STATE OF THE KREBS' CYCLE IN THE KIDNEYS OF RATS WITH CHRONIC PHOSPHORUS POISONING

B. U. Dzharbusynov, B. U. Shalekenov, and N. V. Merkusheva

UDC 616.61-008.93:577.121.4]-092.9-02:[615.916:546.18

Key words: Krebs' cycle, kidneys, phosphorus poisoning.

The mechanism of the toxic action of yellow phosphorus has not yet been explained. We know that changes in the kidneys occupy an important place in the syndrome of chronic phosphorus poisoning after changes in the liver [1]. The central role in cell metabolism is played by the tricarboxylic acid cycle, but its function in phosphorus poisoning has virtually not been studied.

EXPERIMENTAL METHOD

An investigation was carried out on male August rats. Chronic phosphorus poisoning was produced by feeding the rats perorally with an oily solution of yellow phosphorus in a dose of 0.3 mg/kg body weight, daily for 5 days a week, for 3 months. Animals of the control group received pure sunflower oil in the corresponding volume. The experimental groups each consisted of seven rats. The material for investigation consisted of the kidneys of the animals, from which 5% homogenates were prepared in 0.1 M phosphate buffer (pH 7.4) to determine activity of NAD-dependent isocitrate dehydrogenase, malate dehydrogenase, and succinate dehydrogenase [6], on a "Spectonic" spectrophotometer. In a parallel series of experiments the kidney tissues were treated in the appropriate manner for gas chromatographic analysis by the method in [5], in order to determine carboxylic acids linked with the Krebs' cycle. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Reduction of NAD-dependent isocitrate dehydrogenase and succinate dehydrogenase activity and accumulation of virtually all oxidation substrates characterized the working of the Krebs' cycle under conditions of phosphorus poisoning (Table 1). The isocitrate dehydrogenase and succinate dehydrogenase reactions are the velocity-limiting reactions of the tricarboxylic acid cycle, and reduction of their activity is evidently the main cause of accumulation of oxidation substrates in the cycle. Weakening of succinate dehydrogenase activity is probably also a factor promoting accumulation of succinic and α-ketoglutaric acids. During hypoxia, the Krebs' cycle receives more acetyl-CoA from fatty acids than through glycolysis, and the excess of acetyl-CoA inhibits the decarboxylation of pyruvate, thus covering the flow of acetyl-CoA from carbohydrates. The increase in acetyl-CoA, together with propionyl-CoA (on account of oxidation of fatty acids and amino acids) leads to the formation of malonyl-CoA and methylmalonyl-CoA (the latter is easily converted into succinyl-CoA and, in that way, the Krebs' cycle can be replenished "in the middle") [2]. Under the conditions of phosphorus poisoning, both these processes probably take place. Meanwhile, the accumulation of succinic acid guarantees maximal relative power of the supply of high-energy compounds and creates the conditions for intensive operation of the cycle [2].

Alma-Ata State Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 8, pp. 161-162, August, 1991. Original article submitted December 5, 1990.

0007-4888/91/0008-1122$12.50

TABLE 1. Activity of Dehydrogenases (ncat/kg) and Content of Carboxylic Acids (mg/g) Linked with the Tricarboxylic Acid Cycle in Kidney Homogenates of Rats with Chronic Phosphorus Poisoning

Acid	Control, $M_{av} \pm m$	Experiment, $M_{av} \pm m$	Dehydrogenase	Control, $M_{av} \pm m$	Experiment, $M_{av} \pm m$
Citric	0,252±0,01	1,47±0,2*			
cis-Aconitic	0,72±0,07	1,01±0,05*	NAD — ICDH	433±33	321±18*
α-Ketoglutaric	0,87±0,04	4,76±0,4*			
Succinic	0,082±0,004	0,22±0,02*	SDH	335±16	244±20*
Fumaric	0,023±0,002	0,12±0,005*			
Malic	0,046±0,004	0,075±0,004*	MDH	296±18	373±15*
Oxaloacetic	1,014±0,06	3,3±0,06*			
Latice	0,023±0,002	0,094±0,009*			
Pyruvic	0,035±0,003	0,1±0,008*			
Oxalic	0,056±0,005	0,27±0,01*			
Butyric	0,017±0,001	0,05±0,004*			
Glutaric	0,006±0,0008	0,1±0,004*			

Fumaric acid participates in transamination and deamination reactions of amino acids, which are then incorporated into the Krebs' cycle [4]. The reduction of succinate dehydrogenase activity ought to lead to a block at the stage of the succinate dehydrogenase reaction and to a decrease in the fumarate content, but in the kidney tissues of rats with phosphorus poisoning there was a fivefold increase in accumulation of fumaric acid, probably as a result of intensification of amino-acid metabolism.

Malate dehydrogenase is the last dehydrogenase in the cycle, and with its participation malic acid is dehydrogenated and electrons transferred to the respiratory chain. Under physiological conditions, because of the rapid utilization of oxaloacetic acid, equilibrium of the reaction is shifted toward oxidation of malic acid. In the presence of an energy deficiency in the mitochondria, increased synthesis of oxaloacetic acid takes place [3]. The increase in malate dehydrogenase activity is probably directed toward intensifying synthesis of oxaloacetic acid from malic acid in the kidney tissues of rats with phosphorus poisoning.

The high lactate content indicates intensive glycolysis which, in turn, suggests elevation of the pyruvate level and the possibility of carboxylation reactions converting it into oxaloacetic or malic acid [7]. The fourfold accumulation of lactic acid and threefold accumulation of pyruvic acid were evidently caused by stimulation of the glycolytic pathway of energy formation.

Threefold accumulation of butyric acid was probably due to intensification of oxidation of fatty acids and disturbances of their utilization in the Krebs' cycle. Free CoA, formed by interaction of oxaloacetic acid with acetyl-CoA, is required for fatty acid oxidation. Accumulation of citrate of *cis*-aconitate may limit this reaction and cause the accumulation of incompletely oxidized fatty acids, and this probably took place in the kidneys of rats with phosphorus poisoning.

Chronic phosphorus poisoning thus leads to changes in the state of the Krebs' cycle in the kidney tissues of rats.

LITERATURE CITED

1. G. I. Beloskurskaya and B. N. Aitbembetov, Abstracts of Proceedings of an All-Union Foundation Conference on Toxicology [in Russian], Moscow (1980), pp. 138-139.
2. M. N. Kondrashova, Reaction of Living Systems and the State of Energy Metabolism [in Russian], Pushchino (1979).
3. M. G. Kritsman and A. S. Konikova, Induction of Enzymes under Normal and Pathological Conditions [in Russian], Moscow (1968).
4. A. Lehninger, Biochemistry: Molecular Principles of Cell Structure and Function [Russian translation], Moscow (1976).
5. V. M. Mal'tsev and V. K. Orlov, Method of Determination of Mono-, Di-, and Tricarboxylic Acids in Food Products and Animal and Human Tissues [in Russian], Alma-Ata (1983).
6. A. A. Pokrovskii, Biochemical Methods of Investigation in Clinical Practice [in Russian], Moscow (1969).
7. E. M. Khvatova, Cell Respiration under Normal and Hypoxic Conditions [in Russian], Gor'kii (1973).