Quebec Cooperative Study of Friedreich's Ataxia

Purine Metabolism in Friedreich's Ataxia

P. DRAPER, B. LEMIEUX, I. H. FOX AND D. SHAPCOTT

SUMMARY: *In a detailed investigation of nucleotide synthesis, interconversion and degradation, no difference was found between subjects with Friedreich's Ataxia and normal controls. It appears improbable that this disorder is related to a primary defect in purine metabolism.*

RÉSUMÉ: *Une investigation détaillée de la synthèse, de l'interconversion et de la dégradation des nucléotides montre qu'il n'existe aucune différence entre les sujets avec l'ataxie de Friedreich et les sujets contrôles normaux. Il semble donc improbable que cette maladie soit reliée à un défaut primaire du métabolisme des purines.*

From Le Centre Hospitalier Universitaire de Sherbrooke and the Purine Research Laboratory, Wellesley Hospital, Toronto.

Reprint requests for the complete supplement on Friedreich's ataxia (Phase Two, Part One) to: Dr. André Barbeau, Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal, Quebec, Canada H2W 1R7.

Inborn errors of purine metabolism are an important source of new discoveries in general genetic diseases as were the inborn errors of amino acid metabolism two decades ago. At least 23 enzymes are know to be involved in purine metabolism and more than 10 enzyme deficiencies have been described, both in man and animal (Henderson et al., 1974) (Table 1). Further discoveries should be expected with the new advances in methodology.

TABLE 1

Inborn Errors of Purine Metabolism

Purine Synthesis "De Novo":
1. PRPP Synthetase Mutant
2. Glutamic PRPP Aminotransferase Mutant

Nucleotide Interconversion:
?

"Salvage" Pathway:
1. Lesch-Nyhan Syndrome: total HGPRT deficiency (EC 2.4.2.8.)
2. Lesch-Nyhan Variants: partial HGPRT deficiency
3. APRT deficiency (EC 2.4.2.7.)

Purine Catabolism:
1. Adenosine Deaminase (EC 3.5.4.4.) deficiency
2. Xanthine Oxidase deficiency (EC 1.2.3.2.)
3. Increased xanthine oxidase activity

Purine syndromes associated with central nervous system (CNS) diseases are being reported with increasing frequency since the first publication by Lesch and Nyhan (1964) of a deficiency of the enzyme hypoxanthine-guanine — phosphoribosyltransferase (HGPRT; EC 2.4.2.8.). A review by Coleman et al. (1974) revealed more than 12 distinct entities with different CNS involvement, including spino-cerebellar and cerebellar signs (Kelley et al., 1968; Rosenberg et al., 1970; Haslam and Clark, 1971) (Fig. 1).

Farstad et al. (1965a, 1965b) have shown an increase of uric acid in cerebrospinal fluid (CSF) of patients with atrophic progressive CNS degenerations, but without specific mention of the various spino-cerebellar degenerations.

In search for a possible derangement of purine metabolism in Friedreich's ataxia, we undertook to screen blood, CSF and urine for changes in purines and their metabolites and altered activity of the enzymes involved in regulation of purine metabolism (Table 2).

SUBJECTS AND METHODS

Fasting urine and blood samples were taken from fifteen typical Friedreich's ataxia patients (group Ia), seventeen intra familial controls, twelve external controls, four atypical ataxia patients (group II) and two atypical intra familial controls. The subjects had been on a high carbohydrate diet for three days in preparation for the glucose tolerance test.

Plasma and urine uric acid, as well as plasma and urine creatinine values, were obtained for these subjects. Plasma and urine uric acid were determined by the alkaline phosphotungstate method (Tietz, 1970). Plasma and urine creatinine were determined by the Jaffe reaction (O'Brien et al., 1968 and Henry et al., 1974, respectively).

Incubations with radioactive purines were done on red cells from five of the typical Friedreich's ataxia patients (four females, one male, mean age eighteen years) and from five of the external control subjects (four females, one male, mean age twenty-four) using the method described by Henderson et al. (1974). Nucleotide profiles were measured for six of the typical Friedreich's ataxia patients (four females, two

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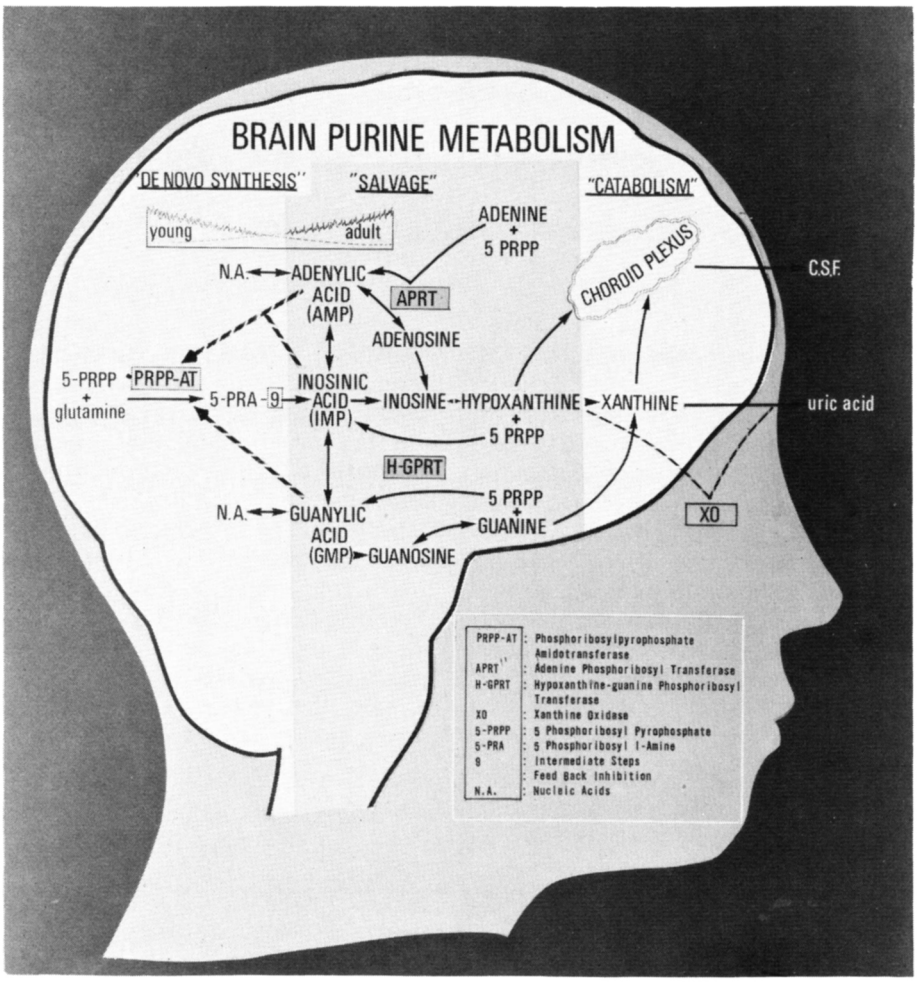
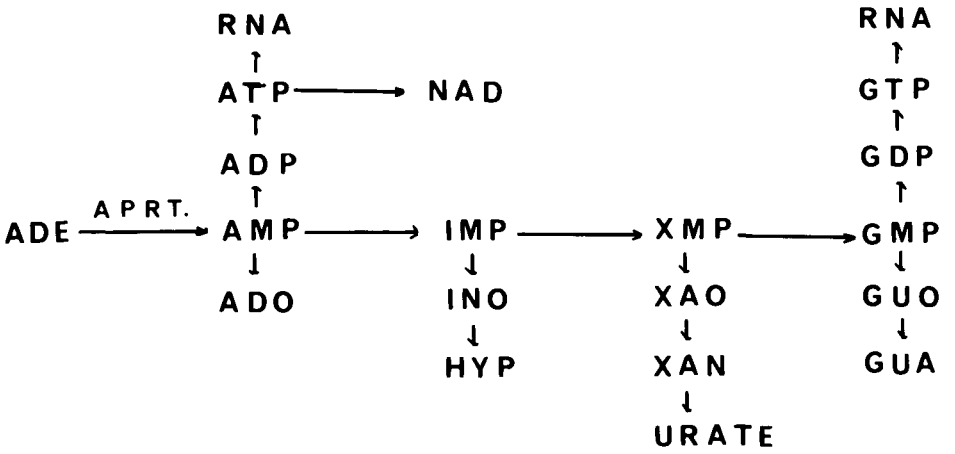


Figure 1—Schematic representation of brain purine metabolism.

TABLE 2 ADENINE METABOLISM



males, mean age sixteen years) and for six of the external control subjects (four females, two males, mean age twenty-five. Purine profiles in CSF were determined for thirteen Friedreich's ataxia patients, five atypical (group II) and for three controls. Some of the group Ia and group II subjects were from those described above, while others along with the controls were different.

Radioactive purines, nucleosides and nucleotides from the incubations were separated by high pressure liquid chromatography (HPLC). The nucleotide profiles of red cells and the oxypurine profiles of CSF were also determined by HLPC. Experimental details will be discussed in a future publication (Draper et al., 1978, to be published).

APRT and HGPRT determinations were performed at the Purine Research Laboratory, Wellesley Hospital, Toronto (Dr. I. H. Fox).

RESULTS

Uric acid and creatinine results for serum and urine of the various groups are shown in Table 3. The serum uric acid values are within normal limits and no significant variations in any of the determinations were observed.

Results for purines in CSF as determined by HPLC are shown in Table 4. No significant differences are seen.

In Table 5, results are given for nucleotide concentrations in red cells for six typical Friedreich's ataxia patients and for six apparently healthy external controls. Again, no major difference in the nucleotide profiles was noted.

Table 6 shows the results for the APRT-HGPRT determinations, and the results of the radioactivity measurements for the estimation of apparent activities of enzymes of purine metabolism are summarized in Table 7. These figures suggest that activities of APRT and HGPRT in Friedreich's ataxia patients are within normal limits, and also that the apparent activities of some other enzymes of purine metabolism are similar for Friedreich's ataxia and external controls.

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TABLE 3

URIC ACID-CREATININE (MEAN ± S.D.)

	PLASMA		URINE
	URIC ACID, mg%	CREATININE, mg%	URIC ACID/CREATININE
Typical Friedreich's Ataxia (Group Ia)	5.43 ± 1.14 (n = 14)	0.64 ± 0.11 (n = 15)	0.36 ± 0.13 (n = 13)
Intra Familial Controls	5.12 ± 1.33 (n = 17)	0.68 ± 0.13 (n = 15)	0.37 ± 0.13 (n = 16)
External Controls	5.1 ± 0.89 (n = 12)	0.80 ± 0.10 (n = 12)	0.36 ± 0.12 (n = 12)

TABLE 4

PURINES IN CSF (µg/ml)

GROUPS	URIC ACID	HYPOXANTHINE	XANTHINE
A. Friedreich's ataxia (group Ia)			
mean (n=15 determinations)	2.9	0.68	0.29
range	1.3 - 5.9	0.30 - 1.26	0.12 - 0.67
B. Ataxia (Group II)			
mean (n=5 determinations)	5.2	0.51	0.25
range	0.9 - 8.7	0.17 - 0.89	0.06 - 0.36
C. Non neurological controls			
mean (n=3 determinations)	1.9	0.61	0.31
range	1.2 - 2.7	0.09 - 1.07	0.12 - 0.48

DISCUSSION

Inborn errors (recessive-autosomal or X-linked) of purine metabolism which have been reported are listed in Table 1. In view of the complexity of the metabolic pathways and the number of enzymes involved, it appears probable that, with increased awareness, other syndromes will be defined.

Study of purine metabolism in the brain is further complicated by the localisation of specific enzymes in certain regions of the CNS (Fig. 1). In particular, HGPRT is located in the basal ganglia and xanthine oxidase — the final enzyme of purine metabolism — is almost absent from the CNS. In the CNS, the end products of purine metabolism the oxypurines (xanthine and hypoxanthine) are excreted by the choroid plexus (Berlin, 1969). From animal experiments, it appears that the blood-brain-barrier is not freely permeable to purines, although whether there is some degree of active transport into the CNS remains to be established (Nakagawa and Guroff, 1973; Silbernagel et al., 1977).

Coleman et al. (1974) have recently reviewed patients with hyperuricemia or hyperuricosuria and CNS symptoms. In most cases, the specific metabolic defect awaits definition. Two cases with spinocerebellar degeneration (Rosenberg et al., 1970) and which were considered to be variants of the Lesch-Nyhan syndrome, have since been shown to have no progression of their clinical state (Nyhan, 1977). The HGPRT activity was also normal in cases of olivo-pontocerebellar ataxia. Nyhan considers there is probably no relationship between HGPRT and spinocerebellar disease. In other neuromuscular diseases (Huntington's Chorea and Muscular Dystrophy). (Lemieux and Shapcott, 1973; Thomson and Smith, 1976), purine metabolism may be implicated, possibly as a secondary factor. Although raised CSF uric acid levels have been reported in atrophic progressive degenerative disease of the CNS (Farstad et al., 1965a, 1965b), it appears unlikely that this represents *de novo* formation within the brain, in view of the low xanthine oxidase activity in CNS tissue.

As Seegmiller has emphasized (1974), normal uric acid levels do not necessarily mean normal brain purine metabolism and the possibility of intracellular deficiency of nucleotide levels and catabolism must be considered (see also Rosembloom et al., 1967).

In our patients there was no evidence for impairment of purine synthesis as shown by normal blood levels and urinary excretion of uric acid. Normal activity of the key salvage pathway enzymes (APRT and HGPRT) in erythrocytes confirm that this pathway is not altered in Friedreich's ataxia. While it was not possible to measure the activity of each enzyme involved in purine metabolism, the interconversion of radioactive purine bases into inter-

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TABLE 5

CONCENTRATION OF NUCLEOTIDES IN RED CELLS (μmoles/ml)

	FRIEDREICH'S ATAXIA (n = 6)		CONTROLS (n = 6)	
	MEAN	(RANGE)	MEAN	(RANGE)
AMP	0.032	(0.011-0.063)	0.044	(0.010-0.082)
ADP	0.25	(0.14-0.45)	0.26	(0.16-0.32)
ATP	1.01	(0.80-1.22)	1.02	(0.83-1.11)
GDP	0.013	(0-0.024)	0.014	(0.009-0.019)
GTP	0.049	(0.023-0.076)	0.048	(0.026-0.060)
IMP	0.174	(0.08-0.26)	0.186	(0.08-0.39)
NAD	0.047	(0.022-0.079)	0.044	(0.013-0.083)
NADP	0.040	(0.030-0.062)	0.044	(0.031-0.056)

TABLE 6

APRT AND HGPRT ACTIVITIES (n mole/h/mg) IN FRIEDREICHS ATAXIA

FRIEDREICHS ATAXIA	APRT	HGPRT
F.P.	15.0	67.5
T.J.	16.5	65.9
F.D.	14.9	63.1
M.Y.	12.6	76.2
F.B.	14.1	51.8
F.S.	16.9	61.5
F.A.	19.1	62.4
F.L.	19.6	64.1
M.G.	15.8	74.6
H.G.	24.1	85.9
H.M.	14.3	54.4
H.M.	19.7	69.2
NORMAL RANGE	10.8 - 38.0	54.1 - 120.5

TABLE 7

RADIOACTIVE METABOLITES IN ERYTHROCYTES (MEAN, RANGE, n moles/g HEMOGLOBIN)

IN 5 FRIEDREICH'S ATAXIA AND 5 CONTROL SUBJECTS

PRECURSOR:	ADE-8-^{14}C		GUA-8-^{14}C		HYP-8-^{14}C	
METABOLITE	FRIEDREICH'S	CONTROL	FRIEDREICH'S	CONTROL	FRIEDREICH'S	CONTROL
ATP	214 (95-356)	339 (143-439)	11 (3-32)	9 (3-16)	6 (1-16)	9 (7-12)
ADP	124 (38-201)	154 (70-240)	3 (0-7)	4 (0-8)	4 (0-11)	4 (0-13)
GTP	11 (3-19)	8 (3-16)	415 (169-578)	661 (561-733)	12 (2-21)	10 (6-17)
GDP	15 (7-29)	18 (9-23)	77 (27-191)	124 (93-148)	1 (0-5)	1 (0-4)
GMP	6 (1-10)	7 (4-12)	16 (3-28)	22 (8-34)	10 (8-13)	17 (9-36)
IMP	16 (4-33)	16 (8-29)	70 (32-110)	116 (88-150)	283 (210-337)	341 (230-505)
XMP	3 (0-6)	4 (2-7)	2 (0-5)	7 (0-13)	2 (0-5)	5 (1-16)
ADO					13 (3-22)	4 (0-7)
ADE					9 (0-17)	4 (0-14)
GUO					10 (6-24)	16 (2-24)
GUA					12 (0-39)	8 (0-22)
INO					81 (28-114)	104 (34-141)
XAO					12 (0-28)	14 (0-27)
XAN			137 (99-160) n=3	97 (50-157) n=4		

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mediates was normal. Finally, the CSF levels of the end products of purine metabolism in the CNS — xanthine and hypoxanthine — were normal in Friedreich's ataxia.

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