

These changes in the ultrastructure of the muscle cells in the myocardium of rats receiving riboxine and exposed to hypoxia in the pressure chamber were less marked and were focal in character. Meanwhile, many areas of the myocardium were observed to have a normal ultrastructure and a high content of ribonucleic granules and glycogen.

Morphometric investigations (Fig. 1) showed that a less-marked degree of hypertrophy of the heart and of the right ventricular myocardium developed in animals exposed to high-altitude hypoxia and receiving riboxine, evidence of the beneficial effect of this substance on the supply of energy and structural materials for the processes of hyperfunction and hypertrophy of the heart muscle. It can be concluded from these results that administration of riboxine can be recommended as a prophylactic and therapeutic measure capable of reducing the harmful action of high-altitude hypoxia on the myocardium.

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