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Levels and Significance of Erythrocyte Purine Enzymes in Hyperuricemia (39555)

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The pathogenesis of hyperuricemia and gout is still largely undefined (1, 2) but it must depend ultimately upon an alteration in the synthesis and/or disposal of uric acid. Several enzymatic abnormalities have been related to an increased uric acid production. These include a deficiency in hypoxanthine-guanine phosphoribosyltransferase, a deficiency of glucose-6-phosphatase, increased activity of phosphoribosylpyrophosphate synthetase, increased activity of glutathione reductase, and increased activity of xanthine oxidase (1).

Recent studies (3, 4) on purine interconversion and salvage in mammalian cells reported an inadequate activity of purine salvage and interconverting enzymes led to excessive production and excretion of purines. These investigators introduced the concept of the "adenosine cycle" whose functioning at a high rate was predicted to lead to an increased concentration of uric acid precursors such as hypoxanthine and xanthine. The activity of the cycle was regulated by adenosine kinase (3, 4). The presence of most of the enzymes of the adenosine cycle, adenosine deaminase (5–9), adenosine kinase (5–7), hypoxanthine-guanine phosphoribosyltransferase (10–15), and 5′-nucleotidase (16), has been reported in the human erythrocyte.

In view of the possible association between the activity of the adenosine cycle and the rate of purine synthesis, this report describes the activities of the following enzymes: adenosine kinase (EC 2.7.1.20), adenosine deaminase (EC 3.5.4.4), hypoxanthine phosphoribosyltransferase (EC 2.4.2.8), adenine phosphoribosyltransferase (EC 2.4.2.7), and 5′-nucleotidase (EC 3.1.3.5) in erythrocytes from young adults who were normouricemic, hyperuricemic, or had a family history of gout. The activities of these enzymes were correlated with plasma uric acid concentrations to evaluate whether inappropriate activities of purine salvage and interconverting enzymes in the erythrocyte might be associated with hyperuricemia and the development of clinical gout.

Materials and methods. Blood samples (10 ml) were collected in EDTA from 1174 University of California, Davis, students during their entering physical examination who willingly consented to participate in a study assessing the factors which may predispose them to premature coronary heart disease. The plasma was separated from the red blood cells by centrifugation at 2000*g*.

Erythrocytes from hyperuricemic (plasma uric acid >7 mg/100 ml), normouricemic (plasma uric acid <7 mg/100 ml), and those with a family history of gout (response to a questionnaire) were washed three times with physiologically buffered saline at 4° and the washed erythrocytes were again isolated by centrifugation. At each washing the buffy layer containing leukocytes was discarded with the washing. The washed erythrocytes were sonicated for 30 sec and centrifuged at 17,000*g* for 20 min at 4°. Two-and-one-half milliliters of 0.15 *M* phosphate buffer, pH 6.8, was added to 0.5-ml aliquots of the supernatant from sonicated erythrocytes and these extracts were stored at −90° until all the purine enzyme assays were performed. All enzyme assays were completed within 4 months after the samples were collected.

Plasma uric acid was determined by an enzymatic spectrophotometric method (17) and the protein content of the erythrocyte extracts by the method of Munro and Fleck (18). The protein concentration in the extracts was approximately 50 mg/ml.

Purine enzyme activities were measured as described by Shenoy and Clifford (19) and the methods are briefly summarized here. The activity of 5′-nucleotidase in hemolysates was measured by the production of adenosine, inosine, and hypoxanthine from [U-^{14}C]AMP; inosine and hypoxanthine

from [U-^{14}C]IMP; and guanosine and guanine from [U-^{14}C]GMP. The reaction mixture contained three different final concentrations of AMP, GMP, or IMP: 0.0125 m*M* (sp act, 40 mCi/mmol), 0.0625 m*M* (sp act, 8 mCi/mmol), and 0.15 m*M* (sp act, 3.35 mCi/mmol); three different concentrations of $MgCl_2$: 0, 40, and 80 m*M*; phosphate (50 m*M*) or Tris-maleate buffer (100 m*M*, pH 6.8), and 5–30 μl of erythrocyte extract in a total volume of 200 μl (made up with deionized water). The reaction mixture was incubated for 10 min at 37°. The reaction was stopped with 15 μl of ice-cold 70% $HClO_4$ and allowed to stand in ice for 15 min before being neutralized with 30 μl of 7 *N* KOH. The mixture was centrifuged for 10 min (3000*g* at 4°) and 10 μl of the supernatant was spotted on cellulose chromatographic sheets with appropriate standards. The plate was chromatographed (ascending) using deionized water as the developing solvent. Then the reactants and products were located under ultraviolet light, transferred to a vial containing 10 ml of scintillation fluid, and the radioactivity was measured.

Adenosine kinase, hypoxanthine phosphoribosyltransferase, and adenine phosphoribosyltransferase were assayed by measuring the amount of radioactive purine base or nucleoside incorporated into the product nucleotides which were isolated as lanthanum salts after precipitation with 0.5 *M* $LaCl_3$. The final reaction mixture for adenosine kinase contained 0.05 m*M* adenosine (sp act, 10 mCi/mmol), 50 m*M* phosphate buffer (pH 6.8), 2.5 m*M* ATP, 0.25 m*M* $MgCl_2$, and 10 μl of erythrocyte extract (approximately 50 mg of protein per ml), in a total volume of 200 μl (made up with deionized water).

The final reaction mixture for hypoxanthine phosphoribosyltransferase and adenine phosphoribosyltransferase contained 0.45 m*M* hypoxanthine or adenine (sp act, 1.1 mCi/mmol), 1 m*M* phosphoribosyl-1-pyrophosphate (dimagnesium salt), 0.625 m*M* $MgCl_2$, 50 m*M* phosphate buffer (pH 6.8), and 10 μl of erythrocyte extract, in a total volume of 200 μl (made up with deionized water). A reaction time of 10 min was used for these enzyme assays.

Adenosine deaminase was assayed by isolating and measuring inosine, hypoxanthine, and IMP formed from labeled adenosine. The final reaction mixture contained 0.1 m*M* adenosine (sp act, 5 mCi/mmol), 50 m*M* phosphate buffer (pH 6.8), and 10 μl of erythrocyte extract in a total volume of 200 μl (made up with deionized water). After incubation for 8 min at 37°, the reaction was terminated with 15 μl of 70% $HClO_4$, giving a final concentration of 1 *N* in the reaction mixture. The mixture was then heated for 1 hr in a boiling water bath in order to transform nucleotides and nucleosides to purine bases. The acid mixture was neutralized to pH 7.0 with 30 μl of 7 *N* KOH, centrifuged at 3000*g*, and hypoxanthine was separated from adenine by thin-layer chromatography (ascending) using water as the developing solvent. The adenosine deaminase activity was determined from the radioactivity recovered in hypoxanthine. The accuracy of the procedure was checked by measuring the radioactivity remaining in adenosine or recovered in inosine, hypoxanthine, and IMP (without acid-heating) using the two-dimensional thin-layer chromatography method described by Henderson, *et al.* (20). Chemicals used in the present study were as we have described previously (19).

Results. For the population from which subjects were selected for the present study, a plasma uric acid level of 7.0 mg/100 ml was chosen as the upper limit of the normal range, and individuals with levels above this limit were defined as hyperuricemic. Using this definition, 4.1% of the total population were hyperuricemic and 7.4% had a family history of gout. Hyperuricemia occurred in 14.6% of those with a family history of gout.

From the total population of 1174 subjects (742 males, 432 females, mean age 23 ± 5 years), 99 were selected for erythrocyte enzyme measurements. The selection was based upon plasma uric acid values and presence of a family history of gout. The distribution of the population into normouricemic, hyperuricemic, and family history of gout groups, and the purine enzyme activities are presented in Table I. The data demonstrate an absence of 5′-nucleotidase, a lack of striking differences in enzyme activities among the groups except for HPRT

which was lower (14 and 17%) in the hyperuricemic and family history of gout groups, respectively, compared with the normouricemic group and APRT which was higher (15%) in those with a family history of gout.

When the ratio APRT/HPRT was calculated, it was elevated by 19 and 36% in the hyperuricemic and subjects with a family history of gout, respectively, compared with the normouricemics. Sixty-six percent (4/6) of the hyperuricemic subjects who also had a family history of gout had an elevated APRT/HPRT ratio when compared with normouricemic subjects without a family history of gout. Only 33% (2/6) of the hyperuricemic subjects who also had a family history of gout had an elevated APRT/HPRT ratio when compared with normouricemic subjects with a family history of gout. The mean APRT/HPRT ratio for the six hyperuricemic subjects with a family history of gout did not differ significantly from that of the normouricemic subjects in this group (Table II). Plasma uric acid levels and erythrocyte enzyme activities did not differ between males and females within each group.

All active enzyme assays resulted in a linear formation of product within 8- to 10-min assay periods and a linear product formation at different enzyme concentrations.

Discussion. The purpose of this investigation was to correlate the activity of purine enzymes and plasma uric acid in an attempt to evaluate the hypothesis that inappropriate patterns of activities of purine salvage and interconverting enzymes might be associated with hyperuricemia and the development of clinical gout.

TABLE I. ERYTHROCYTE PURINE ENZYME ACTIVITIES AND PLASMA URIC ACID LEVELS IN YOUNG ADULTS WHO WERE NORMOURICEMIC, HYPERURICEMIC, OR HAD A FAMILY HISTORY OF GOUT.

	Control	Hyperuricemic	Family history of gout
Number of subjects	27	31	41
Male subjects	21	29	23
Female subjects	6	2	18
Plasma uric acid (mg/100 ml)	4.9[a] ± 1.1	8.1 ± 0.9[b]	5.1 ± 1.8
Adenosine deaminase[c]	29.9 ± 13.7	31.4 ± 12.9	26.5 ± 8.8
Adenine phosphoribosyltransferase[c]	5.1 ± 1.1	5.5 ± 1.0	5.9 ± 1.1[d]
Hypoxanthine phosphoribosyltransferase[c]	89.4 ± 15.2	79.3 ± 12.3[b]	75.9 ± 17.2[b]
Adenosine kinase[c]	11.2 ± 1.6	11.9 ± 1.8	11.5 ± 1.7
5′-Nucleotidase[c]	None	None	None
APRT/HPRT × 10^3	59 ± 16	70 ± 16[b]	80 ± 19[b]

[a] Values are means ± SD.

[b] Horizontal values different from control ($P < 0.001$).

[c] Enzyme activities are micromoles of product produced per gram of protein per hour. The products were adenosine deaminase = inosine + hypoxanthine; adenine phosphoribosyltransferase, hypoxanthine phosphoribosyltransferase, and adenosine kinase = $LaCl_3$-precipitated nucleotides; 5′-nucleotidase = adenosine + inosine + hypoxanthine or guanosine + guanine or inosine + hypoxanthine.

[d] Horizontal values different from control ($P < 0.025$).

TABLE II. APRT/HPRT RATIO[a] IN HYPERURICEMIC SUBJECTS WITH A FAMILY HISTORY OF GOUT.

Subject no.	Plasma uric acid (mg/100 ml)	APRT (μmol of product/g of protein/hr)	HPRT (μmol of product/g of protein/hr)	APRT/HPRT × 10^3
0112	7.6	6.43	72.7	88
1947	9.7	5.16	76.9	67
1846	8.0	6.19	62.5	99
1668	9.7	5.16	76.9	67
1730	7.3	5.15	88.5	58
1036	7.9	4.13	85.1	48
Control[b]	4.5 ± 1.2	5.95 ± 1.09	75.7 ± 18.4	82 ± 19

[a] Adenine phosphoribosyltransferase to hypoxanthine phosphoribosyltransferase ratio.

[b] Mean of the 35 remaining normouricemic subjects with a family history of gout.

On the basis of the selection criteria used, 31 subjects were hyperuricemic (mean plasma uric acid 8.0 mg/100 ml). The normouricemic (controls) and the group with a family history of gout had similar mean plasma uric acid levels (Table I) which were within the range expected for young adults of this age (2, 21, 22). This was somewhat surprising since in gout, a genetically linked disease having as a first-step hyperuricemia (21, 22), a somewhat higher plasma uric acid level in this group than in the controls might be expected. Since this was not the case, one might speculate that this group was composed of normouricemic individuals with a high probability of latent hyperuricemia and gout.

Changes in adenine phosphoribosyltransferase and hypoxanthine phosphoribosyltransferase activities in young adults with hyperuricemia or a family history of gout extend to this type of subject for the first time, observations already made on Lesch-Nyhan and gouty patients (23). Although the biological significance of the above altered APRT/HPRT ratio is not yet clear, a high adenine phosphoribosyltransferase activity in subjects with low hypoxanthine phosphoribosyltransferase activity might reflect enzyme stabilization by increased phosphoribosylpyrophosphate levels (24). The APRT/HPRT ratio which was elevated in cases of high risk of gout [hyperuricemic or family history of gout (21)] could, if confirmed in a larger population of young adults, represent a diagnostic indicator of preclinical gout. This idea is supported by the data presented in Table II. It is especially noteworthy that the highest ratio of APRT/HPRT occurred in normouricemic subjects with a family history of gout.

Although the lack of correlation between erythrocyte hypoxanthine phosphoribosyltransferase and adenine phosphoribosyltransferase activity and serum uric acid has been reported previously (23), the lack of correlation between plasma uric acid and erythrocyte adenosine deaminase and adenosine kinase activities has not been reported previously.

The low activity of erythrocyte 5′-nucleotidase in this study, together with the lack of an enzymatic system for the conversion of IMP to AMP in the human erythrocyte (25), further suggests that the adenosine cycle (3) is incomplete in the human erythrocyte. An incomplete adenosine cycle probably explains the lack of correlation between the activity of purine enzymes and plasma uric acid levels, and suggests the need for utilizing other tissues, in addition to the erythrocyte, to search for this correlation. The higher ratio of APRT/HPRT in erythrocytes from subjects of high risk of gout could, if confirmed in a larger population of young adults, be of diagnostic value in preclinical gout.

Summary. To examine possible relationships among purine enzyme patterns in hyperuricemia and gout, purine enzyme activities were measured in erythrocytes from young adults who were either normouricemic, hyperuricemic, or had a family history of gout. The purine enzymes whose activities were measured included adenine phosphoribosyltransferase (APRT), hypoxanthine phosphoribosyltransferase (HPRT), adenosine deaminase (AD), adenosine kinase (AK), and 5′-nucleotidase. AD and AK activities were not different among the normouricemic, hyperuricemic, or family history of gout groups. The activity of APRT was significantly higher in the group with a family history of gout compared with the normouricemics, and the activity of HPRT was significantly lower in the hyperuricemic and family history of gout groups compared with the normouricemics. The ratio of APRT/HPRT was significantly greater in 66% of those subjects who had a family history of gout and were also hyperuricemic compared with normouricemics without a family history of gout. No measurable 5′-nucleotidase was found in human erythrocyte hemolysates. The lack of correlation between the activities of AD and AK in erythrocytes and plasma uric acid levels may be due to the lack of 5′-nucleotidase in this tissue since in the absence of 5′-nucleotidase the erythrocyte has a minimal capacity to break down nucleotides.

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