

95

Simple screening methods for disorders of purine metabolism using dried blood and/or urine spots on filter paper

Y. Nishida and T. Miyamoto  
Department of Medicine and Physical Therapy  
Faculty of Medicine, University of Tokyo,  
Bunkyo-ku, Tokyo, 113, Japan.

Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and adenine phosphoribosyltransferase (APRT) function in the metabolic salvage of purines. Although these enzyme deficiencies are extremely rare, an early diagnosis is required to prevent renal damage. Therefore simple methods for the detection of HGPRT and/or APRT deficiencies using dried filter paper blood and/or urine spots were studied. Enzyme activity in the eluate from dried filter paper blood spots stored for 4 weeks at room temperature were shown to be quite stable. DE-81 paper allows immobilization of the nucleotides, while removing purine bases by washing. Autoradiographs prepared from dried filter paper blood spots and DE-81 paper soaked with enzyme reaction mixtures containing  $^{14}\text{C}$ -hypoxanthine and/or  $^{14}\text{C}$ -adenine showed sharp radioactive spots in normal subjects. No activity was seen in the case of the Lesch-Nyhan syndrome and/or APRT deficiency. The concentration of uric acid, creatinine and 2,8-dihydroxyadenine (2,8-DHA) in the eluates from dried filter paper urine spots were also shown to be measurable. The high ratio of uric acid to creatinine and/or the demonstration of 2,8-DHA in these eluates was also useful for the screening of HGPRT and/or APRT deficiencies. This method is very convenient for sample collection and simplifies the transfer of samples.

96

Enzyme therapies for hyperuricemia  
Y. Nishida and T. Miyamoto  
Department of Medicine and Physical Therapy  
Faculty of Medicine, University of Tokyo,  
Bunkyo-ku, Tokyo, 113, Japan.

The relationship between dietary factors and the prevalence of gout is well known. Many gouty patients eat food rich in purines and excrete increased urinary urate. The dietary purines may contribute to hyperuricemia. When enzyme activity related to purine metabolism in the human digestive juice was measured, nucleotidase, purine nucleoside phosphorylase and xanthine oxidase activity was shown in bile and pancreatic juice, although they were not so high. Therefore, the effect in chickens of oral administration of enzymes related to purine catabolism on plasma uric acid concentrations was studied. After oral administration of inosine to chickens, plasma uric acid concentrations increased markedly. Prior oral administration of the enzymes purine nucleoside phosphorylase, xanthine oxidase and uricase prevented the rise in plasma uric acid due to inosine intake. It seems that these enzymes administered orally degrade the dietary purines during gastrointestinal passage. Secondly, liposomal-entrapped methoxypolyethyleneglycol (PEG) modified uricase was used to study its plasma urate lowering effect after oral administration to chickens. Plasma uric acid concentrations fell gradually and were accompanied by a rise in plasma uricolytic activity. Oral administration of purine enzymes and/or PEG-modified uricase liposomes may have a useful effect as a new enzyme therapy for hyperuricemia and gout.

97

High-Performance Liquid Chromatographic method for Simultaneous Screening of the Deficiencies of APRT and HGPRT  
Toshihiro NISHINA, Ryozo SAKUMA,  
Tsukasa KOJIMA, Motoshi KILAMURA (Toranomon  
Hosp. Tokyo, JAPAN) Naoyuki KAMATANI, Kusuki  
NISHIOKA (Tokyo Women's Medical College)

A rapid and convenient screening method using high-performance chromatography (HPLC) for the simultaneous detection of deficiencies of adenine phosphoribosyltransferase (APRT, EC 2.4.2.7) and hypoxanthine guanine phosphoribosyltransferase (HGPRT, EC 2.4.2.8) is presented.

The enzyme reactions were started by the addition of 0.1 ml of the red cell lysates (2-5g Hb/dl) treated with charcoal-dextran into 0.5 ml of the reaction mixture A (2 mM PRPP, 6 mM  $\text{MgCl}_2$ , 1 mM HX and 0.2 mM adenine in 50 mM Tris-HCl pH7.4). After incubation at 37°C, 30 min, 0.5 ml of reagent B (20 U/ml ALP, 4 mM  $\text{MgCl}_2$  in 1 M Tris) were added and incubated for 30 min. at 37°C for the conversion of AMP and IMP into adenosine and inosine. ALP reaction was stopped by adding 1.0 ml of 0.5 M  $\text{HClO}_4$ . The mixture centrifuged at 3,000 x g for 10 min. The supernatants were used as samples for HPLC analysis. Normal ranges of APRT and HGPRT activities in erythrocytes obtained 28 healthy subjects were  $0.40 \pm 0.06$  and  $1.97 \pm 0.19$   $\mu\text{mol/min/g Hb}$ , respectively. The method could detect down to 1% of normal APRT activity and 0.3% of normal HGPRT activity.

98

STRUCTURES OF XANTHINE DEHYDROGENASES FROM CHICKEN AND RAT LIVERS.

Tomoko Nishino, Takeshi Nishino and Keizo Tsushima.  
Yokohama City University, School of Medicine,  
Department of Biochemistry, Yokohama 236, Japan

Chicken and rat liver xanthine dehydrogenases have similarity in their molecular weights and composition of cofactors. But rat enzyme is known to be convertible from NAD-dependent to  $\text{O}_2$ -dependent type, while chicken enzyme is not convertible easily. In order to know the structural differences between rat and chicken enzymes, limited digestion of both enzymes with protease and chemical modification of NAD-binding site of both enzymes were carried out. Chicken enzyme was modified with 5'-FSBA with the stoichiometry of one mole per mole of enzyme bound FAD and the modified residue was identified to be a tyrosine. Limited digestion of chicken enzyme with subtilisin produced 81 k, 32 k and 20 k dalton of main peptides, and the  $^{14}\text{C}$ -FSBA modified residue was found to be located in 32 k dalton. After further digestion of  $^{14}\text{C}$ -FSBA modified peptide with  $\text{V}_8$  protease,  $^{14}\text{C}$ -FSBA peptide of 14 amino acids was obtained and sequenced. On the other hand, rat enzyme was modified with 5'-FSBA with the stoichiometry of 1.5 moles per mole of enzyme bound FAD. Compared to chicken enzyme, the modified enzyme was not reactivated by DTT, suggesting that the modified residue is a lysine. The acid hydrolysate of modified enzyme contained two kinds of modified amino acid residues, CBS-lysine and CBS-tyrosine. The limited digestion of rat enzyme with subtilisin yielded three main peptides of 89 k, 44 k and 19 k dalton. When  $^{14}\text{C}$ -FSBA modified enzyme was digested with protease, radioactivity was incorporated into 89 k and 44 k peptides.

99

LATE ONSET EPISODIC LOSS OF CONSCIOUSNESS IN PATIENTS WITH GOUT AND HYPOXANTHINE GUANINE PHOSPHORIBOSYL TRANSFERASE (HGPRT) DEFICIENCY: ASSOCIATED WITH MARKEDLY RAISED CEREBROSPINAL FLUID (CSF) HYPOXANTHINE CONCENTRATIONS

George Nuki<sup>1</sup>, Angus Harkness<sup>2</sup>, Harry L Rylance<sup>1</sup> and Ruth C Wallace<sup>1</sup>  
University of Edinburgh Rheumatic Diseases Unit, Edinburgh EH5 2EO  
Scotland, and MRC Clinical Research Centre<sup>2</sup>, Middlesex HA1 3UU, UK.

Two men (ages 24 and 45) with gout and HGPRT deficiency (RBC extracts <1% normal) but no previous evidence of neurological disturbance began to develop episodes of loss of consciousness without movement disorder 19 and 22 years after starting treatment with allopurinol. Cardiovascular and neurological examination were normal, as were EEGs, CT scans and plasma glucose, electrolytes, urate and creatinine. CSF amino acid concentrations were normal. HPLC measurements of CSF and plasma purines off allopurinol therapy are shown in the table.

As in previous studies of CSF oxypurines in Lesch-Nyhan syndrome CSF hypoxanthine concentrations were 4-6 fold higher than those in plasma suggesting local CNS production. Brain concentrations of hypoxanthine may be high enough in patients with gout and HGPRT deficiency to trigger seizures by interfering with diazepam receptor binding and gamma-aminobutyric acid (GABA) function<sup>1,2</sup>.

Oxypurine concentrations in CSF and plasma (mmol/l)

Patient	CSF			Plasma		
	Hx	X	Urd	Hx	X	Urd
SF	21.0	3.7	5.7	3.5	1.8	7.2
JB	28.2	8.1	5.7	4.7	1.4	-
Normal mean (SD)	1.8 (1.1)	1.7 (1.0)	1.6 (2.0)	2.0 (1.5)	0.8 (0.1)	2.9 (1.0)

References

1. Kish, S.J. et al, Brain Res., 336, 117-123: 1985.
2. Olson, R.W. et al, Adv. Neurology, 44, 365-378: 1986.

100

PERSISTENT PRODUCTION OF HYPOXANTHINE IN RAT SKELETAL MUSCLE CAUSES PROLONGED HYPERURICEMIA AFTER AN EXHAUSTIVE EXERCISE  
MASASHI OGASAWARA, TETSUYA SEINO, SHINJI HADANO\*, AKIRA ITOH, HIROSHI GOTOH AND MITSUO ITAKURA\*

Institute of Health and Sports Science, \*Institute of Clinical Medicine, University of Tsukuba, Tsukuba-shi, Ibaraki, 305 JAPAN

In previous studies on exercise-induced hyperuricemia, we have reported that serum uric acid (UA) increases at 1-2 through 24 hours after an exhaustive exercise in human subjects, and that plasma oxypurine levels increase 1 through 7 hours after the end of an exhaustive exercise in human subjects taking allopurinol. To study the mechanism of such prolonged exercise-induced hyperuricemia, we have used an animal model in this study. The maximal physical activity was enforced for 21 minutes on a treadmill to adult male Wistar rats to which allopurinol was administered beforehand. The concentrations of purine metabolites in plasma and muscle were assayed by HPLC. Plasma UA was consistently low due to the administration of allopurinol. Plasma hypoxanthine (HX) and xanthine reached the peak, respectively, at 5 and 30 min, and they showed respectively, a higher tendency and a statistically higher level compared to the control rats without an exercise for 5 hours. In plantaris muscle, ATP decreased with increased IMP at 5 min. Inosine (Ino) increased significantly at 5 min and showed a higher tendency for 1 hour. HX increased significantly at 5 min through 2 hours. These data suggest that the production of HX from Ino in skeletal muscle persists after the end of an exhaustive exercise leading to the continuous release of HX to blood for at least 2 hours. Based on these, it is concluded that persistent production of HX in skeletal muscle even after the end of an exhaustive exercise is responsible for prolonged exercise-induced hyperuricemia.