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ELEVATED ASPARTATE TRANSCARBAMYLASE AND DIHYDROOROTASE ACTIVITIES IN ERYTHROCYTES FROM PATIENTS WITH HYPOXANTHINE GUANINE PHOSPHORIBOSYLTRANSFERASE DEFICIENCY

James Kennedy

Department of Medicine, University of Kansas Medical Center, Kansas City, Kansas 66103 and the Research Service, Veterans Administration Hospital, Kansas City, Missouri 64128

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SUMMARY: Activities of aspartate transcarbamylase and dihydroorotase are increased 6 to 8-fold in erythrocytes from individuals with hypoxanthine guanine phosphoribosyltransferase deficiency. The increased enzyme activities do not appear to be due to enzyme activation.

Hypoxanthine guanine phosphoribosyltransferase (HGPRTase) (E.C. 2.4.2.8) deficiency is an X-linked disorder characterized by a variety of neurological symptoms and excessive production of uric acid (1,2). The latter is due to acceleration of the overall pathway for purine snythesis de novo (2). In addition, several other biochemical abnormalities have been identified, not all of which are limited to purine metabolism per se. Activities of orotate phosphoribosyltransferase (E.C. 2.4.2.10) and orotidylate decarboxylase (E.C. 4.1.1.23), two of six sequential enzymes required for biosynthesis of pyrimidines de novo, are increased in erythrocytes from patients deficient in HGPRTase (3). The mechanism responsible for the increased activity of orotate phosphoribosyltransferase or orotidylate decarboxylase has not been established. It apparently is not due to enzyme stabilization. I report here that activities of two addiitional pyrimidine pathway enzymes, aspartate transcarbamylase (E.C. 2.1.3.2) and dihydroorotase (E.C. 3.5.2.3) are increased in circulating erythrocytes from patients with HGPRTase deficiency.

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METHODS

Two brothers, A, 9 years old and B, 19 years old, presented with symptomatic uric acid crystalluria and gout, respectively. Both boys are mildly retarded and have mild motor dysfunction. Neither has evidence of choreoathetosis or self-mutilation characteristic of the Lesch-Nyhan syndrome. Twenty-four hour uric acid excretions for patients A and B were 1.3 and 1.5 grams, respectively. Erythrocytes for enzyme assays were obtained prior to initiating allopurinol therapy in the two boys. Blood was drawn into tubes containing EDTA and chilled immediately. After removal of the plasma and buffy coat, the erythrocytes were washed twice in 0.9% NaCl. The packed erythrocytes were suspended in an equal volume of 0.2% NaCl and rapidly frozen and thawed twice. All enzyme assays were performed on fresh blood and in duplicate. HGPRTase was assayed using a modification of the method of Flaks (4) in which the quantity of hypoxanthine remaining after termination of the HGPRTase reaction is measured spectrophotometrically in the presence of xanthine oxidase. The assay mixture contained 100 mM Tris-HCl buffer, pH 7.5, 20 mM MgS_4, 0.2 mM hypoxanthine, 4 mM phosphoribosylpyrophosphate and hemolysate in a final volume of 2.5 ml. After incubation at 37°C for 30 min, the reaction was stopped by addition of 0.1 ml of 70% $HClO_4$ and the protein removed by centrifugation. A 1.5 ml aliquot of the supernatant fluid was adjusted to pH 7 by addition of 1.0 M NaOH followed by addition of water to bring the volume to 3 ml. Residual hypoxanthine was determined at room temperature in an assay mixture containing 100 mM Tris-HCl buffer, pH 7.5, 2 ml of the neutralized incubation mixture and 0.05 units of xanthine oxidase (Grade I, Sigma Chemical Co.) in a final volume of 3 ml. The absorbance of 290 nm was recorded 0.5 min after addition of xanthine oxidase and the decrease in A_{290} was followed to completion. The volume of hemolysate selected for HGPRTase assay was one that would convert 100-200 nmoles of hypoxanthine to IMP under the conditions outlined, generally 0.02 ml for normals. The assay system for aspartate transcarbamylase contained 100 mM Tris-HCl buffer, pH 8.5, 10 mM sodium aspartate, pH 7, 1.4 mM disodium carbamylphosphate and hemolysate in a final volume of 2 ml. After 20 min at 37°C, the reaction was stopped by addition of 0.2 ml of 4 M $HClO_4$. Protein was removed by centrifugation and carbamylaspartate estimated colorimetrically (5). Assays lacking aspartate served as controls. Volumes of hemolysates were selected that would convert 10-20% of the carbamylphosphate to carbamylaspartate. Dihydroorotase was assayed as described previously (6).

RESULTS AND DISCUSSION

Results of enzyme assays performed on erythrocyte lysates from the patients and their mother are listed in Table 1. Levels of HGPRTase activity in erythrocyte lysates from the two affected males were below the level of detection by the spectrophotometric assay employed. Whatever HGPRTase activity, if any, was present was less than 0.1% of normal. Activity in erythrocytes from the mother, an obligate heterozygote, was slightly less than normal. Of particular interest is the striking increase in the specific activites of the two pyrimidine pathway enzymes, aspartate transcarbamylase and dihydroorotase, in hemolysates from the HGPRTase-

TABLE 1

Enzyme activites in erythrocytes from two HGPRTase-deficient males and their mother

Subject	HGPRTase	ATCase*	DHOase*
	(nmoles/mg protein/hr)		
Patient A	< 0.1	45.0	8.9
Patient B	< 0.1	45.2	9.3
Mother	70	13.8	2.6
Controls†	104.4 ± 6.6	6.7 ± 0.4	1.1 ± 0.1

*Mean values from erythrocytes obtained on two separate occasions.
†Mean (± s.d.) of control subject values (n = 7).
ATCase, aspartate transcarbamylase; DHOase, dihydroorotase.

deficient males. Aspartate transcarbamylase activity was increased some 6-fold and dihydroorotase activity was 8 times higher than normal. Levels of activity of both pyrimidine pathway enzymes in the mother's erythrocytes were twice normal.

One possible cause for the increased activities of aspartate transcarbamylase and dihydroorotase in mutant erythrocytes is activation by phosphoribosylpyrophosphate (PRPP), a compound known to be present in increased amounts in such cells (7). Pre-incubation of normal hemolysates in 5×10^{-5} M MgPRPP, the concentration present in HGPRTase-deficient erythrocytes, failed to stimulate either of the two enzymatic activities. Another mechanism that might account for the elevated enzyme activities is lack of a normal inhibitor or presence of an unidentified activator in HGPRTase-deficient cells. Such was not the case. Activities of aspartate transcarbamylase and dihydroorotase in assays containing a mixture of normal and HGPRTase-deficient hemolysates equaled the sum of the activities of each hemolysate assayed independently. A third possibility, increased enzyme stability, was examined. Neither aspar-

tate transcarbamylase nor dihydroorotase in erythrocytes from the two affected males was more stable to heat than the respective enzymes from a normal subject (Fig. 1). If anything, aspartate transcarbamylase from HGPRTase-deficient erythrocytes was inactivated more rapidly than the control. In other experiments, PRPP did not protect against heat inactivation of aspartate transcarbamylase or dihydroorotase from normal erythrocytes.

Activites of four of the six enzymes for pyrimidine biosynthesis *de novo* have now been shown to be elevated in HGPRTase-deficient erythrocytes. The mechamism responsible for the increased activites of orotate phosphoribosyltransferase and orotidylate decarboxylase has not been established (3). The same can be said for aspartate transcarbamylase and dihydroorotase. In all cases, the increased enzyme activities do not appear to be due to absence of a normal inhibitor or the presence of an activator in the mutant cells. Furthermore, the increased activities cannot be attributed to differences in stability to heat. In the case of orotate phosphoribosyltransferase and orotidylate decarboxylase, the rate of inactivation during aging of HGPRTase-deficient erythrocytes *in vivo* did not differ from that observed in normal subjects (3). It remains to be determined whether the increased activities of the four pyrimidine pathway enzymes studied thus far represent increased enzyme synthesis or are due to other factors.

Since the fourth enzyme, dihydroorotate dehydrogenase, in the pathway for pyrimidine biosynthesis is present only in mitochondria (8), one would not anticipate an increase in the overall rate of pyrimidine formation in mature erythrocytes. Measurement of enzyme activities in cells containing the entire complement of pyrimidine enzymes might provide a clue as to whether or not a net increase in pyrimidine biosynthesis in HGPRTase-deficient subjects is to be expected. The two enzymes of particular interest are orotate phosphoribosyltransferase, the probable rate-limiting enzyme for the pathway (9), and the glutamine-dependent carbamylphosphate synthetase, a potentially critical site for control of pyrimidine synthesis via end-product inhibition (10) and

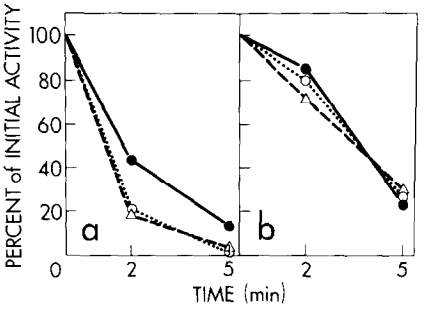

Fig. 1. Inactivation of aspartate transcarbamylase at 63°C, panel a and dihydroorotase at 65°C, panel b. Aliquots of erythrocyte lysates were removed at the times indicated and assayed for enzymatic activity. ●, control; O, 9 year old HGPRTase-deficient male; Δ, 19 year old HGPRTase-deficient male.

activation by PRPP (11). Beardmore et al. (3) were unable to demonstrate increased orotate phosphoribosyltransferase in leukocytes from patients with the Lesch-Nyhan syndrome. In fact, in two of three patients studied, leukocyte orotate phosphoribosyltransferase activity was less than 5% of normal. If such low values are a reflection of orotate phosphoribosyltransferase activity in all nucleated cells, oroticaciduria would probably occur. Using a colorimetric method (12), I was unable to detect orotic acid in the urine of the two patients with HGPRTase deficiency. A careful study of orotate phosphoribosyltransferase activities in fibroblasts and leukocytes might help to clarify this problem.

The first three enzymes in the pyrimidine pathway, glutamine-dependent carbamylphosphate synthetase, aspartate transcarbamylase and dihydroorotase probably exist in the form of a multienzyme complex in mammalian cells (13), perhaps even as a single multifunctional protein (14). Activities of the three enzymes increase or decrease in parallel in a variety of situations where tissue growth rates vary (15). In Drosophila the structural genes for these enzymes are clustered in a single complex genetic locus (16). Thus, the finding that aspartate transcarbamylase and dihydroorotase activvities are elevated in HGPRTase-deficient erythrocytes makes it likely that

carbamylphosphate synthetase activity may be similarly increased, particularly if the increases in aspartate transcarbamylase and dihydroorotase are due to increased enzyme synthesis. In terms of pyrimidine biosynthesis overall, it might be very helpful to determine the level of glutamine-dependent carbamylphosphate synthetase activity as well as orotate phosphoribosyltransferase in nucleated cells.

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