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Purine metabolism abnormalities in a hyperuricosuric subclass of autism

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Abstract

A subclass of patients with classic infantile autism have uric acid excretion which is >2 S.D.s above the normal mean. These hyperuricosuric autistic individuals may comprise approx. 20% of the autistic population. In order to determine the metabolic basis for urate overexcretion in these patients, de novo purine synthesis was measured in the cultured skin fibroblasts of these patients by quantification of the radiolabeled purine compounds produced by incubation with radiolabeled sodium formate. For comparison, de novo purine synthesis in normal controls, in normouricosuric autistic patients, and cells from patients with other disorders in which excessive uric acid excretion is seen was also measured. These experiments showed that de novo purine synthesis is increased approx. 4-fold in the hyperuricosuric autistic patients. This increase was less than that found in other hyperuricosuric disorders. No unusual radiolabeled compounds (such as adenylosuccinate) were detected in these experiments, and no gross deficiencies of radiolabeled nucleotides were seen. However, the ratio of adenine to guanine nucleotides produced by de novo synthesis was found to be lower in the cells of the hyperuricosuric autistic patients than in the normal controls or the cells from patients with other disorders. These results indicate that the hyperuricosuric subclass of autistic patients have increased de novo purine synthesis, and that the increase is approximately that expected for the degree of urate overexcretion when compared to other hyperuricosuric disorders. No particular enzyme defect was suggested by either gross deficiency of a radiolabeled compound or the appearance of an unusual radiolabeled compound, and no potentially neurotoxic metabolites were seen. Although an enzyme defect responsible for the accelerated purine synthesis was not identified, the abnormal ratio of adenine to guanine nucleotides suggests a defect in purine nucleotide interconversion.

Keywords: Autism; Hyperuricosuria; Purine metabolism; Uric acid

1. Introduction

Given the number of well-defined metabolic disorders which are associated with behavioral and neurological abnormalities [1,2], it is reasonable that a subset of patients with classic infantile autism might have some metabolic disorder as the cause of their autistic symptoms. Indeed, a number of patients with phenylketonuria display classic autism [3,4]. Other metabolic disorders, including adenylosuccinate lyase deficiency [5], dihydropyrimidine dehydrogenase deficiency [6], histidinemia [7], and 5′-nucleotidase superactivity [8] display autistic symptoms, but do not fulfill the DSM-IV criteria [9] for classic infantile autism.

A number of cases have been reported describing patients with classic autistic symptoms combined

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with prominent hyperuricosuria [10–17]; although diagnosed before standardized diagnostic criteria were established, many of these patients fulfill the DSM-IV criteria for classic autism. A number of these patients exhibited neurological symptoms as well, including seizures, ataxia, and spasticity. Despite the prominent increase in uric acid production, plasma concentration of urate in most cases is within the normal range, and there is no evidence of urate pathology such as gout or kidney stones. In some of these reports the autistic and/or neurological symptoms seem to have been ameliorated by antihyperuricosuric metabolic therapy. Two patients [10,15] were observed to benefit from a low purine diet, which decreased their purine excretion and made them more alert and sociable. Two other patients [11,12] were shown by double-blind crossover studies to have reduced seizures, less ataxia, and improved behavior with the anti-gout drug allopurinol. Studies undertaken to determine the percentage of the autistic population which overproduces uric acid arrived at a figure of 11–28% [13,16,17] as compared to 0–3% [16,18] of the non-autistic population. These were clinic-based studies, not population-based epidemiological surveys.

Two well-studied defects of purine metabolism which are associated with uric acid overproduction are hypoxanthine phosphoribosyltransferase (HPRT) deficiency [19] and phosphoribosyl pyrophosphate synthetase (PRPPS) superactivity [20]. In both of these disorders it is possible to demonstrate increased de novo purine synthesis, either by increased incorporation of radiolabeled glycine into uric acid in the urine of affected individuals or by increased incorporation of radiolabeled glycine or formate into purine compounds in the cultured cells of patients. The present study was undertaken to determine if increased de novo purine synthesis could be demonstrated in the cultured fibroblasts of hyperuricosuric autistic patients as well, and if so, to compare the rate of purine synthesis with that of these other hyperuricosuric metabolic disorders. A second objective was to detect any abnormal accumulation of intermediates (such as succinylaminoimidazole carboxamide ribonucleotide) during de novo synthesis or any abnormal distribution of purine compounds produced by de novo synthesis which might suggest an enzyme defect.

2. Materials and methods

The subjects for this study were chosen for the presence of both classic autistic symptoms and uric acid production greater than 2 S.D.s above the normal mean.

The clinical presentation of nine patients is summarized in Table 1. These patients were diagnosed before the DSM-IV criteria were established; when reevaluated all subjects fulfilled the criteria for autistic disorder [9]. All the patients exhibited typical autistic symptoms, including lack of interest in social contact, severely restricted communicative ability, and lack of imaginative activity. In every case these symptoms were apparent by the third year. Stereotypic behavior was seen in all patients and consisted of twirling, repetitive motions, toe-walking, and hand-flapping. Though accurate assessment of intellectual capacity was complicated by impaired communicative ability, six of the nine patients were judged to be mildly retarded, though two of these retarded individuals were thought to have savant skills. All showed some type of abnormal sensory response, such as increased sensitivity to auditory stimuli or decreased sensitivity to pain. Self-injurious behavior was seen in four of the nine patients and included eye-poking, head-banging, or hair-pulling. Clinical seizures and abnormal EEGs were seen in six of the nine patients and were of varied types. As is often noted with hyperuricosuric autistic patients [16] dietary sensitivities were present; ingestion of certain foods seemed to exacerbate both behavioral and neurological symptoms. All patients were put

Table 1
Clinical features of hyperuricosuric autistic subjects

Symptom	Patient								
	1	2	3	4	5	6	7	8	9
Early onset of symptoms	+	+	+	+	+	+	+	+	+
Autistic aloneness	+	+	+	+	+	+	+	+	+
Communication deficit	+	+	+	+	+	+	+	+	+
Stereotypic behaviors	+	+	+	+	+	+	+	+	+
Apparent mental retardation	+	+	+	−	+	−	+	−	+
Abnormal sensory response	+	+	+	+	+	+	+	+	+
Self-injurious behavior	−	+	+	−	+	+	+	+	−
Seizures	+	−	−	+	+	+	−	+	+
Savant skills	+	−	−	−	+	−	−	+	+
Dietary sensitivities	+	−	+	+	−	−	+	+	−

on the low purine diet between the ages of 3 and 8 (see below). Although all patients showed excessive urate excretion by 24 h urate assay, all had normal plasma urate values between 1.4 and 5.2 mg/dl.

Although 24 h urate values were not obtained for the normal control subjects from whom the skin biopsies were taken, the normal values for 24 h urate excretion with respect to age and sex as well as the rarity of excessive urate excretion in the normal population have been extensively studied and reported previously [16,18].

^{14}C-Sodium formate (55 mCi/mmol) was obtained from Moravek Radiochemicals (Brea, CA, USA). All other reagents were obtained from Sigma (St. Louis, MO, USA).

Uric acid was measured by the uricase method on a 24 h urine sample [21]. For the urine collection patients and controls were not following any special diet, nor were they receiving any medication.

Fibroblasts from punch biopsies were grown in Coon's F12 medium with 10% fetal bovine serum (FBS) in 100 mm plates. Cells were harvested in the log phase of growth between passages 6 and 10, replated at a density of 5×10^5 cells per plate and switched to Earl's MEM with 10% dialyzed FBS. After 72 h, the cells were harvested, replated at 10^6 cells per plate, and 7 ml of MEM containing 10 μCi ^{14}C-sodium formate was added. Cells were incubated for an additional 24 h and harvested by trypsinization. The cell pellet was extracted with 0.8 M perchloric acid, precipitated protein and cell debris were removed by centrifugation at $20\,000\times g$, and the supernatant was neutralized to pH 5–7 with 2 M potassium phosphate buffer, pH 7.5. Labeled and unlabeled purine bases, nucleosides, and nucleotides were identified and quantified by HPLC with continuous scintillation counting by the method of Ryll and Wagner [22], in which all these compounds are quantified in a single analysis. De novo purine synthesis was calculated as nmol purine nucleotides synthesized+radiolabeled purines excreted into medium/100 nmol purines present in the cell/24 h as previously described [23]. The adenine to guanine nucleotide ratio was calculated from the amounts of radiolabeled nucleotides as (AMP+ADP+ATP)/(GMP+GDP+GTP). For each subject three different incorporation experiments were performed.

3. Results

The urate excretion and de novo purine synthesis in the cultured fibroblasts of the hyperuricosuric patients are shown in Table 2. For comparison, de novo purine synthesis was measured by the same method in the cultured fibroblasts of normal controls, two normouricosuric autistic patients, a Lesch-Nyhan patient, a neurologically normal HPRT-deficient hyperuricosuric patient, and a patient with PRPP synthetase superactivity. Owing to the difficulty of finding age-matched normal control cell lines, urate excretion was not measured in the individuals from whom these lines were established but is assumed to be normal; instead, the literature average normal value [16] is shown in Table 2.

Urate excretion was elevated approx. 2–3-fold in the hyperuricosuric autistic subjects; de novo synthesis in the fibroblasts of these patients was found to be elevated 3–4-fold; this increase was both consistent and statistically significant ($P<0.01$). Subjects with other defects of purine metabolism showed both a higher excretion of uric acid and a greater increase in de novo purine synthesis. Two autistic subjects with normal urate excretion had de novo purine synthesis comparable to the normal controls.

All subjects produced both adenine and guanine nucleotides from formate. No unusual radiolabeled intermediates of the de novo pathway (such as succinylaminoimidazole carboxamide ribonucleotide) or purine interconversion pathways (such as adenylosuccinate) were seen in the cells of the hyperuricosuric autistic subjects. Nor were any gross deficiencies of labeled purine compounds which would suggest an enzyme deficiency noted in these subjects. However, careful analysis of the ratio of labeled adenine to guanine nucleotides revealed that this ratio was on average lower in the hyperuricosuric autistic subjects than in the normal controls (Table 2). The nucleotide ratios (AMP:ADP:ATP and GMP:GDP:GTP) did not differ significantly from those of normal controls (data not shown). The difference in the adenine to guanine ratio reached statistical significance ($P<0.05$) in the case of the hyperuricosuric autistic subjects. In the case of the cells of the subject with other defects of purine metabolism and in the cells from the normouricemic autistic subjects this ratio was comparable to normal controls. Although the

Table 2
Urate excretion and de novo purine synthesis

Subject	Age (years)	Urate excretion (mg urate/kg/day)	De novo purine synthesis (nmol/100 nmol/24 h)	Adenine/guanine nucleotide ratio
NC 1	12	(8.3 ± 2.0)	8.238 ± 6.47	6.47
NC 2	4	(8.3 ± 2.0)	10.907 ± 7.41	7.41
NC 3	6	(8.3 ± 2.0)	8.701 ± 7.96	7.97
NC 4	3	(8.3 ± 2.0)	10.105 ± 6.29	6.29
NC 5	7	(8.3 ± 2.0)	6.929 ± 7.62	7.62
			8.976 ± 1.56	7.15 ± 0.73
HA 1	5	26.5	28.588 ± 3.264	4.14
HA 2	7	19.8	34.531 ± 3.819	4.42
HA 3	12	17.9	34.528 ± 3.754	4.04
HA 4	4	16.2	34.935 ± 0.637	5.67
HA 5	5	35.4	34.258 ± 3.784	6.38
HA 6	9	18.1	34.448 ± 6.918	4.65
HA 7	4	14.7	23.756 ± 1.879	5.87
HA 8	10	17.9	32.018 ± 2.502	6.71
HA 9	3	22.4	38.429 ± 2.825	6.41
			32.832 ± 4.289	5.36 ± 1.0
NA 1	6	5.9	8.348 ± 0.715	7.23
NA 2	4	8.7	6.254 ± 0.284	6.69
PRPPS superactivity	3	43	49.374 ± 1.858	8.66
HPRT deficiency gout	4	37	54.916 ± 3.360	6.53
Lesch-Nyhan	6	48	61.141 ± 1.336	5.76

NC, normal control; HA, hyperuricosuric autistic; NA, normouricosuric autistic. The adenine/guanine nucleotide ratio was calculated as (AMP+ADP+ATP)/(GMP+GDP+GTP).

conversion of radiolabeled IMP to adenine and guanine nucleotides was abnormal in the cells of the hyperuricosuric autistic cells, the steady-state concentration of purine nucleotides was comparable to normal controls (data not shown).

4. Discussion

In the hyperuricosuric subclass of autistic patients, urate excretion is elevated 2–3-fold compared to normal controls. A 3–4-fold increase in de novo purine synthesis which accounts for this increased urate excretion can be demonstrated in the cultured skin fibroblasts of these patients. An abnormal ratio of adenine to guanine nucleotides synthesized by the de novo pathway suggests that a defect in purine nucleotide interconversion may be responsible for the observed increased de novo purine synthesis.

In the cells of all the subjects with urate overproduction, a roughly proportionate increase in de novo purine synthesis was noted. Most elevated were the HPRT-deficient patients, with a nearly 5-fold increase in purine excretion and a more than 6-fold increase in de novo purine synthesis. These increases have been found to be typical of HPRT-deficient patients [19]. A somewhat smaller elevation in both urate excretion and de novo purine synthesis is found in subjects with PRPP synthetase superactivity [20]. Both HPRT-deficient and PRPP synthetase superactive subjects typically have elevated plasma urate [19,20]. These individuals often experience the pathological consequences of urate overproduction, including gout and kidney stones.

The hyperuricosuric autistic subjects appear to have a more modest 2–3-fold increase in urate production and 3–4-fold increase in de novo purine synthesis, as measured in their cultured fibroblasts. These individuals do not usually have elevated plasma uric acid, and rarely experience any type of urate pathology. Apparently, removal of urate by the kidneys is adequate in the case of this smaller increase.

The relationship between urate overproduction and the autistic symptomatology seen in the hyperuricosuric subclass of autism is unlikely to be a direct one, despite circumstantial evidence linking the two.

Some of these patients benefit from allopurinol, a xanthine oxidase inhibitor which decreases production of xanthine and uric acid; allopurinol was found to decrease seizures and improve learning and attention in some patients [11,12]. Several of the hyperuricosuric autistic patients [10,15] were also observed to benefit from a low purine diet [24] which also lowers urate excretion. Although it is difficult to account for placebo effects in experimental diets, dramatic improvement is seen occasionally. The patient reported by Hooft [10] was said to have 'an undeniable improvement' in both behavioral and neurological symptoms. However, no crossover phase was reported. In another case [15] the hyperuricosuric autistic subject showed improvements in speech, attention, and social interaction on a low purine diet; a trial at replacement of the low purine diet with a high purine diet resulted in extreme hyperactivity and decreased social interaction within 2 days. Reinstatement of the low purine diet restored the subject to her pretrial condition. These findings might suggest that uric acid itself may be responsible for autistic and/or neurological symptoms. Although some patients with HPRT deficiency and PRPP synthetase superactivity do show a variety of behavioral and neurological symptoms, these symptoms show no similarity to autism. Some of these patients have uric acid overproduction greater than that seen in the hyperuricosuric autistic patients and no neurological or behavioral symptoms whatsoever. Glycogen storage disease type I (glucose 6-phosphatase deficiency) is another disorder with urate overproduction similar to that of the patients described here, but with no neurological or behavioral symptoms [25].

The exact enzyme defect responsible for increased de novo purine synthesis in purine autism is thus far not known, but the finding of an abnormal adenine/guanine nucleotide ratio in these patients suggests that it is one of the enzymes of purine nucleotide interconversion. Experimental models of defects in purine nucleotide interconversion are known to produce both increased de novo purine synthesis and increased purine excretion [26,27]. These models include decreased adenylosuccinate synthetase, decreased IMP dehydrogenase, and greatly increased IMP dehydrogenase. In each case, the conversion of IMP to adenine and guanine nucleotides is abnormal although the steady-state concentration of nucleotides is normal. This finding is typical of cells of patients with severe deficiencies in one of the enzymes of purine metabolism, such as $HPRT^-$ cells; although incorporation of hypoxanthine into purine nucleotides is grossly abnormal, steady-state concentrations of nucleotides are normal [19]. In at least one case [28], an abnormality of purine nucleotide interconversion was shown to result in clinical gout; in this case a mutant liver AMP deaminase was found to be insensitive to inhibition by GTP, resulting in increased adenine nucleotide catabolism and increased de novo purine synthesis.

Unlike the case of adenylosuccinate lyase deficiency, in which large quantities of potentially neurotoxic substances are produced, here, there is no obvious relationship between increased de novo synthesis or altered nucleotide interconversion and autistic symptoms; knowledge of the enzyme defect and its metabolic consequences in hyperuricosuric autism might shed some light on this relationship and perhaps aid in the design of a rational metabolic therapy. These patients seem amenable to metabolic therapy; if the enzyme defect were known and its metabolic consequences better understood it might be possible with purine dietary supplements to reverse these consequences and bring about improvement of their autistic symptoms.

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