# EFFECT OF GLUTAMIC ACID ON ACTIVITY OF GLUTAMATE DEHYDROGENASE AND $Mg^{++}$- AND DINITROPHENOL-DEPENDENT ADENOSINETRIPHOSPHATASES IN MITOCHONDRIA OF KIDNEYS, HEART, AND LIVER OF ALBINO RATS DURING ACUTE AND CHRONIC HYPOXIA

N. A. Glotov

UDC 616-008.922.1.04-085.272.6:547.466.64]-07:
616-008.931:577.158.429-074

The effect of glutamic acid on activity of glutamate dehydrogenase and of $Mg^{++}$- and dinitrophenol (DNP)-ATPases in the mitochondria of the liver, heart, and kidneys of albino rats was investigated during acute and chronic hypoxia. Acute hypoxia inhibits the activity of both ATPases in the mitochondria of these organs as well as glutamate dehydrogenase in the liver and kidneys. During adaptation to hypoxia, $Mg^{++}$- and DNP-dependent ATPases of the heart mitochondria and DNP-dependent ATPase of the liver mitochondria decreased their activity. However, the activity of glutamate dehydrogenase of the kidney mitochondria is increased. Under the influence of glutamic acid, in experiments with acute hypoxia the activity of glutamate dehydrogenase and of both ATPases in the mitochondria of the kidneys, heart, and liver increases. During adaptation of animals receiving glutamic acid to hypoxia, the activity of both ATPases and of glutamate dehydrogenase in the heart mitochondria, of glutamate dehydrogenase and of DNP-dependent ATPase in the liver mitochondria, and of $Mg^{++}$-dependent ATPase of the kidney mitochondria was increased.

This investigation is a continuation of previous work [1, 2] to study the mechanism of action of glutamic acid on aerobic energy-yielding oxidative processes during acute hypoxia and during adaptation to it.

## EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 180-250 g. Hypoxia was produced in a pressure chamber where, in the experiments with acute hypoxia, the air pressure was reduced to correspond to an altitude of 8000 m. The animals remained under these conditions for 1 h. Adaptation to hypoxia was produced by keeping the rats in a pressure chamber at an "altitude" of 6000 m for 6 h daily for 2 weeks. In the experiments with acute hypoxia sodium glutamate was injected subcutaneously, while during adaptation to hypoxia it was given daily by gastric tube in a dose of 1 mg/g body weight 30 min before the rats were placed in a pressure chamber. Glutamate dehydrogenase (1·4·1·2) activity was determined spectrophotometrically [4] and expressed in $\mu$moles/ml mitochondrial protein/min. ATPase (3·6·1·4) activity was determined from the increase in inorganic phosphorous during hydrolysis of ATP [5]. The incubation mixture (2 ml) contained: 3 mmoles ATP; 0.05 mmole tris buffer (pH 7.4); 0.1 mmole 2,4-dinitrophenol

Sverdlovsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 71, No. 5, pp. 61-64, May, 1971. Original article submitted July 8, 1970.

TABLE 1. Effect of Glutamic Acid on Activity of $Mg^{++}$- and DNP-dependent ATPases in Mitochondria of Liver, Heart, and Kidneys in Acute and Chronic Hypoxia

| Group No. | Exptl. conditions | Liver mitochondria | | Heart mitochondria | | Kidney mitochondria | |
|---|---|---|---|---|---|---|---|
| | | $Mg^{2+}$ | $Mg^{2+}$+ДНФ | $Mg^{2+}$ | $Mg^{2+}$+ДНФ | $Mg^{2+}$ | $Mg^{2+}$+ДНФ |
| 1 | Normal, physiological saline given | 4,3±0,3 (10) | 6,5±0,3 (9) | 13,1±0,6 (9) | 16,0±0,9 (10) | 7,2±0,4 (9) | 10,3±0,5 (9) |
| 2 | Acute hypoxia, physiological saline given | 3,4±0,2 80% $P<0,05$ (11) | 5,4±0,2 82% $P<0,01$ (12) | 8,1±0,3 62% $P<0,001$ (12) | 9,9±0,7 61,9% $P<0,001$ (12) | 5,9±0,3 81,7% $P<0,01$ (11) | 7,5±0,4 73% $P<0,01$ |
| 3 | Acute hypoxia, glutamic acid given | 4,3±0,3 124% $P<0,05$ (11) | 7,3±0,2 135% $P<0,001$ (10) | 11,9±0,5 132% $P<0,001$ (11) | 15,1±0,5 151% $P<0,001$ (11) | 7,7±0,4 131% $P<0,02$ (9) | 10,0±0,5 132% $P<0,01$ (10) |
| 4 | Normal, physiological saline given | 3,8±0,1 (8) | 7,4±0,4 (8) | 15,0±1,7 (8) | 18,4±2,0 (8) | 8,4±0,4 (8) | 11,8±0,6 (8) |
| 5 | Adaptation to hypoxia, physiological saline given | 3,3±0,1 85% $P>0,05$ (18) | 5,6±0,3 60% $P<0,001$ (19) | 8,5±0,5 56% $P<0,001$ (21) | 12,5±0,7 67% $P<0,01$ (21) | 8,8±0,5 104% $P>0,5$ (19) | 11,1±0,5 94% $P>0,5$ (17) |
| 6 | Adaptation to hypoxia, glutamic acid given | 3,8±0,3 115% $P>0,05$ (9) | 6,9±0,4 123% $P<0,01$ (12) | 10,6±0,7 124% $P<0,01$ (14) | 15,5±1,1 124% $P<0,05$ (12) | 12,1±0,5 137% $P<0,001$ (11) | 12,0±0,7 109% $P>0,5$ (14) |

Note. 1) Activity of enzymes expressed in μmoles phosphorus/mg mitochondrial protein/min; 2) number of experiments given in parentheses.

(DNP), and 3 mmoles $MgSO_4$. Inorganic phosphorus was determined by the Fiske-Subbarow method and mitochondrial protein by Lowry's method. Mitochondria were isolated from the organs by differential centrifiguation in 0.25 M sucrose solution, made up in 0.01 M tris buffer with 0.001 M EDTA (pH 7.4).

## EXPERIMENTAL RESULTS AND DISCUSSION

The results given in Table 1 (groups 1 and 2) show that keeping the animals for 1 h under hypoxic conditions inhibits the activity of both $Mg^{++}$- and DNP-dependent ATPases. Under acute hypoxic conditions, a decrease in the activity of $Mg^{++}$- and DNP-dependent ATPases was observed in the mitochondria of the liver, heart, and kidneys. During adaptation to hypoxia, on the other hand, this inhibition was detected only in the mitochondria of the heart for $Mg^{++}$- and DNP-dependent ATPases, and also for DNP-dependent ATPase in the mitochondria of the liver.

ATPase activity is regulated by the ATP:ADP ratio, a decrease which has an inhibitory effect on activity of the enzyme [8]. Since ADP accumulates in the tissues and the content of ATP is reduced in acute hypoxia [3, 6], this shift in content of the adenine nucleotides is evidently the reason for the decrease in activity of the ATPases detected in these experiments. During adaptation to hypoxia, the compensatory biochemical and physiological mechanisms of the organism help to restore energy metabolism in the liver and kidneys, thus restoring activity of the enzymes in these organs. In the heart muscle, which is subjected to intensive physiological loading during exposure to hypoxia, the energy-yielding biochemical oxidative processes cannot maintain the ATP:ADP ratio at a steady level, with the result that the ATPases of the heart mitochondria are inhibited.

Acute hypoxia affects glutamate dehydrogenase activity of the mitochondria of different organs differently (Table 2, groups 1 and 2). Activity of the enzyme when oxygen is deficient in the organism is re-

TABLE 2. Effect of Administration of Glutamic Acid on Glutamate Dehydrogenase Activity in Mitochondria of Liver, Heart, and Kidneys (in μmoles/mg mitochondrial protein/min)

| Group No. | Exptl. conditions | Liver mitochondria | Heart mitochondria | Kidney mitochondria |
|---|---|---|---|---|
| 1 | Normal, physiological saline given | 19,0±1,1 (7) | 7,5±0,5 (10) | 12,4±0,7 (10) |
| 2 | Acute hypoxia, physiological saline given | 15,0±1,3 (9)<br>−21%<br>$P<0,05$ | 7,8±0,5 (8)<br>+4%<br>$P>0,5$ | 10,8±0,7 (8)<br>−13%<br>$P<0,05$ |
| 3 | Acute hypoxia, glutamic acid given | 23,0±1,0 (9)<br>+53%<br>$P<0,001$ | 10,6±1,0 (8)<br>+35%<br>$P<0,01$ | 13,5±0,9<br>+25%<br>$P<0,05$ |
| 4 | Normal, physiological saline given | 16,0±1,5 (10) | 7,7±0,4 (17) | 12,1±0,7 (17) |
| 5 | Chronic hypoxia, physiological saline given | 14,1±0,6<br>−10%<br>$P>0,5$ (18) | 8,8±0,4<br>+14%<br>$P>0,05$ (20) | 14,5±0,9<br>+19%<br>$P<0,05$ (12) |
| 6 | Chronic hypoxia, glutamic acid given Liver mitochondria | 17,9±1,4<br>126%<br>$P<0,01$ (10) | 17,4±1,9<br>197%<br>$P<0,001$ (14) | 14,8±0,6<br>102%<br>$P>0,5$ (11) |

Note. 1) Two control groups of animals (1 and 4) are included in this table. This is because experiments of groups 1-3 and groups 4-6 were performed at different times of year. 2) Number of experiments given in parentheses.

duced in the mitochondria of the liver (−21%) and kidneys (−13%), while in the mitochondria of the heart muscle it remains unchanged. These differences in the changes in glutamate dehydrogenase activity are probably due not only to the specificity of the functions of these organs, but also to differences in the relative quantitative role of oxidative deamination in glutamic acid metabolism in them. In the heart mitochondria, for example, only about 10% of glutamic acid is metabolized with the participation of glutamate dehydrogenase [7], so that this enzyme evidently has no major role to play in heart muscle metabolism, and it does not change its activity significantly under the influence of acute hypoxia. Glutamic acid metabolism in the mitochondria of the liver and kidneys, on the other hand, is largely dependent on glutamate dehydrogenase, and requires oxidized forms of pyridine nucleotides (NAD and NADP), which are present mainly in the reduced form when there is a deficiency of oxygen. This fact, in all probability, is the reason for the lowered activity of glutamate dehydrogenase in the mitochondria of the liver and kidneys.

Whereas the glutamate dehydrogenase activity in the mitochondria of liver and kidneys falls in acute hypoxia, after adaptation to hypoxia (Table 2, groups 4 and 5) its activity in the mitochondria of the kidneys was 19% higher than in the control group of animals, while in the mitochondria of the liver it was close to the same level.

In the experiments with acute hypoxia, glutamic acid increased the activity of glutamate dehydrogenase (Table 2, groups 2 and 3) and of both ATPases in the mitochondria of the liver, heart, and kidneys (Table 1, groups 2 and 3). During adaptation to hypoxia, this amino acid stimulated activity of glutamate dehydrogenase (Table 2, groups 5 and 6) in the mitochondria of the heart and liver, of $Mg^{++}$- and DNP-dependent ATPases in the mitochondria of the heart, of DNP-dependent ATPase in the mitochondria of the liver, and of $Mg^{++}$-dependent ATPase in the mitochondria of the kidneys (Table 1, groups 5 and 6). The enzyme activity in mitochondria isolated from the organs of rats exposed to acute and chronic hypoxia and receiving glutamic acid thus approached that observed in the intact animals.

The increase in glutamate dehydrogenase activity under acute and chronic hypoxic conditions can evidently be explained by the fact that on administration of glutamic acid the increase in its concentration in the organs and tissues was accompanied by inductive stimulation of the activity of this enzyme.

Large quantities of glutamic acid are metabolized in the mitochondria through the tricarboxylic acid cycle. Transport of glutamic acid through the mitochondrial membranes involves expenditure of energy and

the participation of ATPase. The entry of larger quantities of glutamate inside the mitochondria after its administration to the animal therefore stimulates ATPase activity. In addition, by promoting oxidative processes in the body, glutamic acid increases the content of high-energy compounds in the organs and tissues [1-3], and this, in turn, must be accompanied by an increase in the ATP:ADP ratio and by stimulation of ATPase activity in the mitochondria. The hypotheses regarding the stimulating effect of glutamate on ATPase activity require experimental verification.

## LITERATURE CITED


1. A. M. Genkin and N. A. Glotov, Byull. Éksperim. Biol. i Med., No. 2, 50 (1967).
2. N. A. Glotov, in: The Biological Action of Glutamic Acid on the Organism under Experimental and Clinical Conditions [in Russian], Sverdlovsk (1966), p. 15.
3. A. V. Zakharova, in: Effect of Oxygen Deficiency on Metabolism in the Tissues [in Russian], No. 2, Leningrad (1962), p. 53.
4. A. A. Pokrovskii, A. I. Archakov, and A. M. Gerasimov, in: Modern Methods in Biochemistry [in Russian], Moscow (1968), p. 43.
5. V. P. Skulachev, The Accumulation of Energy in the Cell [in Russian], Moscow (1969).
6. L. L. Khachatryan and N. A. Abaturova, Vopr. Med. Khimii, No. 3, 26 (1965).
7. P. Borst, Biochim. Biophys. Acta, 57, 256 (1962).
8. T. Ozawa, J. Biochem. (Tokyo), 65, 317 (1969).