EFFECT OF NONACHLAZINE ON ATP, NAD, AND NADH CONTENT AND TRANSAMINASE ACTIVITY IN THE HEART OF CATS WITH MYOCARDIAL INFARCTION

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Myocardial infarction is known to give rise to considerable changes in oxidative and energy metabolism, accompanied by inhibition of the contractile function of the heart. It has also been shown that because of necrotic changes and depression of energy metabolism in the myocardium in ischemia, permeability of the cell membranes is disturbed [2]. These factors cause various enzymes to be transported from heart muscle cells into the blood stream. Changes in the activity of some of them, especially transaminases, constitute a highly sensitive and specific test for the presence and for tracing the dynamics of a myocardial infarct [2].

It was shown previously that nonachlazine improves the functional state of an ischemic focus in the myocardium, and so improves its contractile function and oxygen reserves [1, 6, 11]. There is also much clinical evidence of the high efficacy of nonachlazine in the treatment of ischemic heart disease [4, 5, 13]. However, there is no information in the literature on the effect of nonachlazine on biochemical parameters during the development of myocardial infarction.

It was accordingly decided to study the effect of nonachlazine on transaminase activity as an indicator of the efficacy of the drug in the treatment of myocardial infarction and also on the content of ATP, NAD, and NADH, indicators of energy and oxidative metabolism in the myocardium.

EXPERIMENTAL METHOD

Experiments were carried out on waking cats weighing 2.5-3.5 kg. Under pentobarbital anesthesia the descending branch of the left coronary artery was ligated in its middle third. The experiments began 2 h after the operation. Nonachlazine was injected intravenously in a dose of 6 mg/kg 3 times a day for the 2 days after ligation. On the assumption that the maximal decrease in aspartate- and alanine-transaminase activity and also the most marked changes in the system of nicotinamide coenzymes and ATP are observed during the first 48 h after the development of a myocardial infarct [10], the effect of nonachlazine on the above-mentioned biochemical parameters was studied 48 h after development of the myocardial infarct. Tissue for testing — the left ventricle — was quickly cut into small pieces with scissors in the cold, and part of the material was frozen in liquid nitrogen for subsequent determination of ATP, NAD, and NADH. The rest was homogenized in the cold in 0.1 M K-phosphate buffer, pH 7.8, and centrifuged at 12,000g for 15 min. The supernatant was the source of enzymes. The ATP content was determined with the aid of kits from "Boehringer," NAD by the method in [8], and NADH as in [15]. Activity of aspartate and alanine transaminases was investigated by the method in [14].

EXPERIMENTAL RESULTS

The experiments showed that on the 3rd day after development of a myocardial infarct the

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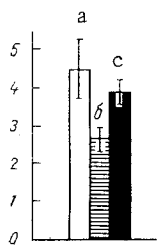

Fig. 1

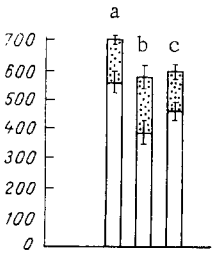

Fig. 2

Fig. 1. Effect of nonachlazine on ATP content (in μmoles/g wet weight of tissue) in myocardium of cat on 3rd day after ligation of coronary artery. a) Control, b) myocardial infarction, c) myocardial infarction + nonachlazine.

Fig. 2. Effect of nonachlazine on NAD and NADH content (in μg/g wet weight of tissue) in myocardium of cat on 3rd day after ligation of coronary artery. Unshaded parts of columns denote NAD, shaded parts NADH content. Remainder of legend as to Fig. 1.

TABLE 1. Changes in Aspartate and Alanine Transaminase Activity (in μmoles pyruvic acid/mg protein/g) after Administration of Nonachlazine Myocardial Infarcts (mean results of five experiments; M ± m)

Experimental conditions	Aspartate transaminase	Alanine transaminase
Control	7,74±0,127	3,72±0,471
Infarcts	5,69±0,478	2,03±0,477
Infarcts + nonachlazine	7,07±0,690	3,98±0,165

ATP content in the muscle of left ventricle of the cats was reduced by 42% (Fig. 1). Nonachlazine almost completely prevented the fall in the ATP content observed on the 3rd day after ligation of the coronary artery in the untreated animals. A study of the effect of nonachlazine on the NAD and NADH content showed that the content of the oxidized form in the control animals was reduced on the 3rd day after ligation of the coronary artery (Fig. 2), the content of the reduced form was increased, and there was a corresponding decrease in the NAD/NADH ratio and in the total NAD + NADH. Unlike in the control animals, the content of oxidized form in fat treated with nonachlazine was increased, and that of the reduced form was reduced. This led to normalization of the NAD/NADH ratio (Fig. 2). Under these circumstances the total NAD + NADH remained low.

The experiments to study transaminase activity revealed that aspartate and alanine transaminase activity in the myocardium was reduced on the 3rd day after ligation of the coronary artery (Table 1). Nonachlazine completely prevented the fall in acidity of these transaminases, reflecting the favorable effect of the drugs on the ischemized myocardium.

The data given above are evidence that on the 3rd day of development of a myocardial infarct nonachlazine, when administered repeatedly, increased the ATP content and normalized the NAD/NADH ratio and transaminase activity.

In myocardial infarction sharp changes are known to take place in all stages of the respiratory chain, and as a result the formation of high-energy compounds, especially ATP, is

disturbed. This leads to inhibition of the contractile function of the heart muscle. On the basis of these arguments and allowing for the fact that nonachlazine potentiates myocardial contractility during ischemia, it can be concluded that the increase in ATP content caused by this drug in animals with experimental myocardial infarction is connected with its ability to stimulate ATP synthesis. This conclusion is confirmed by the results of experiments [6, 7] which showed that nonachlazine stimulates oxidative phosphorylation in cardiac mitochondria. In those experiments nonachlazine, in a concentration of 0.05 M, almost completely restored the fundamental parameters of oxidative phosphorylation when disturbed as a result of ischemia. It is also pertinent to mention here the data of Kryzhanovskii et al. [3], who showed by an electron-microscopic method that nonachlazine accelerates normalization of the structure of the mitochondria in the zone surrounding the infarct, evidence of an improvement in its function.

To sum up the results described above it can be concluded that repeated administration of nonachlazine to animals with an experimental myocardial infarct considerably speeds up the normalization of metabolism in the ischemized region compared with the control, as reflected in the recovery of transaminase activity but under the influence of nonachlazine energy formation also is improved in the heart muscle when disturbed as a result of myocardial infarction. This view is confirmed by the data showing an increase in the ATP content and normalization of the NAD/NADH ratios in animals treated with nonachlazine. To judge from the fact that normalization of these biochemical parameters under conditions when the contractile function of the heart is strengthened can take place only when the oxygen supply is increased, these effects of nonachlazine must evidently be due to its ability to improve the blood supply to the ischemized region of the myocardium. This conclusion is supported by data obtained previously, showing the normalizing effect of nonachlazine on the lactate/pyruvate ratio in blood flowing from the ischemic focus [12] and the considerable fall in the lactate content in the ischemized myocardium [9] caused by nonachlazine.

It can be postulated on the basis of these results that nonachlazine may prove to be an effective drug in accelerating the normalization of metabolic processes in the acute state of myocardial infarction.

LITERATURE CITED

1. N. V. Kaverina, G. A. Markova, and G. G. Chichkanov, in: Nonachlazine in Clinical and Experimental Medicine [in Russian], Tbilisi (1976), p. 3.
2. B. F. Korovkin, Enzymes in the Diagnosis of Myocardial Infarction [in Russian], Leningrad (1965).
3. S. A. Kryzhanovskii, N. N. Kleimenova, and V. A. Arefolov, in: Current Problems in the Pharmacology of the Circulation [in Russian], Gor'kii (1980), p. 30.
4. V. G. Kukes, V. V. Buyanov, V. N. Semenov, et al., Kardiologiya, No. 4, 88 (1976).
5. V. I. Metelitsa, L. S. Matveeva, G. A. Borisova, et al., Kardiologiya, No. 7, 48 (1975).
6. V. V. Pichugin, in: New Cardiovascular Drugs [in Russian], Erevan (1979), p. 35.
7. K. M. Reznikov, N. V. Kaverina, and V. V. Alabovskii, in: New Cardiovascular Drugs [in Russian], Erevan (1979), p. 33.
8. S. E. Severin and L. A. Tseitlin, Vopr. Med. Khim., No. 3, 300 (1964).
9. A. I. Turilova, T. N. Azvolinskaya, and S. A. Kryzhanovskii, Byull. Éksp. Biol. Med., No. 7, 49 (1980).
10. T. V. Fetisova and R. A. Frol'kis, The Biochemistry of Myocardial Infarction [in Russian], Kiev (1976).
11. G. G. Chichkanov and A. K. Bogolepov, in: New Cardiovascular Drugs [in Russian], Erevan (1979), p. 29.
12. G. G. Chichkanov, A. K. Bogolepov, A. I. Turilova, et al., in: Theoretical and Clinical Problems in Molecular Cardiology [in Russian], Moscow (1978), p. 52.
13. A. P. Yurenev, V. B. Chumburidze, and O. Yu. At'kov, Klin. Med., No. 5, 50 (1977).
14. S. S. Reitman and S. Frankel, Am. J. Clin. Path., 29, 56 (1957).
15. V. Stollar, Biochim. Biophys. Acta, 44, 245 (1960).