tributed to the extraordinarily high levels of AII that are then observed (*21*). The mechanism by which AII stimulates thirst during these extreme conditions remains to be elucidated (*22*).

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Hypoxanthine-Guanine Phosphoribosyltransferase Mutant Glioma Cells: Diminished Monoamine Oxidase Activity

Abstract. *A defective capability of cultured rat glioma cells to reutilize purine bases (hypoxanthine-guanine phosphoribosyltransferase deficiency) was associated with a reduced capacity to oxidatively deaminate serotonin and tryptamine. The mutant glioma cells were also more sensitive to the cytotoxic effects of serotonin than were normal cells.*

The Lesch-Nyhan syndrome (*1*) is a rare X-linked recessive form of cerebral palsy characterized by an excessive production of uric acid, hyperuricemia, and a severe deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT) (*2*). Patients with this disease exhibit neurological dysfunction which includes choreoathetosis, spasticity, mental retardation, and a compulsive self-mutilation of the lips and fingers. Patients with a less severe deficiency of HGPRT also exhibit an excessive production of uric acid and a hyperuricemia, as well as gouty arthritis, but only a portion of them manifest mild neurological dysfunction (*3*).

Rockson *et al.* (*4*) have shown that the plasma of patients with the Lesch-Nyhan syndrome has elevated activity of the enzyme dopamine-β-hydroxylase, which catalyzes the conversion of dopamine to norepinephrine. In general, however, the lack of neurologic material from affected individuals and the absence of an animal model for this disease make it difficult to study the biochemical sequence responsible for the neurological manifestations present in Lesch-Nyhan patients.

Clonal cell lines derived from nervous tissue and grown in vitro represent homogeneous populations of neural cells which are easy to manipulate in large numbers. Neuroblastoma cells deficient in HGPRT in culture are a potentially useful model system for identifying secondary metabolic abnormalities accompanying this mutation, for example, an increased intracellular concentration of the putative neurotransmitter glycine (*5*). Recent findings that suggest a neurochemical role for glial cells—that is, the metabolism of biogenic amines and acetylcholine (*6*)—make these cells a potentially useful model system as well. We now report that glioma cells deficient in HGPRT exhibit decreased activity of monoamine oxidase (MAO) and an enhanced response to the cytotoxicity of serotonin.

Rat glioma clone C6 (*7*) and HGPRT-deficient clones selected from mutagenized (C6-16) and nonmutagenized ($C1_2$) C6 cells have been described (*8*). Cells were cultured in monolayer at 37°C in sealed plastic culture flasks purged with a mixture of 10 percent CO_2 and 90 percent air, in Dulbecco's modified Eagle's medium supplemented with 10 percent fetal calf serum and 2 m*M* L-glutamine (*5*). All cultures were negative when tested for mycoplasma contamination (*9*).

The HGPRT activity of wild-type clone C6 represents 202 nmole of inosinic acid formed per hour per milligram of protein (*8*). The decreased HGPRT activity in the mutant clones (< 1 percent of normal) is associated with an increased intracellular concentration of 5-phosphoribosyl-l-pyrophosphate, a small increase in the overall rate of the early steps of de novo purine synthesis (measured by [^{14}C]formate incorporation into α-*N*-formylglycinamide ribonucleotide), and a greatly diminished ability to incorporate guanine, but not adenine, into soluble nucleotides (*8*). These characteristics are essentially the same as those observed in fibroblasts and lymphoblasts derived from Lesch-Nyhan patients (*10*, *11*).

Monoamine oxidase activity toward serotonin and tryptamine in normal and HGPRT-deficient glioma clones was determined as described (*12*) (Table 1).

Table 1. Monoamine oxidase activity in HGPRT$^+$ and HGPRT$^-$ glioma cells. Results are expressed as means ± the standard error of the mean. Monoamine oxidase activity was determined as described (*6*, *12*).

Cell line	Cell extracts [nmole hr^{-1}(mg protein)$^{-1}$]		Whole cells [nmole $hr^{-1}(10^6$ cells)$^{-1}$]
	Tryptamine	Serotonin	Tryptamine
C6 (HGPRT$^+$)	36.4 ± 2.8	19.7 ± 0.6	3.5 ± 0.2
C6-16 (HGPRT$^-$)	13.9 ± 0.4*	6.9 ± 0.6†	0.9 ± 0.1*
$C1_2$ (HGPRT$^-$)	22.1 ± 0.5†		

*P < .001 compared to clone C6. †P < .05 compared to clone C6.

Cell-free extracts of the mutant clone C6-16 exhibited 35 to 40 percent of the activity in extracts of normal cells (C6), a highly significant difference (P < .001). The nonmutagenized HGPRT-deficient clone $C1_2$ had a 40 percent reduction in MAO activity, also a significant decrease from normal (P < .05). In addition, the MAO activity measured in intact clone C6-16 cells represented only 25 percent of the MAO activity in whole C6 cells (P < .001). These observations are similar to those reported showing decreased MAO activity in HGPRT-deficient neuroblastoma cells (*13*).

Neuroblastoma and glioma cells cultured in vitro contain various enzymes for the catabolism of catecholamines and serotonin (5-HT) (*6*, *14*). Because 5-HT is metabolized only by MAO, the possible physiological consequences of decreased MAO activity in neural cells were evaluated by culturing HGPRT$^+$ and HGPRT$^-$ glioma cells in the presence of varying concentrations of 5-HT. Growth of normal glioma cells was unaffected by up to 0.3 m*M* 5-HT (Fig. 1). At 0.8 m*M* 5-HT a slight decrease in cell number was noted after 4 days, although overall growth rates were similar. In marked contrast, the HGPRT$^-$ clone C6-16 was far more sensitive to the cytotoxicity of 5-HT, with an 80 percent decrease in cell number at 0.3 m*M* 5-HT and a complete loss of viable cells at 0.8 m*M* 5-HT, after 4 days (Fig. 1). Cells were judged to be nonviable after becoming detached from the culture flask and by their ability to take up trypan blue dye.

In our study we have shown that HGPRT deficiency in cultured glioma cells is associated with a marked reduction in measurable MAO activity. The reason for this association is not yet clear. Cultures of normal and HGPRT$^-$ cells were harvested together; the cells used were always in the late logarithmic phase of growth. Monoamine oxidase activity of cultured glioma cells is independent of the stage of cell growth (*6*). While this phenomenon could result from a multiple mutation, its occurrence in several independently derived HGPRT$^-$ clones, including one selected without mutagenesis, would make this possibility seem unlikely.

Certain purine compounds, for example, uric acid and caffeine, reportedly act as inhibitors of mitochondrial MAO (*15*). Because (i) HGPRT$^-$ glioma cells show a small increase in purine biosynthetic rate (*8*), and (ii) cultured HGPRT$^-$ lymphoblasts excrete significant amounts of newly synthesized hypoxanthine in comparison to normal cells (*16*), it is possible that an increased concentration of one or more intermediates or metabolites of the purine de novo pathway is inhibitory to the glial MAO, resulting in a lower measurable MAO activity in HGPRT$^-$ glioma cells. Indeed, uric acid and caffeine, as well as hypoxanthine, inhibit the MAO activity in extracts of normal glioma cells. However, measurement of the MAO activity in a mixture of HGPRT$^+$ and HGPRT$^-$ glioma cell extracts gave a value only 10 percent less than the predicted intermediate value, making it difficult to identify with any certainty an inhibitor of MAO in HGPRT$^-$ glioma cells.

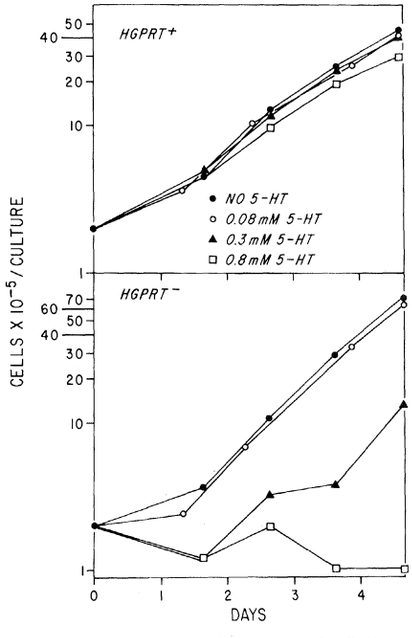

Fig. 1. Effect of serotonin on growth of glioma cells in culture. Glioma cells were grown and harvested as described (*6*), taking cells in late logarithmic growth phase. Cells were pelleted by centrifugation at 100*g* for 5 minutes, then resuspended in culture medium at 0.5 × 10^5 cell/ml. Approximately 2 × 10^5 cells were seeded into 25-cm^2 plastic tissue culture flasks. After allowing several hours for cells to become attached, the medium was replaced with medium containing the following concentrations of serotonin (5-HT): ●, 0 m*M*; ○, 0.08 m*M*; ▲, 0.3 m*M*; □, 0.8 m*M*. The medium was changed daily. Duplicate cultures were counted at the indicated times. (Top) Clone C6 (HGPRT$^+$); (bottom) clone C6-16 (HGPRT$^-$).

A reduction in the capacity of nerve tissue to catabolize 5-HT may be detrimental to certain neurophysiological functions. External concentrations of 5-HT below 1 m*M* were much more toxic to the growth of HGPRT$^-$ glioma cells than to the growth of normal cells. Administration of known MAO inhibitors to laboratory animals has been shown to produce a large rise in brain 5-HT, as well as in dopamine and norepinephrine (*17*). The increase in brain 5-HT is associated with behavioral changes, such as hyperactivity (*18*). Should a similar situation exist with regard to MAO in the nervous system of Lesch-Nyhan patients, these observations may provide some insight into the pathogenesis of the neurological or behavioral abnormalities of this disease.

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Is There Selection Against Wobble in Codon-Anticodon Pairing?

Abstract. *Among amino acid codons that require a third-position pyrimidine, there is a significant bias favoring the use of cytidine over uracil in MS2 phage RNA. This could arise from selection against wobble pairing in the interaction of transfer RNA and messenger RNA. Among amino acid codons with fourfold degeneracy, there is a bias favoring pyrimidines over purines.*

In 1966, Crick (*1*) proposed the wobble hypothesis which permitted a single anticodon to recognize more than one codon. This immediately explained why there are not 61 transfer RNA's (tRNA's) and why coding sometimes differed depending on whether the third-position nucleotide was a purine or a pyrimidine.

One of the intriguing possibilities is that the (negative) binding energies of the adenine : uracil (A : U) and guanine : cytidine (G : C) base pairs are sufficiently larger than that for the G : U base pair in the binding of the anticodon of a tRNA to its complementary codon in the messenger to bring about, selectively, the preferential usage of codons with nonwobble pairing for some reason (for example, that errors are then less frequent). The presence of multiple tRNA's for a single amino acid is a complicating factor in any simple attempt to determine whether this suggestion is valid by any method that does not examine the binding energies directly. There do not, however, appear to be multiple tRNA's with different anticodon specificities for amino acids where the third position of the codon must be a pyrimidine, that is, where a third-position purine would change the amino acid encoded (*2*, *3*). In these cases, the nucleotide involved in pairing with the third nucleotide of the codon is invariably a guanine, albeit sometimes modified. The codons are AAY (Asn), AGY (Ser), CAY (His), GAY (Asp), UAY (Tyr), UGY (Cys), and UUY (Phe), where Y stands for a pyrimidine (*3*). These will be called the pyrimidine-restricted codons. At least one tRNA anticodon is known for each of these amino acids except asparagine, including, in every case, a tRNA from *Escherichia coli*, the host of MS2 phage.

If the hypothesis of selection against wobble pairing is correct, then one should see a preferential utilization of third position C over U in codons for the above cases. This can now be tested since the messenger RNA for the A protein, coat protein, and replicase for the MS2 phage have all been completely sequenced (*4*). This represents a total of 1071 codons, of which 235 code for the cases given above. They are distributed (142 C's plus 93 U's) as shown in Table 1. Aspartate is the only case where more third-position U than C codons are used. The overall bias is significant. Assuming that an equal number of each of the two codons for any one amino acid is expected, we would anticipate the column totals both to be 117.5, on average. On this basis, we find $\chi_1^2 = 10.22$. This means that the probability of this much deviation from expectation is $< .002$. The conclusion must be that there is a bias operating among pyrimidine-restricted codons that favors codons ending in C over those ending in U. Thus, selection has indeed favored the nonwobble pairing. It should be noticed, however, that energy considerations were the basis upon which the question was originally framed, and these results, while consistent with those considerations, do not prove their validity. Any mechanism that would produce the same bias is, a priori, an equally acceptable explanation of that bias. It would therefore be of interest to determine the binding energies of these triplets to their anticodon.

A similar argument and analysis cannot be made for the purine-restricted codons because of the existence, at least in some cases, of tRNA's that specifically recognize codons ending in G only. Apart from any difficulty this imposes in framing a statistical hypothesis, there is no significant difference between the number of purine-restricted codons ending in G and in A.

The remaining codons are, mostly, characterized by fourfold degeneracy. I had no hypothesis regarding them but, for the sake of completeness, they too were examined. They too showed a bias, but in this case it was a preference for third-position pyrimidines over purines (Table 2). Nearly 60 percent (326) of the 565 fourfold degenerate codons contained a third-position pyrimidine. This gives $\chi^2 = 13.4$. The probability of this many pyrimidines in a random sample assuming equal expectations of each is $< .0003$. It is hardly clear why this should be so. It is certainly not the case that the freedom from coding constraint in the fourfold degenerate codons is being used to bring toward random the frequencies of the four necleotides in the third position since that would require a bias in favor of purines rather than pyrimidines in order to compensate for the opposite bias in the restricted codons. There is a slight excess of purines over pyrimidines in the first two codon nucleotide positions, and one might suggest an optimization of secondary structure could be achieved by a corresponding rel-

Table 1. The distribution of pyrimidine restricted codons in MS2 phage. In the first colum shown, the first two nucleotides of those codons that must end in a pyrimidine in order to code for the amino acid in the last column. The center two columns show the frequency of utilization of these codons in MS2 phage according to whether the third position base is cytidine or uracil.

Codons	--C	--U	
AA-	28	17	Asn
AG-	16	8	Ser
CA-	9	6	His
GA-	22	28	Asp
UA-	32	9	Tyr
UG-	6	6	Cys
UU-	29	19	Phe
Total	142	93	

Table 2. The distribution of fourfold degenerate codons in MS2 phage. In the first column, the first two nucleotides of those codons whose amino acid, shown in the last column, is specified regardless of the third nucleotide. The center two columns show the frequency of utilization of these codons in MS2 phage according to whether the third position base is a pyrimidine (Y) or a purine (R).

Codons	--Y	--R	
AC-	39	27	Thr
CC-	28	22	Pro
CG-	41	21	Arg
CU-	41	24	Leu
GU-	47	44	Ala
GG-	53	28	Gly
GU-	42	35	Val
UC-	35	38	Ser
Total	326	239	