THE EFFECT OF COENZYME Q_{10} ON TYROSINE METABOLISM IN GUINEA PIGS

Vincent G. Zannoni, George A. Jacoby and Bert N. La Du

National Institute of Arthritis and Metabolic Diseases
National Institutes of Health, U. S. Public Health Service
Bethesda, Maryland

Received March 5, 1962

p-Hydroxyphenylpyruvic acid oxidase, the enzyme catalyzing the oxidation of p-hydroxyphenylpyruvic acid (pHPP) to homogentisic acid (HGA) is inhibited by excess substrate and by analogues of the substrate, such as phenylpyruvic acid (Hager, et al. 1957; La Du and Zannoni, 1955; Zannoni and La Du, 1959). Inhibition of the oxidase also occurs in vivo following the administration of large amounts of pHPP (Zannoni and La Du, 1960a) or tyrosine (Knox and Goswami, 1960; Zannoni and La Du, 1960b) to scorbutic guinea pigs. However, the inhibition can be prevented with ascorbic acid, analogues of the vitamin, and more effectively by 2,6-dichlorophenolindophenol dye (2,6-DCPP) both in vitro (Knox, 1955; La Du and Greenberg, 1953; La Du and Zannoni, 1961) and in vivo (Zannoni and La Du, 1960b). In recent studies to determine the chemical structure necessary to protect pHPP oxidase from inhibition, it was reported that coenzyme Q_{10} in combination with ascorbic acid protects the oxidase in vitro nearly as efficiently as reduced 2,6-DCPP (Zannoni, 1962). Since there is evidence that ascorbic acid may prevent inhibition of pHPP oxidase indirectly, perhaps by reducing another agent (La Du and Zannoni, 1956; Zannoni and La Du, 1959, 1960a, 1960b), we have evaluated the effect of coenzyme Q_{10} in vivo in two groups of guinea pigs—one frankly scorbutic, and the other less severely deficient in vitamin C.

Two groups of male albino guinea pigs, weighing approximately 200 g, were placed on a vitamin C-free diet as previously described (Zannoni and La Du, 1960a and 1960b). After 5 days on the diet, one group, the less

severely "vitamin C-deficient" group, was still gaining weight, showed no signs of scurvy, and had an average concentration of 6.1 mg. of ascorbic acid per 100 g wet weight of liver. Guinea pigs on the deficient diet supplemented with vitamin C had liver concentrations of ascorbic acid of 15 mg. per 100 g wet weight. The other group, the "scorbutic" group, was maintained on the deficient diet for 2 weeks. They showed typical signs of scurvy: weight loss, swollen joints with hemorrhages in the knee joints and had an average concentration of 1.5 mg. of ascorbic acid per 100 g wet weight of liver. The test animals were fed a total of 400 mg. of L-tyrosine, 100 mg. each hour for 4 hours. A number of guinea pigs were given 10 mg. of either ascorbic acid or coenzyme Q_{10} intraperitoneally one half hour before the first tyrosine feeding, and 10 mg. more, intraperitoneally, 2 hours later. One hour after the last dose of tyrosine, the animals were sacrificed and the activity of the tyrosine oxidizing enzymes determined in liver homogenates. Tyrosine transaminase, pHPP oxidase, and HGA oxidase were assayed and plasma tyrosine and urinary pHPP were measured as previously described (Zannoni and La Du, 1960a).

Coenzyme Q_{10} was as effective as ascorbic acid in protecting pHPP oxidase from inhibition in the "vitamin C-deficient" group (Table I). As was found previously (Zannoni and La Du, 1960b), pHPP oxidase was inhibited over 80% following tyrosine feeding in guinea pigs on a vitamin C-free diet for 5 days. Tyrosine transaminase and HGA oxidase were not altered significantly. Further evidence of the protective effect of coenzyme Q_{10} on pHPP oxidase *in vivo* is the finding that the concentration of tyrosine in the plasma and the amount of pHPP excreted in the urine were elevated only in the vitamin C-deficient animals not given coenzyme Q_{10} or ascorbic acid.

Coenzyme Q_{10} was found to be much less effective when tested in scorbutic guinea pigs (Table II). The intermediate level of tyrosine in the plasma and the amount of pHPP excreted in the urine also indicated

that coenzyme Q_{10} protected the oxidase less completely when the animals were frankly scorbutic.

TABLE I

EFFECT OF INTRAPERITONEAL COENZYME Q_{10} OR ASCORBIC ACID IN GUINEA PIGS MODERATELY DEFICIENT IN VITAMIN C FED L-TYROSINE [a]

	Without protective agents (6) [b]	20 mg. Ascorbic acid (4)	20 mg. Coenzyme Q_{10} (8)
	μmoles of substrate oxidized/hr/g wet weight [c]		
Liver enzymes			
Tyrosine transaminase	17.4 ± 7.0	18.4 ± 2.6	23.4 ± 3.9
pHPP oxidase [d]	3.1 ± 1.6	24.3 ± 4.7	21.3 ± 4.2
HGA oxidase	74.4 ± 18.7	83.5 ± 25.3	73.6 ± 14.2
	mg/100 ml. of plasma (average values)		
Plasma tyrosine	25.7	3.5	3.9
	mg. excreted in 5 hours		
Urinary pHPP	11.0	< 0.1	< 0.1

[a] The animals received a total of 400 mg. of L-tyrosine orally as previously described (Zannoni and La Du, 1960b). Mean ascorbic acid concentrations in liver: group without protective agents, 6.1 mg./100 g wet weight; ascorbic acid group, 15.6; coenzyme Q_{10} group, 4.7.

[b] Numbers in parentheses = number of animals.

[c] Mean, ± standard error.

[d] The average pHPP oxidase activity in 6 vitamin C-deficient animals not fed tyrosine was 25.8 ± 1.9. The level of tyrosine in the plasma was not elevated and no significant amount of pHPP was excreted in the urine.

Several quinones and indophenols have the ability to protect pHPP oxidase from inhibition (Zannoni, 1962). It is of interest, however, that only two of the compounds which are active both *in vivo* and *in vitro* occur normally in mammalian tissue—L-ascorbic acid and coenzyme Q_{10}. The fact that coenzyme Q_{10} is much more effective in the presence of ascorbic acid

TABLE II

EFFECT OF INTRAPERITONEAL COENZYME Q_{10} IN SCORBUTIC GUINEA PIGS [a]

	Without tyrosine feeding (5)	Tyrosine feeding alone (4)	Tyrosine feeding with coenzyme Q_{10} intraperitoneally (6)
	μmoles of substrate oxidized/hr/g wet weight		
Liver enzymes			
Tyrosine transaminase	28.6 ± 8.8	24.6 ± 6.9	29.0 ± 6.1
pHPP oxidase	15.9 ± 2.7	5.3 ± 1.7	9.6 ± 1.7
HGA oxidase	77.4 ± 26.0	72.6 ± 21.0	104.8 ± 19.0
	mg/100 ml. of plasma (average values)		
Plasma tyrosine	1.6	42.3	2.6
	mg. excreted in 5 hours		
Urinary pHPP	< 0.1	27.5	1.0

[a] Mean ascorbic acid concentrations in liver: group not fed tyrosine, 1.7 mg/100 g wet weight; group fed tyrosine, 4.1; group fed tyrosine and given coenzyme Q_{10}, 1.5.

Coenzyme Q_{10} was generously supplied for these studies by Dr. A. L. Smith of the Institute for Enzyme Research, University of Wisconsin.

is in agreement with the finding that the protective agents are required in their reduced forms (Zannoni, 1962).

The exact mechanism by which these reducing agents function with pHPP oxidase is not known. They apparently are not acting as specific coenzymes (La Du and Zannoni, 1961) and are required only under conditions of high substrate concentration. It seems reasonable that an active site of the enzyme, or some component of the enzyme system, must be maintained in a reduced state to prevent alteration by excess substrate or a product derived from the substrate (Zannoni and La Du, 1959). The data presented is compatible with this hypothesis if ascorbic acid maintains normal tyrosine metabolism *in vivo* indirectly by reducing another component, perhaps a quinone of the coenzyme Q family. Further studies on the possible

physiological significance of coenzyme Q_{10} in tyrosine metabolism are in progress.

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