EFFECT OF GLUTAMIC ACID ON RESPIRATION AND OXIDATIVE PHOSPHORYLATION IN THE MITOCHONDRIA OF THE LIVER IN NORMAL AND HYPOXIC CONDITIONS

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Previous investigations in this department have shown that administration of glutamic acid to animals in hypoxic conditions increases their oxygen consumption and stimulates the aerobic oxidation of incompletely oxidized metabolic products, thereby promoting a more economical utilization of the energy resources of the organism [2]. This effect of glutamic acid is largely determined by its ability to activate certain enzymes of the respiratory cycle [8].

Since the enzymes of aerobic oxidation are localized in the mitochondria, it was interesting to investigate the effect of administration of glutamic acid to animals on the intensity of respiration and of oxidative phosphorylation in the mitochondria of the liver in both normal and hypoxic conditions.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 170-230 g. The experimental animals were injected subcutaneously with sodium glutamate in a dose of 1 mg/g body weight, and the control animals received the same volume of physiological saline. One pair of rats (control and experimental) was decapitated 3 h after injection of the solutions, and another pair of animals was placed in a pressure chamber 1 h after injection of the solutions, kept for 2 h at an "altitude" of 7000-8000 m, and then decapitated.

The mitochondria of the liver were isolated by differential centrifugation [9] at 0-5° without subsequent washing.

The isolation medium consisted of a 0.25 M solution of sucrose. The respiration of the mitochondria was investigated by a manometric method in a Warburg's apparatus at 26° for 15 min. The sample contained 0.4 ml of a suspension of mitochondria containing 2.0-2.5 mg nitrogen. The incubation medium (2 ml) included phosphate buffer, pH 7.4 (40 μmoles) and magnesium chloride (10 μmoles). The oxidation substrate was ketoglutaric acid, pH 7.4 (10 μmoles), ATP, pH 7.4 (5 μmoles), and 3 units of yeast [7] or 0.6 mg of crystalline hexokinase. The gaseous environment was air.

The oxygen absorption and the decrease in inorganic phosphate in the medium were expressed as microatoms (μA) oxygen and phosphorus per hour per milligram nitrogen of the mitochondria. The intensity of phosphorylation was judged from the P :O ratio. Yeast hexokinase was obtained by Sols' method [13], the decrease in inorganic phosphate was determined by the Fiske-Subbarow method, and the nitrogen of the mitochondria with Nessler's reagent [1]. The contractile properties of the mitochondria suspended in 0.25 M sucrose solution were investigated nephelometrically [5] at room temperature; the results of the measurements were expressed as percentages of the initial optical density of the mitochondrial suspension.

Experiments were performed on 41 animals.

EXPERIMENTAL RESULTS

The results given in Table 1 show that in rats kept in normal conditions administration of glutamic acid had no significant effect on the respiration and oxidative phosphorylation in the mitochondria of the liver. This conclusion is in agreement with the fact earlier observed in this laboratory, that glutamic

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TABLE 1. Effect of Glutamic Acid on Respiration and Oxidative Phosphorylation in Mitochondria of the Liver of Albino Rats in Normal and Hypoxic Conditions (M ± m)

Oxygen and phosphorus utilization (in μA/mg nitrogen/h)	Normal conditions		Hypoxia	
	Control animals (10)	Experimental animals (10)	Control animals (10)	Experimental animals (10)
Oxygen	9.87 ± 0.61	10.95 ± 0.63	10.12 ± 0.45	12.11 ± 0.43
Phosphorus	33.40 ± 1.82	36.72 ± 1.82	32.59 ± 2.16	36.61 ± 1.88
P :O	3.39 ± 0.11	3.37 ± 0.09	3.22 ± 0.12	3.02 ± 0.09

TABLE 2. Effect of Glutamic Acid on Optical Density of Mitochondria Isolated from the Liver of Rats Kept in Hypoxic Conditions (Mean Results in % of Initial Extinction

Time (in min) after isolation of mitochondria	Control animals (3)	Experimental animals (3)
0	100	100
10	95	99
20	91	93
30	90	90
40	88	82
50	85	83
60	85	85

acid, administered to intact animals, does not affect their oxygen consumption [2, 8]. In mitochondria isolated from the liver of rats kept in hypoxic conditions, respiration was increased by 19.6% over its value in animals kept in the same conditions but not receiving glutamic acid. This difference is statistically significant ($P = 0.01$). Glutamic acid had no effect on the P :O ratio in these experiments.

Investigations [5, 6, 10] have shown that when respiration is coupled with phosphorylation swelling of the mitochondria does not take place, and only slight changes in their volume are observed in association with their physiological state [11]. The work of S. E. Severin [5, 6] has shown that anserine, carnosine, and, to some extent, histidine may prevent aging of the mitochondria, and the impression is gained that these compounds may stimulate oxidative phosphorylation.

No such influence on the mitochondria was observed after administration of glutamic acid, because there was no difference in the optical density of the mitochondria isolated from the liver of the control and experimental animals (Table 2) kept in hypoxic conditions.

Since, according to these findings, in hypoxic conditions glutamic acid causes marked stimulation of respiration but does not change the character of oxidative phosphorylation, it may be supposed that this must lead to an overall increase in the synthesis of high-energy compounds, which may be utilized for restoring normal metabolism in the liver. This has been reported earlier by the authors [2] and confirmed by other workers [3, 4].

The mechanism of the stimulating action of glutamic acid on respiration of the liver mitochondria is not yet clear. Remembering that the oxidation of α-ketoglutaric acid takes place by the action of enzymes on the inner surface of the mitochondrial membranes [7], the permeability factor of these membranes toward various metabolites may have a direct bearing on the intensity of respiration. Finally, glutamic acid, on entering the body, may in some way or other influence the activity of the enzymes of the respiratory cycle. These and other hypotheses require further experimental verification.

LITERATURE CITED

1. V. S. Asatiani, Textbook of Biochemical Methods [in Russian], Tbilisi (1939), p. 350.
2. A. M. Genkin and N. A. Udintsev, Byull. Éksp. Biol., No. 5, 58 (1958).
3. M. A. Dobrinskaya, Transactions of the Department of Biochemistry, First Leningrad Medical Institute [in Russian], No. 2, Leningrad (1962).
4. A. V. Zakharova, Transactions of the Department of Biochemistry, First Leningrad Medical Institute [in Russian], Leningrad (1962), p. 53.
5. S. E. Severin and Yu. Shui-yu, Biokhimiya, No. 6, 862 (1958).
6. S. E. Severin, In the book: Phosphorylation and Function [in Russian], Leningrad (1960), p. 111.
7. V. P. Skulachev, Relationships between Oxidation and Phosphorylation in the Respiratory Chain [in Russian], Moscow (1962).

8. N. A. Udintsev, The Effect of Glutamic Acid on Metabolic Processes in Hypoxic Conditions, Candidate Dissertation, Sverdlovsk (1960).
9. E. Chargaff and J. N. Davidson, The Nucleic Acids [Russian translation], Moscow (1957), p. 113.
10. A. Lehninger, Horizons in Biochemistry [Russian translation], Moscow (1964), p. 326.
11. L. Packer, J. Cell. Biol., 18 (1963), p. 487.
12. A. Sols et al., Biochim. Biophys. Acta., 30 (1958), p. 97.