DEAMINATION OF SOME NITROGEN COMPOUNDS IN THE LIVER MITOCHONDRIA OF RATS WITH VITAMIN K DEFICIENCY

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The rate of deamination of AMP in the liver mitochondria of rats with vitamin K deficiency is approximately twice the normal value. Administration of synthetic vitamin K normalized this process. No decrease in the rate of deamination of monoamines (tyramine, p-nitrophenylethylamine) was found, nor did the ability to deaminate histamine appear in the liver mitochondria of rats with vitamin K deficiency.

Treatment of highly purified preparations of monoamine oxidases by certain oxidizing agents (including oxidized oleic acid) led to the appearance of a qualitatively new property, the ability to deaminate AMP [1]. This phenomenon occurred in experiments with mitochondria [6] and also in the intact organism [2, 7, 15] in pathological states (hypervitaminosis D, radiation sickness) accompanied by the accumulation of lipid peroxides similar in their chemical properties and biological action to oxidized oleic acid [9] in the tissues.

The antioxidizing properties of the substituted p-quinones, which include vitamins K and E, are well known [4, 12].

In this investigation an attempt was made under conditions of vitamin K deficiency to detect disturbances of catabolic reactions of some nitrogenous compounds similar to disturbances found in hypervitaminosis D [15, 16] and in radiation sickness [2, 5, 7].

EXPERIMENTAL

Male albino rats weighing 185-310 g were used in the experiments. Vitamin K deficiency was induced by keeping the animals on the diet described previously [11, 17]. The criterion of development of vitamin K deficiency was an obvious lengthening of the prothrombin time determined by Quick's method. The animals were sacrificed after being kept for 40-45 days on the diet, when their prothrombin time was 30 ± 0.5 sec compared with 17 ± 0.4 sec in rats on the normal animal house diet. Control rats also received the same vitamin K-deficient diet and daily subcutaneous injection of 1 mg of the water-soluble vitamin K analog Vikasol; the prothrombin time in these animals was normal (18 ± 0.6 sec). The control animals were sacrificed at the same time as the experimental. The liver was washed with 0.9% NaCl solution and frozen at −20°C. The material was kept in this form (but for not more than 5 days). The tissues were thawed and a 15% homogenate prepared in 0.25 M sucrose in a Potter-type homogenizer. The method of washing the mitochondria with hypotonic buffer solution was described previously [8]. The residue of mitochondria was suspended in 0.2 M phosphate buffer, pH 7.4. The protein content in the suspension was from 2 to 5 mg/ml. To a sample 1.8 ml in volume were added 1 ml of suspension and 1 ml of one of the

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TABLE 1. Rate of Deamination of Some Nitrogenous Compounds When Incubated with Rat Liver Mitochondria (M ± m)

Nitrogen compound	Rate of deamination (in nmoles NH_3/min per g protein)		
	normal	vitamin K deficiency	vitamin K deficiency+Vikasol
Tyramine	38,3±12,1 (7)	37,5±5,9 (9)	37,8±18,1 (8)
p-Nitrophenylethylamine	35,2±6,3 (8)	43,9±5,7 (10)	42,4±2,3 (9)
Histamine	0 (7)	0 (9)	0 (8)
AMP	1,6±0,7 (7)	5,4±1,3* (9)	2,9±0,9 (8)

*Difference between mean values obtained in vitamin K deficiency and under normal conditions statistically significant: $0.02 < P < 0.05$.

Note: Number of animals in parentheses.

substrates in the following optimal ("saturating") concentrations (in mM): tyramine-HCl 3.2; AMP-Na 10.0, histamine-2HCl 10.0. The samples were incubated at 37°C for 45 min, fixed by the addition of TCA, and the ammonia concentration in the protein-free supernatants was determined by Conway's isothermic distillation method followed by nesslerization. The quantity of ammonia liberated during incubation for 45 min was directly proportional to the duration of incubation and the amount of enzyme added. The rate of deamination of p-nitrophenylethylamine-HCl was determined as described previously [5]. The protein concentration was determined by Lowry's method.

EXPERIMENTAL RESULTS AND DISCUSSION

In vitamin K deficiency no statistically significant changes could be found in the rate of deamination of monoamines (tyrosine or p-nitrophenylethylamine). No deamination of histamine could be detected under these conditions. However, the increase in the rate of deamination of AMP in the liver mitochondria of rats with vitamin K deficiency was clearly marked. Administration of Vikasol to these animals reduced the rate of this reaction to values not statistically significantly different from the level of AMP-deaminase activity of the liver mitochondria of the control rats (Table 1).

The considerable (almost twofold) increase in the rate of the AMP-deaminase reaction in vitamin K deficiency is in agreement with results reflecting the reduced content of adenosine phosphates in the muscles or blood plasma [3, 11], and also the disturbance of oxidative phosphorylation in the muscles [10, 11] of mammals with avitaminosis K. In rats receiving antivitamin K (tromexan) a decrease in the ATP and AMP concentrations in the heart muscle has been described [18] and attributed to inhibition of biosynthesis of the AMP precursors. The present results indicate that increased breakdown of AMP is possible in vitamin K deficiency. In hypervitaminosis D_2, which is accompanied by a disturbance of oxidative phosphorylation [13, 14], the AMP-deaminase activity in the liver mitochondria also rises sharply [16]. However, in the liver mitochondria in hypervitaminosis D_2 the decrease in monoamine oxidase activity is accompanied by simultaneous appearance of ability to deaminate the substrates of diamine oxidase [15]. In vitamin K deficiency, under the conditions described in this paper, it was impossible to observe these phenomena which were expected on the grounds of the results of experiments with purified enzyme preparation [19]. The relationships between the amine-oxidase and adenylate-deaminase activities observed in experiments with purified amine oxidases [1, 19, 20] are possibly much more complex at the mitochondrial level [6] than in the intact organism [7].

LITERATURE CITED

1. Zh. I. Akopyan, R. I. Gvozdev, et al., Vopr. Med. Khimii, No. 6, 654 (1971).
2. Zh. I. Akopyan, V. Z. Gorkin, et al., Radiobiologiya, 10, 826 (1970).
3. L. M. Bronshtein, T. S. Barkagan, and I. I. Matusis, Byull. Éksperim. Biol. i Med., No. 3, 48 (1969).
4. V. Z. Gorkin, Ukr. Biokhim. Zh., No. 2, 270 (1959).
5. V. Z. Gorkin, Zh. I. Akopyan, et al., Vopr. Med. Khimii, No. 5, 538 (1968).
6. V. Z. Gorkin, Zh. I. Akopyan, et al., Biokhimiya, No. 1, 141 (1970).

7. V. Z. Gorkin, Zh. I. Akopyan, et al., Byull. Éksperim. Biol. i Med., No. 11, 42 (1971).
8. V. Z. Gorkin and I. V. Verevkina, Vopr. Med. Khimii, No. 3, 315 (1963).
9. Yu. B. Kudryashov and E. N. Goncharenko, Radiobioligiya, 10, 212 (1970).
10. I. I. Matusis, N. G. Bogdanov, et al., Vopr. Med. Khimii, No. 6, 613 (1966).
11. I. I. Matusis, N. G. Bogdanov, et al., in: Mechanisms of Action of Biologically Active Substances [in Russian], Gor'kii (1970), p. 7.
12. I. I. Matusis and I. I. Ulasevich, Uspekhi Sovr. Biol., 63, No. 3, 415 (1967).
13. L. P. Shipitsyna, Byull. Éksperim. Biol. i Med., No. 9, 60 (1966).
14. L. P. Shipitsyna, Vopr. Pitaniya, No. 2, 38 (1967).
15. Zh. I. Akopyan, N. V. Blazheivich, et al., Int. Z. Vitaminforsch., 40, 497 (1970).
16. V. Z. Gorkin, Zh. I. Akopyan, et al., Abstracts of Communications of the 7th Meeting of the European Biochemical Society (1971), p. 65.
17. M. S. Mameesh and B. C. Johnson, Proc. Soc. Exp. Biol. (New York), 101, 467 (1959).
18. J. Randriamapandry, C. R. Soc. Biol. (Paris), 164, 928 (1970).
19. L. V. Tatyanenko, R. I. Gvozdev, et al., Biochim. Biophys. Acta, 242, 23 (1971).
20. I. V. Veryovkina, M. M. Abdel Samed, and V. Z. Gorkin, Biochim. Biophys. Acta, 250, 234 (1971).