65 MOLECULAR GENETIC STUDIES ON URICASE AND ONE ASPECT OF ITS EVOLUTIONAL BEHAVIOR

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The enzymes responsible for the degradation of urate have been lost one by one during evolution. In order to study the inactivation of genes coding for these enzymes during evolution, we tried to isolate cDNAs for uricase. A full length-cDNA for rat liver uricase was isolated and nucleotide sequence was determined. The predicted amino acids sequence showed a polypeptide of 280 amino acids with a molecular weight of 32,226 daltons. The amino acids sequence deduced was compared with that of soybean nodulin uricase and nine highly homologous stretches of the two enzymes were found.

Then, we searched for the inactivated uricase-gene in chicken. A 35k daltons-protein cross-reacting with antibody against rat liver uricase was recognized in the homogenates of the embryo from 4 days to 12 days after the fertilization and disappeared after that time point. Therefore, chicken might express the enzyme at some stage of embryonic development.

66 MAMMALIAN XANTHINE OXIDOREDUCTASE – A UNIQUE ENZYME AMONG HYPOXANTHINE--HYDROXYLATING ENZYMES IN VERTEBRATES

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Xanthine oxidoreductase preparations were obtained from liver of: fish Cyprinus carpio /ammonotelic/, snake Natrix natrix and chicken Gallus gallus /both uricotelic/, toad Bufo viridis and Wistar rat /both ureotelic/. The course of hypoxanthine → xanthine → → uric acid hydroxylation catalyzed by NAD^+- dependent xanthine oxidoreductases was found to be specific for each species, and especially different in case of the uricotelic ones. Substrate competition between xanthine and hypoxanthine for the enzyme active centers seems to occur for xanthine oxidoreductases from ammono- and ureotelic species, but not from the uricotelic ones.

The enzyme from fish, snake, toad and chicken liver was inhibited by NADH at micromolar concentrations and that from rat liver – by NADH at nanomolar concentrations; this suggests that in vivo the activity of only rat enzyme could be dependent on changes in the NAD^+/NADH ratio in cell. Therefore, only in mammals the inhibition of xanthine oxidoreductase by NADH may spare hypoxanthine for the salvage pathway of purine nucleotide biosynthesis.

67 HYPOXANTHINE AND XANTHINE TRANSPORT THROUGH THE BLOOD-BRAIN BARRIER IN HYPOXANTHINE PHOSPHORIBOSYL-TRANSFERASE (HPRT) DEFICIENCY.

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The transport of purines nucleosides and bases through the blood-cerebrospinal fluid (CSF) barrier is incompletely understood. Allopurinol increases plasma purine but should not elevate CSF purines as a result of xanthine oxidase inhibition due to the absence of this enzyme in the central nervous system (CNS). We have examined the effect of allopurinol (5-10 mg/kg/24 h) on plasma and CSF uric acid (UA), hypoxanthine (Hx), xanthine (X) and inosine (Ino) concentrations in 4 patients with HPRT deficiency (less than 1% of normal erytrocyte HPRT levels). Results (means±SEM) were as follows:

	PLASMA (μM)				CSF (μM)			
	UA	Hx	X	Ino	UA	Hx	X	Ino
Controls (n=4)	286±12	1,7±0,4	0,9±0,2	0,9±0,2	12±9	3,3±1,1	2,0±0,2	0,6±0,2
HPRT deficiency								
Basal state	593±114*	8,7±1,6*	2,0±0,3*	1,6±0,5*	26±6	17,5±2,8*	4,2±0,3*	0,6±0,1
On allopurinol	205±66+	38,6±1,9+	21,0±3,5+	3,9±1,1+	ND*	35,0±4,6+	11,9±1,9+	0,6±0,1

*P<0,01 vs controls; +P<0,01 vs basal state; *not detectable.

Allopurinol increased plasma Hx, X and Ino concentrations 4-fold, 10-fold and 2.4 fold, respectively, compared to base-line values. The mean absolute increase in CSF Hx and X concentrations during allopurinol treatment was 17.5 μM and 7.7 μM, respectively. These results indicate that Hx and X may be transported to the brain through the blood-brain barrier in HPRT deficiency.

68 INCREASED PURINE NUCLEOTIDE DEGRADATION IN THE CENTRAL NERVOUS SYSTEM (CNS) IN PRPP SYNTHETASE SUPERACTIVITY.

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The end products of purine metabolism in the CNS are hypoxanthine for adenine nucleotides and xanthine for guanine nucleotides. Previous reports of two families with PRPP synthetase superactivity associated with neurodevelopmental defects have not documented metabolism of purines in the CNS. Fibroblasts extracts from an eight-year-old male with tophaceous gout and sensorineural deafness and from his mother with gout showed an aberrant PRPP synthetase characterized by resistance to purine nucleotide inhibition of enzyme activity. Hypoxanthine and xanthine concentrations in plasma and cerebrospinal fluid (CSF) were simultaneously measured by HPLC in both patients. In 4 normal subjects hypoxanthine and xanthine levels in plasma were (mean±SEM) 1.7±0.4 μM and 0.9±0.2 μM, respectively, and 3.3±1.1 and 2.0±0.2 μM in CSF. The hemizigous male showed a substantially increased hypoxanthine concentration of 5.6 μM in plasma and of 22.1 μM in CSF. Xanthine levels were 1.8 μM in plasma and 4.5 μM in CSF. The heterozigous female had moderately elevated plasma and CSF hypoxanthine concentrations (3.9 and 10.6 μM) and normal xanthine levels (1.3 and 1.8 μM).

These results suggest an increased purine nucleotide degradation in the CNS of patients with PRPP synthetase superactivity and neurological symptoms. The predominance of hypoxanthine over xanthine may indicate a relatively enhanced adenine nucleotide over guanine nucleotide degradation.

69 THE HPRT PROMOTER.

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The human HPRT promoter, like that of other "housekeeping" genes, lacks the TATA box transcriptional signal which is commonly found 20-30 nucleotides 5' to the RNA start sites of most other characterized genes. Since several viral genes contain regions upstream from the TATA box that can be inverted without impairing transcription, we decided to study the effect of orientation on HPRT promoter activity. Recombinant plasmids containing the human HPRT promoter in either orientation upstream from the firefly luciferase (lux) reporter gene were transfected into HeLa cells and luciferase activity was assayed after 48 hours. Efficient expression of luciferase was detected, independent of the orientation of the HPRT promoter. Studies with other plasmids containing the HPRT promoter flanked by both the luciferase and the chloramphenicol acetyltransferase (CAT) genes demonstrated that the HPRT promoter could function bidirectionally, and drive expression of two reporter genes simultaneously. We are currently examining bidirectional activity of other promoters and determining the degree of bidirectional expression from the HPRT promoter in vivo.

70 EVIDENCE AND EXPLANATION FOR THE PRESENCE OF A COMMON ANCESTOR DISEASE-CAUSING GENE FOR THE JAPANESE-TYPE APRT DEFICIENCY.

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79% of all the Japanese patients with 2,8-dihydroxyadenine urolithiasis have been only partially deficient in APRT and they synthesize mutant enzyme with common altered properties (Japanese-type APRT deficiency). Based on various pieces of evidence, we have predicted that the Japanese-type patients have unique mutant gene (APRT*J) deriving from a common ancestor. Recently, Hidaka et al. identified a nucleotide substitution in exon 5 in a Japanese-type APRT deficient patient. This nucleotide substitution changes the predicted amono-acid sequence from Met to Thr at position 136. We have devised a method by which the change of Met at position 136 to another amino-acid is specifically identified. This method uses BrCN to cleave protein at Met residue and sequence-specific antisera against the probable PRPP-binding site of human APRT. Using this method, we have shown that separate families with the Japanese-type APRT deficiency exclusively synthesize methionine-free APRT. Along with other data such as RFLP of APRT gene among the patients, these data provide evidence at the molecular level for our previously presented hypothesis that the Japanese-type APRT deficiency patients are homozygotes having a unique disease-causing gene APRT*J deriving from a common ancestor gene created in an ancestor of Japanese. Explanation for the expansion of this disease-causing gene among Japanese will be presented.