Biogenic Amine Synthesis Defect in Dihydropteridine Reductase Deficiency

Abstract. *In the enzymatic hydroxylation of aromatic amino acids, tetrahydrobiopterin is the essential cofactor. Regeneration of tetrahydrobiopterin requires dihydropteridine reductase, without which there should be a deficiency of hydroxylated amino acids and their products, biogenic amines. Assay of biopsied brain cortex of a patient with a deficiency of dihydropteridine reductase showed low concentrations of serotonin and dopamine, and this was reflected in the concentrations of their major metabolites measured in cerebrospinal fluid from lumbar, ventricular, and subarachnoid spaces. The metabolite concentrations were restored to normal, or above normal, by treatment with specific amino acids which bypass the metabolic block at the hydroxylation step. It is postulated that the seizures and neurological deterioration of the patient were related to a deficiency in the synthesis of biogenic amine neurotransmitters.*

In this report we present evidence from a single case study that there is a central nervous system disorder involving defective biosynthesis of catecholamine and indoleamine neurotransmitters due to a deficiency in dihydropteridine reductase (DHPR). The subject was a male child who, at 10 days of life, was diagnosed as having phenylketonuria. Despite excellent control of hyperphenylalaninemia by dietary restriction of phenylalanine, seizures and mental deterioration became evident at 7 months of age. Analysis of a liver biopsy at age 15 months showed that the phenylalanine hydroxylase activity was approximately 20 percent of the average normal adult value (*1, 2*), which is much higher than that of patients with classical phenylketonuria or other forms of hyperphenylalaninemia. For optimal hydroxylation of phenylalanine, molecular oxygen and the cofactor tetrahydrobiopterin are needed. Tetrahydrobiopterin is regenerated to its active form by DHPR. There was no detectable DHPR activity in the child's liver or frontal brain cortex (*1*).

Dihydropteridine reductase is essential for the regeneration of tetrahydrobiopterin, and, as expected, essentially no active tetrahydrobiopterin was present in the patient's liver. In turn, tetrahydrobiopterin is a necessary cofactor for the hydroxylation of the aromatic amino acid precursors needed for the biosynthesis of catecholamine and indoleamine neurotransmitters (*3*). The neurotransmitters, dopamine and serotonin, were deficient in the patient, as evidenced by the low concentrations of their metabolites in cerebrospinal fluid (CSF) and their impaired turnover determined after administration of probenecid. These findings were substantiated by the direct measurement of amines in frontal cortical tissue sampled at brain biopsy (*4*).

All studies were performed with the patient on a low-phenylalanine diet to maintain a serum concentration of less than 5 mg/100 ml, and vitamin supplementation including oral folic acid at 50 μg/day. Phenobarbitone, 90 mg/day in divided doses, was continued during neurotransmitter investigations to control seizures. Although studies with animals indicate that barbiturates can depress biosynthesis of neurotransmitters (*5*), the barbiturate dose used in this study was low compared to those in the animal studies and the deficit in CSF metabolites was much more severe than those observed in epileptic patients on anticonvulsants (*6*).

Metabolites in CSF were measured by quantitative gas chromatography–mass spectrometry (GC-MS) (*7*). Lumbar CSF was sampled before and 18 hours after oral administration of probenecid, 175 mg/kg in four divided doses (*8*). The initial 5 ml of lumbar CSF was stored in a glass tube at −70°C with sodium metabisulfite (10 mg/ml). Concomitant with the biopsy of brain cortex at 21 months of age, CSF was taken from cortical subarachnoid spaces and right lateral ventricle. Brain biopsy tissue was processed for routine histology and electron microscopy, and a portion was rapidly frozen in liquid nitrogen and later dissected into gray and white tissue for neurotransmitter analysis by GC-MS (*9*).

Table 1. Concentrations of HVA and 5-HIAA in cerebrospinal fluid before (baseline) and after administration of probenecid. Lumbar CSF was sampled before and 18 hours after oral administration of probenecid, 175 mg/kg in four divided doses. Lateral ventricle and subarachnoid fluid was sampled at brain biopsy. Abbreviations: B., baseline; A.P., after probenecid.

Area sampled	HVA (ng/ml)		5-HIAA (ng/ml)	
	B.	A.P.	B.	A.P.
Lumbar	33	128	4.2	22.4
Lateral ventricle	85		24.6	
Cortical subarachnoid	29		44.5	

Initial investigations showed that, at age 18 months, the basal levels of 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) in lumbar CSF (Table 1) were low compared to the median control values [44 ng/ml for HVA and 40 ng/ml for 5-HIAA (*10*)]. Treatment with probenecid permits determination of the turnover of these amines since it prevents active transport of the amine metabolites out of the CSF (*11*). The metabolite concentrations after probenecid administration were low (Table 1): the 5-fold increase in 5-HIAA was within the normal 4- to 12-fold range, but the 4-fold increase in HVA was low compared to the normal 9- to 40-fold increase (*8, 12*). Low levels of HVA and 5-HIAA were also found in lateral ventricle and cortical subarachnoid fluid (Table 1). For lateral ventricle, normal values of HVA range from 352 to 466 ng/ml and normal values of 5-HIAA from 102 to 105 ng/ml (*13*).

After these baseline measurements were made and after recovery from brain biopsy, the patient (21 months of age) was given tyrosine orally for 6 days at 100 mg/kg per day. Clinically, this treatment did not produce any significant change in the patient. Blood and urinary amino acids were measured during and after the administration of tyrosine. Serum amino acids did not change appreciably with tyrosine, nor was there any significant change in HVA in lumbar CSF before or after probenecid (Table 2). There was, however, a decrease in 5-HIAA in lumbar CSF both before and after probenecid (Table 2). These results support the idea that the blockade of neurotransmitter biosynthesis is at the hydroxylation step, since tyrosine, the precursor of dopamine, does not result in the expected increase in formation of the major metabolite of dopamine, HVA. The fall in the serotonin metabolite, 5-HIAA, may be due to competition of tyrosine with tryptophan for common transport and uptake mechanisms from the periphery (*14*).

To overcome the blockade at the hydroxylation step, oral administration of L-dihydroxyphenylalanine (L-dopa) and 5-hydroxytryptophan (5-HTP) was begun when the patient was 22 months of age; the doses were gradually increased over several weeks to 500 mg/day for L-dopa and 250 mg/day for 5-HTP. Vomiting was observed at the high dose of 5-HTP but stopped when the dose was reduced to 200 mg/day. There was no sig-

nificant clinical improvement at these doses, although lumbar CSF measurements (24 months of age) before and after probenecid showed substantial increases in 5-HIAA and HVA (Table 2). In an attempt to increase clinical effectiveness and obtain higher concentrations of the amine precursor and amines in the central nervous system, peripheral aromatic amino acid decarboxylation was blocked by simultaneously administering α-methyldopahydrazine (carbidopa) in combination with L-dopa (*15*). The amino acids and decarboxylase inhibitor were increased over several weeks, starting at 25 months of age, to 400 mg of L-dopa, 40 mg of carbidopa, and 100 mg of 5-HTP daily in four divided doses. After 3 months of treatment (Table 2), there were even greater increases in the lumbar CSF concentrations of HVA and 5-HIAA. The HVA levels were approximately five times greater than normal and eight times greater than those observed before amino acid therapy. The 5-HIAA levels were approximately equal to those in normal controls and 25 times higher than the value before treatment. During this 3-month period, episodic opisthotonus and irritability appeared to be related to excess hydroxylated amino acids, and the doses of both precursors were intermittently reduced.

The deficiency in neurotransmitter synthesis was substantiated by direct measurement of brain amines in cortical gray matter and white tissue. Serotonin was barely detectable and was not measured. Dopamine concentrations were 3 ng per gram of gray matter and 2 ng per gram of white tissue, which are low compared to the values reported for human cortex, 80 to 190 ng/g (*16*). The data on these two neurotransmitters are therefore consistent with the low levels of CSF metabolites detected. The major metabolite of norepinephrine, 3-methoxy-4-hydroxyphenylethylene glycol, was not determined in CSF. In this patient's cortical tissue the norepinephrine concentrations were 1600 ng per gram of gray matter and 540 ng per gram of white tissue, which are high compared to values reported for human adult controls, 30 to 50 ng/g (cortex) and 270 to 780 ng/g (hindbrain) (*16*). The high norepinephrine values are not consistent with the concept that norepinephrine is dependent on the same hydroxylation biosynthetic pathway as dopamine and serotonin. However, they are consistent with the finding of normal total serum catecholamine (norepinephrine and epinephrine) levels and normal urinary excretion of the epinephrine metabolite metanephrine, and with the fact that there was no clinical evidence of hypotension or blood vessel instability. The implications of these findings are not clear. Possible interpretations include impaired breakdown of norepinephrine by metabolic enzymes, an alternative biosynthetic pathway not involving tyrosine hydroxylase, alteration in the storage mechanism, a second rate-limiting step other than tyrosine hydroxylase (that is, dopamine β-hydroxylase), or a differential turnover rate.

Table 2. Concentrations of HVA and 5-HIAA in lumbar cerebrospinal fluid following amino acid therapy. Lumbar CSF was sampled before and 18 hours after oral administration of probenecid, 175 mg/kg in four divided doses. Details of amino acid therapy are in the text. Abbreviations as in Table 1.

Therapy	HVA (ng/ml)		5-HIAA (ng/ml)	
	B.	A.P.	B.	A.P.
Basal	33	128	4.2	22.4
Tyrosine	26	108	1.9	5.9
L-Dopa and 5-HTP	128	213	7.8	32
L-Dopa, 5-HTP, and carbidopa	264		105	

Despite the increased concentrations of dopamine and serotonin in the brain, as indicated by the lumbar CSF levels of HVA and 5-HIAA, there was minimal improvement in the clinical condition of this patient after treatment with L-dopa and 5-HTP. There was an initial reduction in body and limb hypertonicity and hyperreflexia, and a decrease in the frequency and severity of seizure activity associated with improvement of the hypsarrhythmic pattern on his electroencephalogram (EEG). However, his neurological condition subsequently regressed, although the improvement in the EEG was maintained. Replacement treatment with hydroxylated amino acid precursors was not started until 22 months of age, at which time the patient had severe neurological deficits. Although this treatment may have increased the survival period, we believe that irreversible brain damage occurred before therapy.

This study explicitly documents a deficiency in biogenic amine neurotransmitters due to a lack of DHPR and presumably the cofactor tetrahydrobiopterin, which is needed for hydroxylation of the aromatic amino acids. The data indicate that it is possible to increase the amine concentrations in the central nervous system by treatment with the hydroxylated amino acid precursors L-dopa and 5-HTP. When peripheral biosynthesis of these amines is prevented by concomitant administration of a peripheral decarboxylase inhibitor, there is more efficient uptake and utilization of the precursors by the brain and the dose can be reduced, thereby decreasing the accumulation of toxic by-products of peripheral biosynthetic pathways. It is suggested that patients with a similar biogenic amine deficiency due to this form of hyperphenylalaninemia may be treated with these hydroxylated amino acids if replacement is initiated at an early age.

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4. Informed consent for liver and brain biopsy, lumbar punctures, and probenecid and other treatments was obtained from both parents of the patient after the nature of the procedure had been fully explained. The brain biopsy was performed as part of a protocol designed to evaluate degenerative disorders of the central nervous system in children. After a deficit in cerebral neurotransmitters was demonstrated, informed consent from both parents was obtained before use of precursor amino acids.
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