Journal of Neurochemistry
Lippincott Williams & Wilkins, Inc., Philadelphia

Abnormal Purine and Pyrimidine Nucleotide Content in Primary Astroglia Cultures from Hypoxanthine–Guanine Phosphoribosyltransferase-Deficient Transgenic Mice

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Abstract: Lesch-Nyhan syndrome is a pediatric metabolic–neurological syndrome caused by the X-linked deficiency of the purine salvage enzyme hypoxanthine–guanine phosphoribosyltransferase (HGPRT). The cause of the metabolic consequences of HGPRT deficiency has been clarified, but the connection between the enzyme deficiency and the neurological manifestations is still unknown. In search for this connection, in the present study, we characterized purine nucleotide metabolism in primary astroglia cultures from HGPRT-deficient transgenic mice. The HGPRT-deficient astroglia exhibited the basic abnormalities in purine metabolism reported before in neurons and various other HGPRT-deficient cells. The following abnormalities were found: absence of detectable uptake of guanine and of hypoxanthine into intact cell nucleotides; 27.8% increase in the availability of 5-phosphoribosyl-1-pyrophosphate; 9.4-fold acceleration of the rate of de novo nucleotide synthesis; manyfold increase in the excretion into the culture media of hypoxanthine (but normal excretion of xanthine); enhanced loss of label from prelabeled adenine nucleotides (loss of 71% in 24 h, in comparison with 52.7% in the normal cells), due to 4.2-fold greater excretion into the media of labeled hypoxanthine. In addition, the HGPRT-deficient astroglia were shown to contain lower cellular levels of ADP, ATP, and GTP, indicating that the accelerated de novo purine synthesis does not compensate adequately for the deficiency of salvage nucleotide synthesis, and higher level of UTP, probably due to enhanced de novo synthesis of pyrimidine nucleotides. Altered nucleotide content in the brain may have a role in the pathogenesis of the neurological deficit in Lesch-Nyhan syndrome. **Key Words:** Astroglia cultures—Hypoxanthine–guanine phosphoribosyltransferase deficiency—Lesch-Nyhan syndrome—Hypoxanthine—Xanthine—Adenine nucleotides—Transgenic mice.
J. Neurochem. **72,** 1139–1145 (1999).

Hypoxanthine–guanine phosphoribosyltransferase (IMP: pyrophosphate phosphoribosyltransferase, EC 2.4.2.8; HGPRT) catalyzes the salvage synthesis of IMP and GMP from the purine bases hypoxanthine and guanine, respectively. Complete deficiency of HGPRT activity is associated with the Lesch-Nyhan syndrome (LNS), characterized by abnormal metabolic and neurological manifestations (Lesch and Nyhan, 1964; Seegmiller et al., 1967; Nyhan, 1973; Watts et al., 1982; Breadfield and Cambi, 1987; Rossiter and Caskey, 1995). Metabolically it is associated with excessive purine production, which may result in uric acid nephropathy. Neurologically, LNS is associated with self-mutilation, choreoathetosis, spasticity, and developmental retardation. The cause of the metabolic consequences of HGPRT deficiency is clarified, but that of the neurological manifestations is not yet understood (Hershfield and Seegmiller, 1977; Kelley and Wyngaarden, 1983; Baumeister and Frye, 1985; Rossiter and Caskey, 1995). Apparently, the direct cause for the neurological deficit in LNS is defective arborization of the dopaminergic neurons (Lloyd et al., 1981; Baumeister and Frye, 1985; Janovitch et al., 1988; Palmour et al., 1989; Jinnah et al., 1992, 1994), but the connection between the enzyme deficiency and this abnormality is still not known. Obviously, one or several of the alterations in brain purine metabolism, induced by the HGPRT deficiency, initiate the pathological process leading to the neurological deficit. In search for the identity of these alterations, we have earlier characterized purine metabolism in HGPRT-deficient neurons, using an HGPRT-deficient rat neuroma cell line as a model system (Zoref-Shani et al., 1993). The results of that study demonstrated that the HGPRT-deficient neurons exhibit the alterations in purine nucleotide metabolism described before in various other HGPRT-deficient tissues, including accumulation of 5-phosphoribosyl-1-pyrophosphate (PRPP), enhanced rate of de novo purine synthesis, excessive production and excretion of hypoxanthine, and enhanced turnover rate of adenine nucleotides (Kelley et al., 1970; Fox and Kelley, 1971; Brosh et al., 1976; Zoref-Shani et al., 1980, 1993). The

Received April 17, 1998; revised manuscript received September 24, 1998; accepted September 30, 1998.

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Abbreviations used: HGPRT, hypoxanthine–guanine phosphoribosyltransferase; LNS, Lesch-Nyhan syndrome; PRPP, 5-phosphoribosyl-1-pyrophosphate.

HGPRT-deficient neurons exhibited also elevated cellular content of UTP, probably reflecting enhanced synthesis of pyrimidine nucleotides, due to the increased availability of PRPP. The aim of the present study was to characterize the effect of HGPRT deficiency on purine metabolism in the astroglia, the major cellular component of the brain. The model system for the HGPRT-deficient astroglia, chosen for the present study, was that of primary astroglia cultures prepared from HGPRT-deficient transgenic mice (Hooper et al., 1987). The HGPRT-deficient astroglia cultures exhibited all the abnormalities found before in the HGPRT-deficient neurons, including the elevated cellular UTP content. However, in contrast to the neuronal model, the astroglia cells exhibited also low cellular content of ADP, ATP, and GTP. Altered content of purine and pyrimidine nucleotides in the glia might be the link between HGPRT deficiency and the associated neurological deficit in LNS.

MATERIALS AND METHODS

Materials

[8-^{14}C]Guanine sulfate (56 mCi/mmol), [8-^{14}C]hypoxanthine (50 mCi/mmol), [8-^{14}C]adenine (55.7 mCi/mmol), [8-^{3}H]adenine (28.6 Ci/mmol), and [^{14}C]formic acid, sodium salt (55 mCi/mmol) were purchased from Amersham (Buckinghamshire, U.K.). Purine nucleotides, nucleosides and bases, PRPP, cellulose (Sigmacell type 20), and all other chemicals were purchased from Sigma (St. Louis, MO, U.S.A.). Dulbecco's modified Eagle medium, containing glucose (1 mg/ml) and sodium pyruvate (110 mg/L), antibiotics, and fetal calf serum were obtained from Biological Industries (Kibbutz Beth Haemek, Israel). Culture dishes and flasks were the product of Corning (NY, U.S.A.).

Animals

Astroglia cultures were prepared from an HGPRT-deficient strain of transgenic mice (Hooper et al., 1987). C57BL/6J mice were used as controls.

Astroglia cultures

Astroglia cultures were prepared according to Hertz et al. (1982/3) and McCarthy and Partlow (1976). Brains were removed from newborn mice and cerebral cortex was dissected. Meninges were peeled off and resulting brain was minced thoroughly. The cells were thereafter dissociated by vigorous shaking in Dulbecco's modified Eagle's medium, supplemented with 10% (nondialyzed) fetal calf serum, and seeded in 250-ml flasks, with 20 ml of the above medium (five cerebral cortexes per flask). Cultures were maintained in 10% CO_2 atmosphere at 37°C. After 1 week, each culture flask was split into three 60-mm culture dishes with 5 ml of the above medium. Thereafter, the medium was changed twice a week. Nonastroglia cells were removed by vigorous shaking of the dishes for ~1 min, followed by change of the media, performed for the first time after 1 week and then with every change of media. The cultures were found to contain at least 90% astroglial cells, by staining with the immunocytochemical marker glial fibrillary acidic protein (Dahl and Bignami, 1977). The normal and HGPRT-deficient astroglia cultures exhibited the same rate of multiplication of cells, as manifested in the time needed to cover the culture flasks. Only confluent cultures, 14 days old, were used for all studies.

Enzyme assays

HGPRT and AMP:pyrophosphate phosphoribosyltransferase (EC 2.4.2.7; adenine phosphoribosyltransferase) were assayed radiochemically as described before (Zoref-Shani et al., 1993). Protein determinations for this assay and for all other purposes were done according to Bradford (1976).

Uptake of purine bases and incorporation into nucleotides

Monolayer cultures in 60-mm dishes were incubated at 37°C with 8 μM of the labeled purine base, in 5 ml of fresh culture medium, for 15 min. After incubation, the culture medium was discarded and the cells were washed with cold saline two times. Perchloric acid (0.5 ml 1 *M*) was added, the cells were scraped off, and the total content of the dishes subjected to centrifugation. The purine nucleotides in the acid extract were separated from the corresponding purine bases by chromatography on microcrystalline cellulose, using *n*-butanol/methanol/H_2O/NH_4OH 25% (60:20:20:1, by volume) as solvent (Zoref-Shani et al., 1988). The nucleotide spots were scraped off and counted.

Fate of labeled adenine nucleotides

For the study of the fate of adenine nucleotides, the nucleotide pool of similarly confluent cultures was labeled by incubation of the cells with 10 μM [8-^{14}C]adenine for 30 min. The cells were washed and incubated for 24 h with fresh medium containing dialyzed fetal calf serum. After incubation, the medium was collected, perchloric acid was added to a final concentration of 0.5 *M*, and the purines were hydrolyzed at 100°C for 1 h. The radioactivity of the various purines was determined after chromatography, as described above. The nucleotides in the cells were extracted by the addition of 5 ml of cold 0.5 *M* perchloric acid, immediately after the removal of the medium. The lysed cells were scraped off and the resulting suspension was subjected to centrifugation. The nucleotides in the supernatant were separated by chromatography and the radioactivity was counted, as described above.

Purine synthesis de novo

The rate of purine nucleotide synthesis de novo was gauged in the intact astroglia cells by measuring the rate of incorporation of [^{14}C]formate into purines, as described before (Zoref-Shani et al., 1993). Monolayer cultures in 60-mm culture dishes were incubated for 2 h at 37°C with 10 μCi sodium [^{14}C]formate. After incubation, the culture media were separated from the cells and each fraction treated separately. The total cellular purines and the total purines excreted into the incubation medium were subjected to hydrolysis in 1 *M* perchloric acid, at 100°C for 1 h. The resulting free purine bases were then precipitated as purine–silver complexes and the content of label determined.

Quantification of nucleotides, nucleosides, and bases

Purine nucleotides, nucleosides, and bases in extracts from cells and culture media were determined by using HPLC methodology. The growth medium was separated and the cells extracted immediately in 1 ml of cold 1 *M* perchloric acid. Two minutes after the acid extraction, the cell extract was neutralized with 0.7 ml of a solution of 0.5 *M* $KHCO_3$ in 1.5 *M* KOH and subjected to centrifugation (40,000 *g*, 15 min at 4°C). The growth media was acidified and neutralized as described above. The extracted purines were kept at −70°C until analyzed. The purine nucleotides in the cell extracts were quantified by using a SAX-10 Partisil column (Whatman), using a gradient of

TABLE 1. *Incorporation of labeled purine bases into nucleotides of cultured astroglia cells*

	Incorporation into total nucleotides (nmol/mg of protein/h)	
Purine base	Control	HGPRT(−)
Hypoxanthine	3.66 ± 0.98 (18)	Undetectable
Guanine	2.68 ± 1.14 (27)	Undetectable
Adenine	13.46 ± 1.79 (22)	17.2 ± 4.3 (13)[a]

Values represent means ± SD. Values in parentheses represent numbers of measurements in separate cultures.
[a] $p < 0.001$, by Student's *t* test, in comparison with control cultures.

100% 2 m*M* $NH_4H_2PO_4$ (pH 2.8) to 60% 0.75 *M* $NH_4H_2PO_4$ (pH 3.9), at a flow rate of 2 ml/min (Sidi and Mitchell, 1985). The purine nucleosides and bases in the culture media were separated on Lichrosorb RP-18 columns (Merck), using a linear gradient made from 10 m*M* KH_2PO_4, pH 5.3, and 80% methanol/H_2O, for a 30-min period, with 0–25% of the 80% methanol solution, at a flow rate of 1.5 ml/min (Osborne et al., 1983). The HPLC system used was a Varian 9010 Solvent Delivery System, with a Varian 9050 Variable Wavelength Detector set at 254 μm, and with computerized integrator.

RESULTS

The cultured astroglia from the HGPRT-deficient transgenic mice exhibited complete HGPRT deficiency (undetectable compared with 105.6 ± 15 nmol/mg of protein/h in the cultures prepared from the control mice). In accordance, in these cells, hypoxanthine and guanine incorporation into nucleotides was also undetectable (Table 1). Adenine phosphoribosyltransferase activity in the HGPRT-deficient cultures (185 ± 23.8 nmol/mg of protein/h; eight determinations) did not differ ($p = 0.22$) from that in the control cultures (176.5 ± 23.2 nmol/mg of protein/h; 10 determinations), but the rate of adenine incorporation into the intact cells' nucleotides, gauging the metabolic availability of PRPP, was accelerated in the HGPRT-deficient cells, in comparison with the control cultures, by 27.8% (Table 1).

The rate of de novo purine synthesis in the astroglia was gauged by the rate of incorporation of the labeled precursor formate into purines. In the HGPRT-deficient astroglia, the rate of incorporation of [^{14}C]formate into intracellular purines was accelerated 9.5-fold, and into the extracellular purines 8.2-fold, the rate in the control astroglia cultures (Table 2).

TABLE 2. *Rate of de novo purine synthesis in cultured astroglia cells*

	Incorporation of [^{14}C]formate into purines (dpm/mg of protein/2 h)	
Cell type	Intracellular	Extracellular
Control (37)	97,399 ± 38,694	11,220 ± 6,609
HGPRT(−) (8)	927,300 ± 126,126[a]	92,223 ± 30,251[a]

Values represent means ± SD. Values in parentheses represent numbers of measurements in separate cultures.
[a] $p < 0.001$, by Student's *t* test, in comparison with control cultures.

TABLE 3. *Fate of prelabeled adenine nucleotides in the astroglia cells*

	Distribution of labeling after 24 h of incubation (% of total radioactivity)	
Fraction	Control	HGPRT(−)
Nucleotides	47.3 ± 2.8 (10)	29.0 ± 3.9 (24)[a]
Perchloric acid insoluble	43.6 ± 2.6 (10)	32.6 ± 4.5 (24)[a]
Medium	9.1 ± 1.1 (10)	38.4 ± 5.0 (24)[a]

Values represent means ± SD. Values in parentheses represent numbers of measurements in different cultures.
[a] $p < 0.001$, by Student's *t* test, in comparison with control cultures.

The fate of adenine nucleotides in the cultured astroglia was assessed by tracing the fate of label from prelabeled adenine nucleotides, after incubation for 24 h (Table 3). The two astroglia cultures differed markedly in the rate of decrease in nucleotide labeling. The nucleotide fraction in the control astroglia lost 52.7% of the radioactivity in 24 h, the larger portion being lost into perchloric acid-insoluble substances and a smaller portion into purines excreted into the culture medium. In contrast, the HGPRT-deficient astroglia exhibited a greater decrease (71%) in the nucleotide labeling, due to a 4.2-fold increase in the excretion of label into the medium. In addition to the difference in the rate of excretion of label into the medium, the two astroglia cultures differed also in the distribution of label among the excreted purines (Table 4). Whereas in the control astroglia the highest proportion of label was found in xanthine, in the HGPRT-deficient cells the majority of the label was found in hypoxanthine (2.5-fold the proportion in the control cultures). In accordance, the proportion of label in adenine, guanine, and xanthine in the HGPRT-deficient cells was markedly lower than in the control astroglia (Table 4). These differences reflect the markedly greater excretion of hypoxanthine by the HGPRT-deficient astroglia (Fig. 1). Both the control and HGPRT-deficient astroglia excreted into the medium similar quantities of xanthine, but differed markedly in the excretion of hypoxanthine. After incubation for 3 days, the media of the control cells contained less hypoxanthine (−28.8 ± 7.5 nmol/mg of protein) than at the beginning of incubation, whereas that of the HGPRT-deficient astroglia contained more hypoxanthine (29.5 ± 9.2 nmol/mg of protein) than at zero time. Apparently, the latter value reflects the amount of hypoxanthine produced endogenously (and not reutilized) in the HGPRT-deficient glia during the 3 days of incubation.

The cellular content of ADP, ATP, and GTP in the HGPRT-deficient glia was significantly lower than that in the control cells, whereas the content of UTP was significantly higher (Table 5). Accordingly, the ratios UTP/ATP and UTP/GTP were markedly higher in the HGPRT-deficient astroglia.

TABLE 4. *Distribution of label from prelabeled nucleotides among cellular and extracellular purines*

		Distribution of label 24 h after prelabeling (dpm/mg of protein)			
		Control			
Fraction analyzed	n	Adenine	Hypoxanthine	Guanine	Xanthine
Intracellular	5	611,976 ± 128,757 (88.3)	23,393 ± 7,835 (3.4)	38,407 ± 10,414 (5.6)	20,153 ± 10,414 (2.8)
Extracellular	8	8,623 ± 730 (13.1)	31,195 ± 5,478 (32.4)	5,955 ± 2,445 (8.2)	40,903 ± 11,359 (46.9)
		Distribution of label 24 h after prelabeling (dpm/mg of protein)			
		HGPRT(−)			
Fraction analyzed	n	Adenine	Hypoxanthine	Guanine	Xanthine
Intracellular	5	467,812 ± 145,205 (89.5)	18,043 ± 5,708 (4.1)	17,280 ± 7,786 (4.1)	10,411 ± 6,739 (2.3)
Extracellular	8	9,984 ± 2,919 (2.0)[a]	461,215 ± 202,860 (82.0)[a]	18,264 ± 10,563 (3.2)[a]	86,687 ± 43,440 (12.8)[a]

Values represent means ± SD (percentages are in parentheses).
[a] $p < 0.001$, by Student's t test, significantly different from the respective control cell value.

DISCUSSION

The HGPRT-deficient astroglia were found to exhibit the basic abnormalities in purine metabolism, which were demonstrated in other HGPRT-deficient cells (Kelley et al., 1970; Fox and Kelley, 1971; Brosh et al., 1976; Zoref-Shani et al., 1980, 1993), including neurons (Zoref-Shani et al., 1993). The connection between HGPRT deficiency and these metabolic abnormalities was discussed in detail before (Zoref-Shani et al., 1993).

The primary metabolic defect in HGPRT-deficient cells is the inability to salvage hypoxanthine and guanine, the degradation products of ATP and GTP, respectively. Indeed, in the HGPRT-deficient glia cells, the uptake of hypoxanthine and guanine from the medium was undetectable (Table 1). However, also in the control cells, despite the presence of HGPRT activity and in contradiction to the results presented in Table 1, guanine reutilization appeared to be very poor. This is evident from the finding that the excretion of xanthine (originating in the glia cells only from guanine) into the culture medium in the control cells did not differ from that in the HGPRT-deficient cells (Fig. 1). The apparent contradiction between the results presented in Table 1 and in Fig. 1 may be explained by the different concentrations of guanine used in these experiments. The metabolic fate of guanine depends on the activity of two enzymes, guanase (guanine aminohydrolase; EC 3.5.4.3) and HGPRT. Apparently, under the conditions at which the amount of guanine available to the cells is low (Fig. 1), resembling more the physiological conditions, the degradative activity of guanase dominates over the anabolic activity of HGPRT. Thus, according to the results depicted in Fig. 1, the primary metabolic defect in the HGPRT-deficient glia, in comparison with the control cells, appears to be only the loss of hypoxanthine, not of guanine. In the control glia cells, the different metabolic fate of hypoxanthine from that of guanine probably reflects also the absence of xanthine oxidase (xanthine:oxygen oxidoreductase; EC 1.3.2.3) activity (Zoref-Shani et al., 1995). Similar results concerning the fate of hypoxanthine and guanine in the control and HGPRT-deficient glia were obtained in our laboratory before in neurons (Brosh et al., 1992; Zoref-Shani et al., 1993), and by others in whole brain tissue (Wong and Henderson, 1972). These results are also compatible with findings concerning the content of hypoxanthine and xanthine in the CSF of boys affected with LNS (Rosenbloom et al., 1967; Sweetman, 1968; Harkness et al., 1988).

The guanase-catalyzed deamination of unsalvaged guanine in the HGPRT-deficient glia cells could result in hyperammonemia in the brain regions rich in guanase, which could be toxic to the brain. However, the finding of equal generation of xanthine by the normal and HGPRT-deficient astroglia (Fig. 1) refutes this possibility. In contrast, the finding of the excessive excretion of hypoxanthine in the HGPRT-deficient astroglia cultures supports our former suggestion (Zoref-Shani et al., 1993) that excessive hypoxanthine concentration in LNS brains may exert neurotoxic effects, resulting in the neurological deficit. Several important functions in neurotransmission have been attributed to purine derivatives, such as adenosine and ATP (Stone, 1981; Su, 1983). No data are available presently to indicate that hypoxanthine may interfere with the receptors for adenosine or ATP. However, hypoxanthine has been shown to be an endogenous ligand for benzodiazepine receptors (Skolnick et al.,

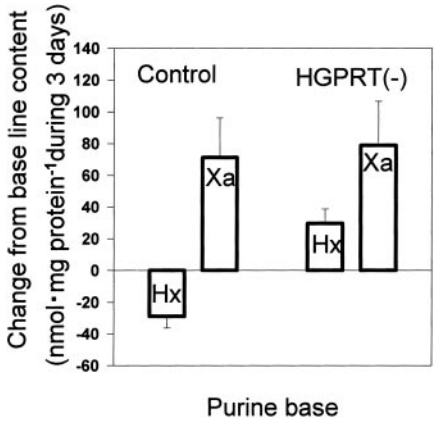

FIG. 1. Change in the culture media content of hypoxanthine (Hx) and xanthine (Xa) by incubation with astroglia cultures for 72 h. The media purine content was determined as described in Materials and Methods. The baseline content of hypoxanthine and xanthine in the fresh medium was 7.212 and 18.215 μM, respectively. The content of these bases per each culture dish was subtracted from the respective values measured in the medium after incubation for 72 h and calculated per milligram of protein.

1980; Kopin, 1981), although with low efficiency (Asano and Spector, 1979).

The absence of salvage of hypoxanthine in the HGPRT-deficient astroglia was reflected in a faster loss of labeling from the prelabeled adenine nucleotides to excreted hypoxanthine (Table 3). Evidently, the normal cycling of the label from adenine nucleotides, through degradation to hypoxanthine, salvage to IMP, and conversion of IMP to adenine nucleotides, does not operate in the HGPRT-deficient glia cells. Similar results were obtained before in our laboratory in an HGPRT-deficient neuroma cell line (Zoref-Shani et al., 1993), and by others in LNS patients, in vivo (Puig et al., 1989).

The most important finding in the present study is that of abnormal nucleotide content in the HGPRT-deficient astroglia (Table 5). Diminished nucleotide availability in HGPRT-deficient brains was one of the first mechanisms considered to underlie the behavioral disturbances in LNS (McKeran, 1977; McKeran and Watts, 1978). As in the brain, the HGPRT-catalyzed salvage pathway, rather than the de novo pathway, was demonstrated to be the major pathway for nucleotide synthesis (McKeran, 1977; McKeran and Watts, 1978; Allsop and Watts, 1980). Nevertheless, until today, this possibility could not be verified. Normal purine nucleotide content was found in whole brains of HGPRT-deficient transgenic mice (see below; Jinnah et al., 1993), as well as in other HGPRT-deficient tissues, or cells such as lymphoblasts, fibroblasts, and erythrocytes (Rosenbloom et al., 1967; Lommen et al., 1971; Nuki et al., 1977*a*,*b*). In contrast, lower ATP content was demonstrated in platelets (Rivard et al., 1975) and lower GTP content was found in erythrocytes from LNS patients (Sidi and Mitchell, 1985; Simmonds et al., 1987). The finding in this study of decreased content of purine nucleotides in the HGPRT-deficient astroglia is the first such demonstration in brain tissue. It is in contrast to the finding of normal content of purine nucleotides in the whole brain tissue of the same HGPRT-deficient transgenic mice used in our study (Jinnah et al., 1993). We have no explanation for this discrepancy, except for the difference in the tissues studied. The normal nucleotide content was demonstrated in whole brain tissue of mature mice (Jinnah et al., 1993), whereas we studied astroglia cultures prepared from newborn mice, after 14 days in culture. It is possible that the low content of purine nucleotides occurs in the HGPRT-deficient astroglia only at a certain stage of maturation of these cells. The finding of low content of purine nucleotides in the HGPRT-deficient astroglia is in accordance with the important role of the salvage pathway in nucleotide homeostasis in the astroglia. In these cells, the ratio [salvage]/[de novo] purine synthesis was demonstrated to increase markedly with maturation in culture (Zoref-Shani et al., 1995). Indeed, nucleotide shortage may occur in HGPRT deficiency, if at all, only in tissues, which depend mainly on the HGPRT-catalyzed salvage pathway for nucleotide generation (Baumeister and Frye, 1985). In view of the quantitative dominance of the glial cells in the brain, the abnormal nucleotide content in these cells, even if confined to a specific stage of development only, may be associated with the neurological deficit in LNS (see below). The finding of the low content of ATP and GTP in the HGPRT-deficient astroglia indicates that the marked acceleration of de novo nucleotide synthesis observed in these cells (Table 2) does not suffice to compensate fully for the absence of hypoxanthine salvage. This could result from consumption of a certain proportion of the accumulating PRPP for synthesis of other nucleotides such as pyrimidine or pyridine nucleotides. Evidently, in the HGPRT-deficient astroglia, some of the PRPP is channeled to synthesis of pyrimidine nucleotides, manifested in the elevated level of cellular UTP content. PRPP is a limiting precursor for pyrimidine nucleotide synthesis and increased cellular UTP content was demonstrated before in HGPRT-deficient tissues (Nuki et al., 1977*a*,*b*). Elevated pyridine nucleotide content was reported in erythrocytes from an

TABLE 5. *Nucleotide content of cultured astroglia cells*

	Nucleotide content (nmol/mg of protein)		
Nucleotide	Control	HGPRT(−)	p^a
ADP	7.72 ± 1.70 (44)	6.32 ± 1.15 (28)	<0.0001
GDP	0.91 ± 0.41 (40)	0.81 ± 0.30 (28)	NS
UTP	10.88 ± 2.51 (40)	12.60 ± 1.83 (28)	<0.001
CTP	8.21 ± 2.19 (44)	7.77 ± 1.59 (28)	NS
ATP	42.63 ± 6.92 (44)	31.92 ± 4.48 (28)	<0.0001
GTP	5.31 ± 1.10 (35)	4.17 ± 0.86 (28)	<0.0001
UTP/ATP	0.28 ± 0.06 (40)	0.40 ± 0.05 (28)	<0.0001
UTP/GTP	2.29 ± 0.35 (35)	3.08 ± 0.50 (28)	<0.0001

Values represent means ± SD. Values in parentheses represent numbers of measurements in different cultures.

[a] p values were calculated according to Student's t test.

HGPRT-deficient subject (Micheli et al., 1995), associated with increased activity of nicotinamide phosphoribosyltransferase (EC 2.4.2.12). This abnormality could also be taken to reflect enhancement of pyridine nucleotide synthesis by the elevated PRPP content. It is not known if the shortage of purine nucleotides accompanies HGPRT deficiency also in the neuronal component of the brain. In a study of an HGPRT-deficient neuroma cell line (Zoref-Shani et al., 1993), we have observed alterations in the cellular content of purine and pyrimidine nucleotides, similar to those detected in the present study in the HGPRT-deficient astroglia (Table 5). However, except for the increase in UTP content, these alterations were statistically insignificant.

Present knowledge assumes that defective dopaminergic transmission is the direct cause for the neurological deficit in LNS (Lloyd et al., 1981; Baumeister and Frye, 1985; Jinnah et al., 1992, 1994; Rossiter and Caskey, 1995). Shortage of nucleotides may cause this abnormality through inhibition of the arborization of the dopaminergic neurons at a developmental stage sensitive to nucleotide availability (Baumeister and Frye, 1985). In a recent study (Yeh et al., 1998), HGPRT-deficient PC12 cells containing dopamine (representing dopaminergic neurons) exhibited impaired differentiation by nerve growth factor, when de novo purine synthesis was inhibited by ~30%. This finding was taken to suggest a possible mechanism for the connection between HGPRT deficiency and the selective injury to the dopaminergic neurons in LNS. According to this suggestion, deficiency of purine nucleotides (mainly ATP) in the HGPRT-deficient dopaminergic neurons in vivo causes dopamine to be more toxic by allowing its leakage from synaptic vesicles to the cytosol.

In conclusion, we have characterized the alterations in purine metabolism in HGPRT-deficient mouse astroglia. These cells exhibited virtually complete deficiency of hypoxanthine salvage, associated with excessive excretion of hypoxanthine, enhanced loss of adenine nucleotides to hypoxanthine, and increased availability of PRPP. The latter abnormality was found to enhance de novo synthesis of purine nucleotides (apparently not adequately compensating for the absence of salvage nucleotide synthesis) and probably also of de novo synthesis of pyrimidine nucleotides. These alterations resulted in lower cellular content of ATP and GTP, and in higher content of UTP. More studies are needed to clarify if the abnormalities in purine and pyrimidine nucleotide content, demonstrated in the HGPRT-deficient astroglia, are associated with the neurological deficit in LNS.

Acknowledgment: This study partially fulfills the requirements of the M.Sc. thesis of D.P. at the Sackler Faculty of Medicine, Tel Aviv University. We gratefully acknowledge the help of Dr. N. Kariv, of the animal facility of the Sackler Faculty, in breeding the mice and the technical assistance of Mrs. H. Oelsner, in the use of HPLC. This study was supported in part by a grant from the Chief Scientist of the Ministry of Health (Israel).

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