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Dietary-Induced Variation of Hypoxanthine-Guanine Phosphoribosyl Transferase Activity in Patients with the Lesch-Nyhan Syndrome

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ABSTRACT We have studied three patients with the Lesch-Nyhan syndrome to assess the effect of dietary purines on erythrocyte hypoxanthine-guanine phosphoribosyltransferase (HPGRT) activity. During dietary purine restriction HPGRT activity rose in all three patients; resumption of normal dietary purine intake or the addition of adenine (10 mg/kg per day) to a purine-free diet resulted in a fall in HPGRT activity. These changes in enzyme activity appeared to be due to an activation or inactivation of the mutant enzyme without a change in the half-life or absolute amount of HPGRT enzyme protein.

INTRODUCTION

The X-linked, virtually complete deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPGRT)¹ activity results in a bizarre neurologic disorder, the Lesch-Nyhan syndrome (1). After the initial description of the enzyme defect, several investigators noted low levels of HPGRT activity in hemolysate from patients with this syndrome (2, 3) while most found HPGRT activity undetectable (4-6). More recently we have noted a wide range of HPGRT activity over time in the same patient suggesting that environmental factors might partly account for this discrepancy. Since the deficiency of HPGRT activity in the Lesch-Nyhan syndrome is associated with a normal level of catalytically-incompetent HPGRT protein (7, 8), activation of the mutant protein by environmental manipulation

represents one possible approach to the therapy of patients with this disease. The present report describes the influence of alterations in dietary purine content on HPGRT activity in erythrocytes from three patients with the Lesch-Nyhan syndrome. The changes observed in HPGRT activity are shown to be due to activation or inactivation of preformed HPGRT protein rather than a change in the absolute amount of the protein.

METHODS

Three patients with the classical Lesch-Nyhan syndrome were hospitalized on the Clinical Research Unit of Duke University Medical Center and received isocaloric diets which, as specified later, were either free of purines, contained a normal quantity of purines or contained the amount of adenine (10 mg/kg per day in four divided doses) present in the normal diet. Allopurinol (150-300 mg/day) was administered orally to each of the patients during the entire period of study.

HPGRT was assayed in hemolysate by a previously described radiochemical technique using guanine as substrate (8, 9). [8-¹⁴C]guanine (60 mCi/mmol) was obtained from New England Nuclear Corp., Boston, Mass. Identification of the product of the reaction as guanosine monophosphate (GMP) after incubation of mutant hemolysate with [¹⁴C]guanine, magnesium, and PP-ribose-P was confirmed in four different chromatographic systems as described previously (8). Background (150-250 cpm) was defined by the radioactivity migrating with carrier GMP after incubation of a dialysed preparation of hemolysate with a reaction mixture which was complete except for the absence of PP-ribose-P. Each increment in specific activity of 0.001 nmol/mg per h represented 75 counts above background. Orotate phosphoribosyltransferase (OPRT), and orotidylidic decarboxylase (ODC) were assayed as described previously (10, 11).

Density fractionation of intact circulating erythrocytes was also performed by a previously described technique (8). Semiquantitative immunodiffusion using a monospecific rabbit antisera to a homogeneous preparation of normal

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¹ Abbreviations used in this paper: HPGRT, hypoxanthine-guanine phosphoribosyltransferase; GMP, guanosine monophosphate; ODC, orotidylidic decarboxylase; OPRT, orotate phosphoribosyltransferase.

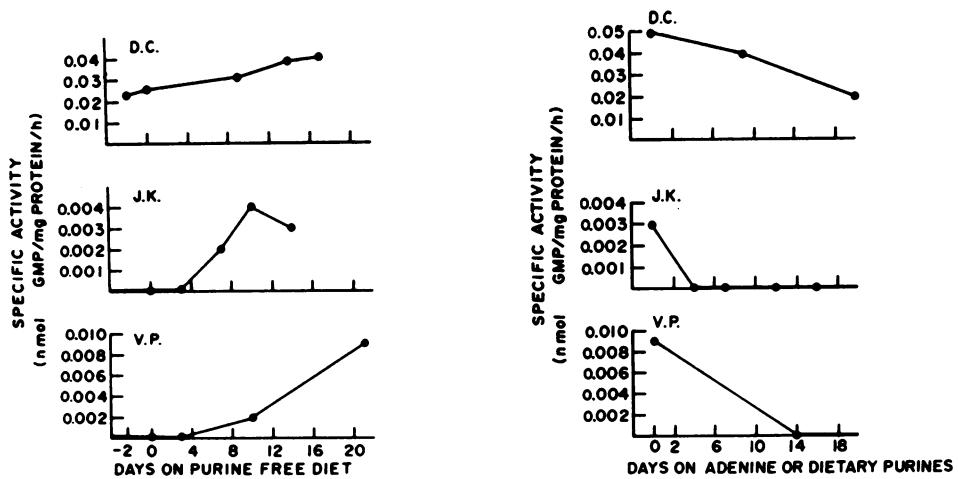


FIGURE 1 The effect of the dietary purines or adenine on erythrocyte HGPRT activity. The panel on the left illustrates the effect of purine restriction on erythrocyte HGPRT activity while the panel on the right illustrates the effect of normal dietary purine content either by the administration of adenine (D. C. and J. K.) or by reinstatement of a diet containing a normal amount of purines (V. P.) on HGPRT activity.

human erythrocyte HGPRT was performed as previously described (8). This technique was standardized by using known quantities of pure human HGPRT. Changes in concentration of 13 $\mu\text{g}/\text{ml}$ (representing approximately 10% of the HGPRT protein present in 1 ml of hemolysate) could be detected (8).

RESULTS

The effect of a purine-free diet on the level of HGPRT activity in hemolysate from three patients with the Lesch-Nyhan syndrome is illustrated in the left hand panel of Fig. 1. Each patient demonstrated an increase in erythrocyte HGPRT activity beginning from 7 to 14 days after withdrawal of purines from the diet. In two of the three patients the activity was initially undetectable and rose at least three- to ninefold; in the third patient an increase to 54% above control values was observed.

The right hand panel of Fig. 1 illustrates the reduction in HGPRT activity seen with the addition of adenine to a purine-free diet (patients D. C. and J. K.) or the resumption of a diet with a normal content of purines (patient V. P.). In two of the patients, erythrocyte HGPRT activity became essentially undetectable (J. K. and V. P.) while in the third patient (D. C.) the HGPRT activity decreased by 58%.

The standard error of the mean observed from 10 different determinations of HGPRT activity in a single sample of hemolysate from D. C. and V. P. was 2.9% and 5.2% of the mean activity respectively. This, as well as the consistent overall trend in all three patients, suggests that the changes in HGPRT activity observed with dietary manipulation cannot be attributed to assay

variability. In all three patients the activity of OPRT and ODC was unchanged during the entire period of study.

The effect of adenine administration on HGPRT activity in circulating erythrocytes fractionated by their density is shown in Fig. 2. HGPRT activity decreased to approximately the same extent in all cell fractions; thus the apparent half-life of HGPRT activity *in vivo* was unchanged.

The effect of dietary purine restriction on the level of immunoreactive HGPRT protein in each of the three patients is illustrated in Table I. Despite the increase in

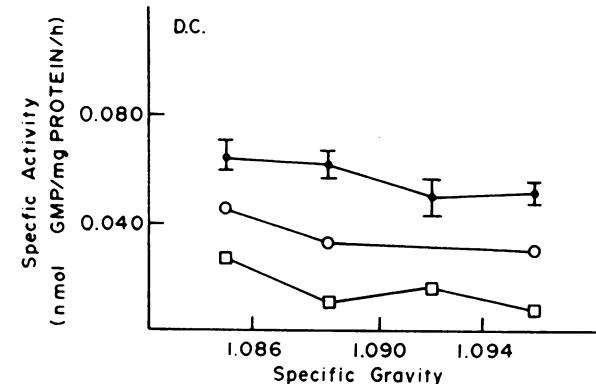


FIGURE 2 The effect of adenine administration (10 mg/kg per day) on HGPRT activity in density-fractionated erythrocytes from patient D. C. Range of two separate determinations during the control period (vertical bars); 9 days after beginning adenine (○-○); 14 days after beginning adenine (□-□).

TABLE I
Comparison of HGPRT Activity and Immunoreactive HGPRT Enzyme Protein (CRM) Before and After Purine Restriction in Three Patients with the Lesch-Nyhan Syndrome

Patient	Diet	HGPRT activity nmol/mg/h	HGPRT protein (CRM)
			μg/ml hemolysate
D. C.	Constant purine	0.022	133
	Purine free*	0.037	133
J. K.	Constant purine	<0.001	130
	Purine free*	0.003	130
V. P.	Constant purine	<0.001	133
	Purine free*	0.003	133

* These values were obtained 14-17 days after the initiation of purine restriction.

enzyme activity, the amount of enzyme protein was unchanged.

DISCUSSION

These studies demonstrate that exogenous influences in the form of dietary purines can alter the phenotypic expression of the basic genetic defect responsible for the Lesch-Nyhan syndrome, i.e., the level of HGPRT activity. The finding that the oral administration of adenine, at roughly the level ordinarily found in the diet, produces essentially the same effect as an unrestricted diet, suggests that the effect of dietary purines can be at least partly attributed to adenine.

The addition of adenine to the diet results in a decrease of HGPRT activity in young as well as old erythrocytes with no change in the apparent half-life of HGPRT. If adenine were reducing HGPRT activity in vivo by repressing the synthesis of HGPRT protein in nucleated erythrocyte precursors one would anticipate a selective decrease of HGPRT activity in the youngest cells. If adenine were reducing HGPRT activity by destabilization of the protein in vivo, this should be reflected in a shortened apparent half-life of HGPRT in circulating erythrocytes during adenine administration. Since neither of these changes were noted, this suggests that the effect of adenine is due, in some manner, to inactivation of HGPRT activity and not due to a change in the rate of synthesis or degradation of the protein itself. This conclusion is further supported by the observation that the level of immunoreactive HGPRT protein remained constant during dietary purine restriction despite a 60-300% increase in HGPRT activity.

At present the mechanism responsible for this alteration in HGPRT activity is obscure. The changes of HGPRT activity observed in this study are difficult to relate to the apparent activation of mutant HGPRT

observed in vitro by Bakay and Nyhan (12) since the activation reported by these investigators required the presence of normal HGPRT protein.

These observations provide one explanation for the variability of HGPRT activity observed with time in a given patient with the classical Lesch-Nyhan syndrome. However, since each of the three patients in this study had quite different maximal levels of HGPRT activity despite similarities in diet and drug ingestion, other factors must contribute to the variability observed between patients. It seems likely that this is a reflection of the genetic heterogeneity previously documented in this disease (13). In addition, this study suggests that dietary purine restriction could be of therapeutic benefit in some patients. Although we did not observe any striking alterations in the clinical course of our patients during purine restriction it is feasible that salutary effects may become evident after more prolonged dietary restriction.

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