EFFECT OF ATP AND COCARBOXYLASE ON ACTIVITY OF MALATE DEHYDROGENASE AND ITS ISOZYMES IN BLOOD SERUM AND MYOCARDIUM

UDC 612.128+612.173.1.015.1]:577.152.111].014.46:615.355

I. M. Nosova

Changes in the activity of malate dehydrogenase (MD) and its isozymes in the blood serum and heart muscle of rats were investigated during hypoxia and under the influence of ATP and cocarboxylase. An additional cathodic isozyme (MD_4) appeared in the blood serum during hypoxia. Under the influence of ATP and cocarboxylase the metabolic changes in the blood and myocardium induced by hypoxia did not correlate fully.

KEY WORDS: *malate dehydrogenase; isozymes; blood serum; heart; hypoxia.*

The study of the isozyme spectrum of malate dehydrogenase (MD) for diagnostic purposes has not proved very popular [4, 11]. However, reports have recently been published on changes in MD isozymes in several pathological states [1, 2, 7, 8, 10].

The object of the present investigation was to study the activity of MD and its isozymes in the blood serum and heart muscle of rats exposed to hypoxia and the effect of ATP and cocarboxylase on the MD isozyme spectrum in these tissues when disturbed by hypoxia. Guidance in the choice of ATP and cocarboxylase as correcting factors was obtained from the fact that both these substances are widely used in clinical practice for the treatment of disturbances of oxidative processes, for ATP is a source of energy and cocarboxylase is a coenzyme that participates in the glycolytic hydrolysis of carbohydrates.

EXPERIMENTAL METHOD

Experiments were carried out on 30 noninbred male rats weighing 120-130 g. Hypoxia was induced by keeping the animals for 30 min in an airtight vessel. Intact animals acted as the control. In the experiments of series I, the animals were killed after exposure to hypoxia for 30 min. In series II the rats were removed from the chamber after 30 min, ATP was injected intraperitoneally in a dose of 0.01 mg/g body weight, and the rats were killed 15 min later. In the experiments of series III, after exposure to hypoxia for 30 min the rats received an intraperitoneal injection of cocarboxylase in a dose of 0.02 mg/g, and they also were killed 15 min later. The blood serum and heart muscle were tested. MD activity was determined spectrophotometrically [3]. Protein was determined by Lowry's method [9]. The isozymes were separated by electrophoresis on agar [5]. They were determined quantitatively by Yurkov's method [6].

EXPERIMENTAL RESULTS AND DISCUSSION

Investigation of the activity of MD and its isozymes in the blood serum showed no

Laboratory of Biochemistry, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. A. Vishnevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 80, No. 8, pp. 48-50, August, 1975. Original article submitted June 17, 1974.

TABLE 1. Changes in Activity of MD and Its Isozymes in Blood Serum (M ± m)

Series of experiments	Total MD activity (in Bucher units units/mg protein)	MD isozymes (in %)			
		MD_1	MD_2	MD_3	MD_4
Control	4,17±0,57	13,37±1,24	12,23±0,99	68,95±2,44	—
Hypoxia for 30 min	3,65±0,41	13,41±0,38	13,94±1,19	57,77±2,05*	14,9±4,19
Hypoxia for 30 min+ ATP	6,2±0,38*	10,62±0,63	16,44±2,92	52,6±4,50*	20,72±2,57
Hypoxia for 30 min + cocarboxylase	3,23±0,16	18,24±2,05	31,94±1,67*	46,8±2,46*	

*P < 0.05 (compared with control).

TABLE 2. Changes in Activity of MD and Its Isozymes in Heart Muscle (M ± m)

Series of experiments	Total MD activity (in Bucher units/mg protein)	MD isozymes (in %)			
		MD_1	MD_2	MD_3	MD_4
Control	206,5±21,9	5,02±0,30	12,9±1,13	47,4±2,94	35,2±2,51
Hypoxia for 30 min	291,2±23,8*	3,90±0,70	6,9±0,77*	45,6±3,39	47,3±3,94*
Hypoxia for 30 min+ ATP	162,3±3,72	4,5±0,44	6,75±0,94*	43,15±1,64	45,8±2,16*
Hypoxia for 30 min+ cocarboxylase	228,8±10,6	4,4±0,34	8,1±1,09*	37,9±0,83*	49,3±1,16*

*P < 0.05 (compared with control).

change in the total activity after hypoxia for 30 min. However, the isozyme spectrum was modified: whereas normally as a rule 3 MD isozymes are present in the serum, after hypoxia an additional cathodic fraction (MD_4) appeared and the MD_3 activity was reduced (Table 1). MD_4 was found in only 2 of 16 intact rats. Administration of ATP led to an increase in the total MD activity, mainly on account of the cathodic isozyme MD_4. After administration of cocarboxylase, although the additional isozyme disappeared, marked discoordination was observed in the distribution of activity of the remaining isozymes: activity of MD_2 rose sharply but that of MD_3 fell.

In heart muscle (Table 2) a marked increase in total MD activity (by 42%) was observed after 30 min of hypoxia; considerable changes also took place in the myocardial isozyme spectrum: activity of MD_2 was reduced almost by half, whereas activity of MD_4 was increased by 34.3%. Injection of ATP reduced the increase in total MD activity produced in the myocardium by hypoxia; under these circumstances the MD_2 activity fell significantly — by 20%. Corresponding changes in the activity of this enzyme and its isozyme spectrum also were observed under the influence of cocarboxylase: total MD activity in the heart muscle returned to normal, the MD_2 level increased slightly, but did not reach normal, and MD_4 activity, just as after administration of ATP, remained at the same high level as during hypoxia, and the MD_3 activity fell by 20%.

The investigation of the effect of hypoxia on the state of the MD isozyme spectrum in the blood serum thus confirmed the appearance of a fourth cathodic isozyme. Since the cathodic fractions are known to be mitochondrial in origin [4], and since under normal conditions MD_4 is present in a small percentage of cases [1], its appearance in the serum may indicate destructive changes in the myocardial cells and it may be used to some extent as an indicator of cell destruction during hypoxia. When the simultaneous action of ATP and cocarboxylase on metabolic changes in the heart muscle and blood induced by hypoxia were studied, the corrective effect of these substances was incomplete. Besides some degree of normalizing action, these preparations

had an additional effect on the processes studied. The fact that the changes in the state of MD and its isozyme profile in the heart muscle under the action of the different substances were similar in direction and equivalent in value will be noted. In addition, no correlation was found between changes in the isozyme activity in the blood serum and myocardium. The changes in membrane permeability produced by hypoxia to allow the various isozymes to escape from the heart muscle evidently differed in degree.

LITERATURE CITED

1. T. Ya. Safonova, Yu. A. Yurkov, and I. P. Elizarova, "Malate dehydrogenase isozymes in the serum and erythrocytes in asphyxia neonatorum," Vopr. Okhr. Mat., No. 11, 15 (1970).
2. T. Ya. Safonova and Yu. A. Yurkov, "Effect of hypoxia on activity of malate dehydrogenase isozymes in newborn rabbits," Vopr. Med. Khim., No. 6, 631 (1972).
3. I. Todorov, Clinical Laboratory Investigations in Pediatrics [in Russian], Sofia (1960).
4. J. Wilkinson, Isoenzymes, Lippincott (1960).
5. Yu. A. Yurkov and V. V. Alatyrtsev, "An electrophoretic method of quantitative determination of lactate dehydrogenase isozymes on agar," Lab. Delo., No. 12, 705 (1966).
6. Yu. A. Yurkov, "A simple method of quantitative measurement of isozymes on the photoelectric colorimeter," Vopr. Med. Khim., No. 5, 547 (1968).
7. V. S. Yakushev, V. B. Slobodin, R. I. Lifshits, et al., "Changes in activity of malate dehydrogenase and its isozymes in the early periods of experimental burns," Vopr. Med. Khim., No. 5, 528 (1973).
8. P. Georgiew et al., "Changes in the isoenzymes of lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and nonspecific esterases in the dog with experimentally induced myocardial infarct," Dokl. Sel'skokhoz. Akad. (Sofia), 6, 67 (1973).
9. O. H. Lowry et al., "Protein measurement with the Folin phenol reagent," J. Biol. Chem., 193, 265 (1951).
10. B. Wieckowski and J. Gregorczyk, "Oceana przydatnosci diagnostycznej izoenzymogramog surowiczych dehydrogenazy jablszy nowej," Diagnost. Lab., 8, 247 (1972).
11. V. J. Yakulis, C. W. Gibson, and P. Heller, "Agar gel electrophoresis for the determination of isoenzymes of lactic and malic dehydrogenase," Am. J. Clin. Path., 38, 378 (1962).