ROLE OF p-QUINONES IN REGULATION OF GLUCOSE-6-PHOSPHATE METABOLISM

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KEY WORDS: glycolysis, vitamins, p-quinones.

There have been many communications devoted to the effect of the supply of p-quinones (naphthoquinones, tocopherols) to the body on the state of tissue respiration and oxidative phosphorylation coupled with it [1, 12, 13]. It has been shown that p-quinones, like retinol, are constituents of the lipid component of the biomembrane and have fixation points on it [4]. It has recently been suggested that they may participate in regulation of the structural state of the biomembrane [2, 10]. Consequently, it was logical to suggest that in p-quinone (naphthoquinone, tocopherol) deficiency, in vivo the direction of tissue metabolism may undergo secondary changes due to a primary disturbance of biomembrane structure. These metabolic changes are evidently compensatory in character and may reflect the state of activity of alternative pathways for the conversion of one of the principal oxidation substrates (glucose-6-phosphate) in the tissues.

To study this problem it was decided to investigate the state of the pentose phosphate shunt (PPS) in rats with p-quinone (naphthoquinone, tocopherol) deficiency.

EXPERIMENTAL METHODS

Experiments were carried out on 74 male albino rats and 24 Chinchilla rabbits. Clinical material (erythrocytes, blood plasma) from 29 normal healthy subjects (control) and patients undergoing long-term treatment with antivitamin K (pelentan) in a dose of up to 300 mg daily for 8-12 days, was used in some of the investigations. A vitamin K deficiency was induced in albino rats by keeping them on a synthetic diet without vitamin K [11], and in some animals by feeding with pelentan (30 mg/kg daily for 12 days). Vitamin E deficiency was induced by keeping the rats for 4-6 months on Lonis' vitamin E-deficient diet [9]. Fructose-1,6-diphosphate aldolase acivity was investigated by Bruns' method in the modification of Tovarnitskii and Voluiskaya [5] and glucose phosphate isomerase activity by Bodensky's method [7]. Glucose-6-phosphate dehydrogenase (G6PD) activity was determined by Dische's method [7]. The

TABLE 1. Fructose-1,6-diphosphate Aldolase Activity (in conv. units/g wet weight of tissue) and Glucose Phosphate Isomerase Activity (in μmoles/g wet weight of tissue/h) in Albino Rats with Avitaminosis K or E (M ± m)

Tissue	Fructose-1,6-diphosphate aldolase			Glucose phosphate isomerase		
	control	avitaminosis K	avitaminosis E	control	avitaminosis K	avitaminosis E
Liver	6,05±0,3	11,1±1,3*	12,0±1,2*	85,0±1,9	124,0±12,9*	122,0±3,2*
Myocardium	3,8±0,2	6,1±0,1*	5,9±0,2*	78,0±2,5	123,0±1,7*	110,0±4,5*
Skeletal muscles	2,1±0,3	4,5±0,06*	5,1±0,1*	82,0±1,3	105,0±4,0*	93,0±2,3*
Spleen	5,1±0,3	8,6±1,1*	7,8±0,1*	55,0±1,9	68,0±1,5	71,0±2,3
Small intestine	2,1±0,3	4,4±0,1	3,6±0,1*	64,0±1,5	76,0±1,6*	82,0±1,8*
Adrenals	9,0±0,4	13,2±0,4*	11,2±0,2*	81,0±1,7	83,0±1,82	84,0±2,9
Lungs	6,7±1,4	14,0±3,6*	12,6±1,7*	23,0±1,1	34,0±1,4*	38,0±1,6*
Pancreas	3,1±0,3	6,0±0,1*	5,9±0,2*	74,0±1,9	79,0±1,9	88,0±2,7*

Legend. Here and in Table 2: *P < 0.05 compared with control.

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TABLE 2. G6PD Activity (in μmoles/ml/h) in Erythrocytes and Blood Plasma of Male Albino Rats and Rabbits with Dietary Avitaminosis K and E (M ± m)

Tissue	Albino rats		Rabbits	
	control	avitaminosis K	control	avitaminosis E
Erythrocytes	0,25±0,01 (8)	0,37±0,03* (7)	0,27±0,08 (6)	0,55±0,02* (6)
Blood plasma	0,025±0,009 (4)	0,14±0,03* (6)	0,037±0,004 (4)	0,054±0,009* (6)

Legend. Number of animals shown in parentheses.

lactate concentration was determined as in [6] and pyruvate by reduction of NAD, using a kit from Boehringer, West Germany; glucose was determined as in [3]. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that under the conditions of p-quinone deficiency developing in the rats changes were found in activity of fructose-1,6-diphosphate aldolase, a key enzyme of glycolysis, in all tissues studied ($P < 0.05$). The increase in the activity of this enzyme is evidence of activation of glycolysis as the principal metabolic pathway for G6PD conversion. The adrenals ($P > 0.05$) were the exception. This feature of adrenal metabolism was probably due to some distinguishing feature of their metabolism and the complexity of their morphological structure. This hypothesis was confirmed by investigations of the state of metabolism of the adrenals under extreme conditions, due to various causes [8]. Further evidence of increased activity of glycolysis in rats with p-quinone deficiency was given by the results of a study of another key enzyme – glucose phosphate isomerase (Table 1). Activity of this enzyme was increased in all tissues studied ($P < 0.05$). Again the adrenals were the exception ($P > 0.01$). Activity of this enzyme was increased in all tissues studied ($P < 0.05$). Again the adrenals were the exception ($P > 0.01$). These aspects of adrenal metabolism are in agreement with data in the literature [8].

As was to be expected, there was a parallel increase in the concentrations of the most important metabolites of glycolysis, namely pyruvate and lactate ($P < 0.01$), in the blood plasma of animals with avitaminosis K and E and patients undergoing long-term treatment with pelentan. This was accompanied by a simultaneous fall in the blood plasma glucose level ($P < 0.05$) and the development of hypoglycemia.

Incidentally, similar changes also were observed in patients receiving long-term treatment with pelentan, an oxycoumarin derivative with antivitamin K properties. The uniform character of the change in glycolytic activity in primary (albino rats) and secondary (patients) p-quinone deficiency was established.

It follows from Table 2 that changes in glycolytic activity in p-quinone deficiency were accompanied by increased G6PD activity, characterizing the state of the aerobic branch of the PPS. This was evidence of compensatory increase in PPS activity in the tissues of animals with developed (primary or secondary) deficiency of p-quinones (naphthoquinones or tocopherols). Incidentally, an increase in G6PD acivity in p-quinone deficiency was discovered both in the erythrocytes and blood plasma of animals and man. An increase in G6PD activity in the erythrocytes in developed avitaminosis K or E, in the writers opinion, is compensatory in character and is indirectly confirmed by data in the literature [1, 8, 10]. Changes in G6PD activity in the blood plasma of these same animals were evidently the result of a disturbance of the unique structure of the erythrocyte membrane and hemolysis of some erythrocytes. Changes in the direction of G6PD metabolism discovered during the development of p-quinone deficiency in vivo are in the same direction and are evidence that there are certain common mechanisms concerned in the realization of the physiological action of natural p-quinones and tocopherols. At the same time, these changes in G6PD metabolism in p-quinone deficiency were discovered not only in animals, but also in patients treated with antivitamins K of the oxycoumarin series (pelentan).

The development of naphthoquinone and tocopherol deficiency in vivo is thus accompanied by changes in the direction of metabolism of G6PD, one of the principal oxidation substrates. Under these circumstances activity of gycolysis and of the aerobic branch of PPS increases,

which confirms the view that these disturbances of metabolism in avitaminosis K or E are secondary in character.

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ISOLATION AND PARTIAL PURIFICATION OF AN ENDOGENOUS INHIBITOR OF ^{3}H-L-GLUTAMATE RECEPTOR BINDING

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The existence of endogenous substances which inhibit receptor binding of brain neurotransmitters in vitro is very interesting because these substances can claim the role both of true ligands and of modulators of synaptic transmission [7]. Reports on the identification of endogenous inhibitors of GABA [9], benzodiazepine [6], and acetylcholine [10, 12] receptors have recently been published. The hypothesis that there is an endogenous inhibitor of L-glutamate receptor binding was first put forward by Sharif and Roberts [13] in connection with the discovery of increased specific ^{3}H-L-glutamate binding during successive washing of synaptic membranes isolated from brain. The dipeptide N-acetylaspartylglutamate, which inhibits ^{3}H-L-glutamate binding with synaptic membranes, was isolated from a perchlorate extract of rat brain [14]. According to their hypothesis, this isolated dipeptide can perform the function of neurotransmitter in synapses previously classified as glutamergic or aspartatergic. The present writers showed previously [2] that an aqueous extract of synaptosomes contains a thermostable factor of peptide nature, which inhibits specific binding of ^{3}H-L-gluamate.

The aim of the present investigation was to isolate, purify, and determine certain characteristics of this factor.

EXPERIMENTAL METHODS

Specific binding of ^{3}H-L-glutamate (33 Ci/mmole, from Izotop, USSR) with synaptic membranes isolated from the rate cerebral cortex was determined by the method described previously [3].

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