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Monoamine Oxidase Activity Decreased in Cells Lacking Hypoxanthine Phosphoribosyltransferase Activity

Abstract. *The Lesch-Nyhan syndrome in humans is characterized by lack of hypoxanthine phosphoribosyltransferase activity and neurologic abnormalities that suggest changes in catecholamine metabolism. Monoamine oxidase, which degrades biogenic amines, has decreased activity in noradrenergic murine neuroblastoma cell lines lacking hypoxanthine phosphoribosyltransferase activity and in skin fibroblasts from patients with the Lesch-Nyhan syndrome.*

The primary biochemical lesion in the Lesch-Nyhan syndrome is lack of hypoxanthine phosphoribosyltransferase activity (HPRT; E.C. 2.4.2.8), an enzyme involved in purine salvage (*1*). It is not clear why this lesion produces neurologic dysfunction, including mental retardation, spasticity, choreoathetosis, and compulsive self-mutilation (*2*). Other findings suggest that catecholamine metabolism may be altered in this disease: potentiation of catecholamine pathways in the brains of experimental animals leads to chorea, compulsive gnawing, and self-mutilation (*3*); and Lesch-Nyhan patients show peripheral changes in noradrenergic functioning (*4*). Our finding that monoamine oxidase (MAO; E.C. 1.4.3.4) activity is reduced in cells lacking HPRT activity could account for altered catecholamine metabolism in this disease. Monoamine oxidase is one of the major enzymes involved in degradation of biogenic amines throughout the body, and its decreased activity results in increased tissue levels of these neurotransmitters (*5*).

Murine neuroblastoma cells in culture have many properties of normal neurons (*6*) and provide a model system to explore the effects of HPRT deficiency on neuronal metabolism. We have used clonal lines of neuroblastoma that differ in their abilities to synthesize various neurotransmitters (*7*). Line N1E-115 is classified as noradrenergic in that it has high activities of two enzymes needed in norepinephrine synthesis, tyrosine 3-hydroxylase (E.C. 1.14.16.2) (*8*) and dopamine β-hydroxylase (E.C. 1.14.17.1) (*9*), as well as the ability to store catecholamines (*10*). This line also shows high MAO activity of the A type, which is the predominant form in sympathetic neurons (*11*). Cholinergic line NS-20 has high levels of choline acetyltransferase (E.C. 2.3.1.6) activity and synthesizes acetylcholine (*8*). Lines N-4 and N-18 are "inactive" with respect to synthesis of these transmitters as they have low levels of both tyrosine 3-hydroxylase and choline acetyltransferase (*8*).

6-Thioguanine (TG) resistant lines derived from these neuroblastoma clones (*7*) were found to have less than 1 percent of the HPRT activity (*12*) of the pa-

Table 1. Catecholamine metabolism in neuroblastoma lines with and without HPRT activity. The HPRT activities were measured in two different cultures of each line; 100 percent corresponds to 32 to 161 pmole/min per milligram of protein (*12*). Tyrosine hydroxylase (TH) and MAO activities (*13*) are expressed as the means (picomoles per minute per milligram of protein) ± standard mean error (S.E.): The numbers in parentheses refer to the number of separate homogenates tested; each homogenate was assayed in duplicate; only duplicates varying by < 20 percent were averaged and used here. Chromatographic identification of the metabolic products of [^{3}H]dopamine (*15*) are expressed as the percentage of radioactivity recovered from strips which comigrated with authentic standards; values are expressed as the means ± S.E., averaged from two separate experiments. Abbreviations: DA, dopamine; NE, norepinephrine; and MT, 3-methoxytyramine.

Cell line	HPRT (%)	MAO	TH	[^{3}H]Dopamine metabolism, radioactivity migrating as:			
				DOPAC (%)	NE (%)	MT (%)	DA (%)
			Noradrenergic				
N1E-115	100	103 ± 9 (5)	59 ± 7 (6)	9 ± 2	24 ± 1	4 ± 1	56 ± 1
N1E-115TG1	< 1	3.3 ± 2.8 (3)	64 ± 37 (4)	0	12 ± 2	9 ± 1	71 ± 4
N1E-115TG2	< 1	4.9 ± 3.3 (3)	48 ± 10 (3)	0	22 ± 9	7 ± 1	56 ± 8
N1E-115TG3	< 1	0.8 ± 0.5 (3)	21 ± 2 (4)	0	19 ± 6	7 ± 2	67 ± 8
N1E-115TG4	< 1	1.3 ± 0.6 (3)	50 ± 7 (2)	0	22 ± 3	7 ± 3	61 ± 2
N1E-115TG5	< 1	1.1 ± 0.5 (3)	24 ± 2 (3)	0	20 ± 4	6 ± 1	67 ± 3
N1E-115TG6	< 1	1.0 ± 0.5 (3)	29 ± 12 (5)	0	30 ± 8	7 ± 1	58 ± 9
N1E-115TG7	< 1	0.02 ± 0.01 (2)	40 ± 10 (4)	0	21 ± 5	6 ± 1	66 ± 8
N1E-115TG8	1	1.9 ± 1.1 (3)	17 ± 5 (4)	0	26 ± 3	3 ± 1	63 ± 4
			Cholinergic				
NS-20	100	132 ± 16 (4)	8 ± 3 (2)	8 ± 1	18 ± 1	4 ± 1	67 ± 2
NS-20TG7B	< 1	133 ± 18 (4)	15 ± 8 (2)	6 ± 2	10 ± 1	1 ± 1	78 ± 1
NS-20TG11E	< 1	85 ± 15 (4)	5 ± 3 (2)	10 ± 1	5 ± 1	1 ± 1	81 ± 2
			"Inactive"				
N-18	100	138 ± 20 (4)	9 ± 2 (3)	47 ± 6	4 ± 1	5 ± 2	35 ± 3
N-18TG2	< 1	75 ± 6 (5)	9 ± 4 (4)	42 ± 9	2 ± 1	9 ± 1	41 ± 6
N-4	100	112 ± 5 (2)	10 ± 8 (2)	31 ± 1	0	8 ± 1	50 ± 2
N-4TG1	< 1	136 ± 27 (4)	6 ± 3 (3)	32 ± 5	0	10 ± 3	45 ± 6

rental clones (Table 1). In addition, all eight of the HPRT deficient lines derived from noradrenergic clone N1E-115 had reduced levels of MAO activity (Table 1) (*13*). This decrease in MAO activity was not observed in the TG resistant lines derived from cholinergic and "inactive" clones. In contrast, there was no consistent change in tyrosine 3-hydroxylase activities of HPRT deficient lines derived from clone N1E-115; all these noradrenergic lines did have tyrosine 3-hydroxylase activities several times greater than the other lines (Table 1) (*14*).

By examining chromatographically the metabolic products of [^{3}H]dopamine formed by intact cultures, we can assess the intracellular activities of MAO, dopamine β-hydroxylase, and catechol methyltransferase (E.C. 2.1.1.6) (*15*). These enzymes convert dopamine to 3,4-dihydroxyphenylacetic acid (DOPAC), norepinephrine, and 3-methoxytyramine, respectively. Almost all of the radioactivity recovered from the chromatograms was accounted for by these compounds (1 to 8 percent of the radioactivity appeared as an unidentified peak near the origin). Parental lines and the HPRT deficient lines derived from them showed similar cellular metabolism of dopamine, except that, in accord with the MAO activities measured in vitro, DOPAC formation was observed in N1E-115, but not in the N1E-115TG lines (Table 1). By this method MAO activity appeared to be lower, and dopamine β-hydroxylase activity higher, in parental lines N1E-115 and NS-20, as compared to N-18 and N-4; catechol methyltransferase activities were similar in all lines.

It is not clear why the reduction in MAO activity associated with HPRT deficiency was only observed in mutant lines derived from clone N1E-115. These lines differed from the other neuroblastoma lines examined in that they showed higher activities of both tyrosine 3-hydroxylase and dopamine β-hydroxylase; they may differ also in other undetermined ways. Although the eight lines derived from N1E-115 are similar by the biochemical criteria presented, some are distinct by morphological criteria, including length of neurites and the tendency of cells to aggregate. Still, we cannot rule out the possibility that they are all progeny of a single cell in which, for other reasons, both MAO and HPRT activities were decreased.

Patients with the Lesch-Nyhan syndrome have less than 1 percent of normal HPRT activity in cells throughout the body; this deficiency is maintained in fibroblasts cultured from skin biopsies (*16*). We measured MAO activities in homogenates prepared from fibroblasts cultures (*13*, *17*) of six Lesch-Nyhan patients and six controls (Table 2). The mean MAO activity for Lesch-Nyhan lines was 9.4 pmole/min per milligram of protein (S.D. ± 6.0), whereas the mean for controls was 27.4 (±13.3). This represents an average threefold lower MAO activity in the Lesch-Nyhan fibroblasts.

Table 2. Monoamine oxidase activity (picomoles per minute per milligram of protein) of human fibroblast lines from Lesch-Nyhan patients and controls. Values for each individual are expressed as means ± S.E. The numbers in parentheses refer to the number of separate homogenates assayed. Each homogenate was assayed in duplicate; only duplicates varying by < 20 percent were averaged and used. The total population mean is given ± standard deviation.

Lines	Sex	Age (years)	MAO
		Control	
Ro Bel	M	14	46.4 ± 11.4 (5)
Rid Mor	M	15	17.6 ± 2.0 (5)
El San	M	8	18.8 ± 1.3 (2)
87	M	12	27.1 ± 5.9 (5)
82	F	30	40.4 ± 8.8 (4)
237	M	30	13.9 ± 1.8 (3)
Total population mean			27.4 ± 13.3*
		Lesch-Nyhan	
Sal Mat	M	14	8.1 ± 3.0 (5)
Mi Ten	M	12	4.6 ± 0.6 (2)
To Ser	M	9	6.5 ± 1.0 (5)
On Ser	M	7	18.1 ± 5.5 (6)
115	M	13	3.7 ± 0.9 (5)
28	M	14	15.7 ± 2.7 (5)
Total population mean			9.4 ± 6.0*

*Significantly different at $P \sim .014$ by Student's *t*-test.

Both noradrenergic murine neuroblastoma cells and human fibroblasts showed decreased MAO activity occurring in conjunction with HPRT deficiency. The reduction of MAO activity in noradrenergic neuroblastoma cells was much larger than in fibroblasts, and other neuroblastoma lines showed no decrease in activity. This suggests that different cell types may be more sensitive to this inhibitory effect than others. In addition, the reduced MAO activity observed in noradrenergic neuroblastoma cells lacking HPRT activity was not associated with other changes in catecholamine metabolism, including the activities of tyrosine 3-hydroxylase, dopamine β-hydroxylase, and catechol methyltransferase. Wood *et al.* (*18*) have reported that neuroblastoma line N-4 and the HPRT deficient line N-4TG1 derived from it are similar with respect to neurite formation, electrical excitability, and acetylcholinesterase (E.C. 3.1.1.7) activity. These findings suggest that HPRT deficiency may depress MAO activity specifically, leaving many other neuronal properties intact.

The reduction of MAO activity in some HPRT deficient cells points to an as yet undefined link between the regulation of purine and catecholamine metabolism. The highest levels of HPRT activity in the body are observed in the brain, especially in the region of the basal ganglia (*19*). This area of the brain controls involuntary movements and receives catecholaminergic input. Reduction of HPRT activity in the basal ganglia of Lesch-Nyhan patients may be associated with reduced MAO activity, which in turn could affect neurotransmission mediated by biogenic amines.

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14. Tyrosine hydroxylase activities for N1E-115, NS-20, N-4, and N-18 have been reported as 980, 0, 4 and 2 pmole of product formed per minute per milligram of protein, respectively [T. Amano, E. Richelson, M. Nirenberg, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 258 (1972)]. We have no explanation for the 16-fold lower activity seen here for N1E-115, but presume it results from a higher subculture number of 20 to 30.
15. The cellular metabolism of [^{3}H]dopamine was determined by exposing cultures to it for 30 minutes, extracting washed cells, and identifying labeled metabolites chromatographically by their comigration with authentic standards (*10*). In our study here, we used samples containing 100,000 count/min for the thin-layer chromatograms, which were run in solvent system A (*10*). The following compounds migrate together in system A and cannot be distinguished: 3,4-dihydroxyphenylacetic acid, 3-methoxy-4-hydroxyphenylglycol, and 3-methoxy-4-hydroxymandelic acid.
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Ultrastructure of the Auditory Regions in the Inner Ear of the Lake Whitefish

Abstract. *Hair cell polarization patterns were investigated on the sensory macule of the sacculus and lagena of the lake whitefish. The saccular hair cells are divided into four groups, with all of the cells within a group having the same orientation. Saccular orientations are anterior, posterior, dorsal, and ventral with respect to the axis of the animal. Two groups, one dorsal and one ventral, are found on the lagena. The saccular orientations are significantly different from those in tetrapods. Since this organ appears to have different functions in fish and tetrapods it is likely that the orientation patterns in fish are adapted to some aspect of audition—perhaps directional localization of sound.*

Sound detection in many species of teleost fish involves the sacculus and lagena, two otolithic regions of the inner ear (*1*, *2*). Stimulation of the sensory hair cells in these regions probably results from differential movement between the sensory macula (hair cell containing neuroepithelium) and a single overlying dense calcareous otolith (*2*, *3*). Physiological recordings along the length of the saccular macula show that different regions respond differently to the same signal, and that the level of microphonic response in any particular region may vary, depending on the direction of the sound field (and otolith movement) (*4*, *5*). These data suggest a complex interaction between the hair cells and the otolith, and may indicate mechanisms in the teleost ear for various types of signal analysis (for example, frequency) as well as directional localization of sound

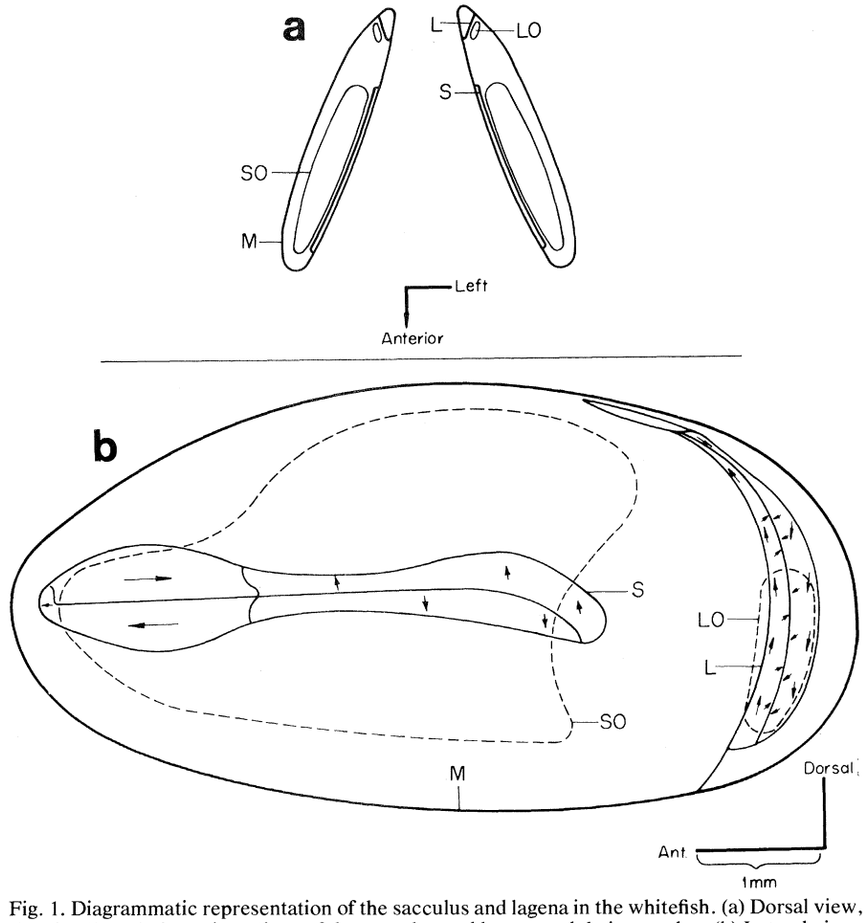

Fig. 1. Diagrammatic representation of the sacculus and lagena in the whitefish. (a) Dorsal view, showing the relative orientations of the sacculus and lagena and their maculae. (b) Lateral view, showing the sacculus and lagena with the orientation patterns of the hair cells on the different regions of macula. The arrows indicate the directions of orientation of the hair cells. The positions of the otoliths are indicated by dashed lines since they normally lie lateral to the maculae. It should be noted that the lower 95 percent of the lagena is at an angle of about 45° to the saccular macula, while the top of the sensory region is turned so that the sensory area faces ventrally. Abbreviations: *SO*, saccular otolith; *LO*, lagenar otolith; *S*, saccular macula; *L*, lagenar macula; and *M*, membranous chamber of the ear.