

## Rapid Communication

# Monoaminergic Effects of Folinic Acid, L-DOPA, and 5-Hydroxytryptophan in Dihydropteridine Reductase Deficiency

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**Abstract:** Plasma and CSF concentrations of endogenous L-DOPA, catecholamines, and metabolites of monoamines were assayed in a patient with atypical phenylketonuria due to absent dihydropteridine reductase (DHPR), before and during treatment with folinic acid, Sinemet, and 5-hydroxytryptophan. The patient had low but detectable levels of L-DOPA, 3,4-dihydroxyphenylacetic acid (DOPAC), and 3,4-dihydroxyphenylglycol (DHPG) in plasma and low but detectable levels of these compounds and of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in CSF, with approximately normal plasma and CSF levels of norepinephrine [noradrenaline (NA)]. Folinic acid treatment approximately doubled plasma levels of L-DOPA, NA, DOPAC, and DHPG, compared with values during dietary phenylalanine restriction alone. Detection of L-DOPA, catecholamines, and monoamine metabolites in this patient indicates that monoamine synthesis in humans does not absolutely require DHPR. The results are consistent with the existence of an alternative biochemical pathway, with folinic acid treatment augmenting activity along this pathway. Low plasma levels of L-DOPA, DOPAC, and DHPG may reflect decreased catecholamine synthesis and turnover in sympathetic nerves, with compensatory increases in exocytotic release normalizing plasma NA levels. **Key Words:** Dihydropteridine reductase—Tetrahydrobiopterin—Phenylketonuria—Catechols—DOPA—Norepinephrine—Dopamine—Serotonin—Dihydroxyphenylacetic acid—Dihydroxyphenylglycol—Homovanillic acid—5-Hydroxyindoleacetic acid—5-Hydroxytryptophan.

*J. Neurochem.* **64**, 2810–2813 (1995).

Dihydropteridine reductase (DHPR) is an essential enzyme in the hydroxylating systems for phenylalanine (Phe), tyrosine, and tryptophan. Deficiency of DHPR causes a severe variant form of phenylketonuria (PKU), where dietary restriction of Phe alone fails to prevent neurological impairments (Kaufman et al., 1975).

Because DHPR catalyzes production of tetrahydrobiopterin (BH<sub>4</sub>) from quinonoid dihydrobiopterin (q-BH<sub>2</sub>) and because BH<sub>4</sub> is a key cofactor for tyrosine hydroxylase and tryptophan hydroxylase, DHPR deficiency would be ex-

pected to cause low or absent levels of catecholamines, serotonin (5-HT), and their metabolites.

DHPR deficiency is also associated with low levels of folates, and the combination of monoaminergic and folate abnormalities could explain the progression of neurological failure. Patients with DHPR deficiency can benefit from treatment with monoamine precursors and folinic acid (Kaufman, 1991; Irons et al., 1987).

We report here levels of endogenous L-DOPA, other catechols, and monoamine metabolites in a patient with absent DHPR activity and the monoaminergic effects of treatment with folinic acid, L-DOPA (as Sinemet, a combination of L-DOPA with the peripheral inhibitor of L-aromatic amino acid decarboxylase, carbidopa), and 5-hydroxytryptophan (5-HTP).

## MATERIALS AND METHODS

The patient had a positive neonatal test for PKU. Despite a well-controlled low Phe diet instituted within 2 weeks after birth, he failed to develop normally and by 6 months of age showed loss of milestones and neurological abnormalities, including extreme irritability and truncal hypotonia.

At 7 months of age, the patient's levels of urinary pterins were measured. Almost none of the biopterin was present

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Resubmitted manuscript received February 23, 1995; accepted February 27, 1995.

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*Abbreviations used:* BH<sub>4</sub>, tetrahydrobiopterin; DA, dopamine; DHPG, 3,4-dihydroxyphenylglycol; DHPR, dihydropteridine reductase; DOPAC, 3,4-dihydroxyphenylacetic acid; EPI, epinephrine; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; 5-HTP, 5-hydroxytryptophan; HVA, homovanillic acid; 3-MT, 3-methoxytyrosine; NA, noradrenaline; Phe, phenylalanine; PKU, phenylketonuria; q-BH<sub>2</sub>, quinonoid dihydrobiopterin.

**TABLE 1.** Concentrations of monoamines and metabolites in plasma and CSF in a DHPR-deficient patient, during dietary Phe restriction alone and after additional treatment with folinic acid (F), L-DOPA/carbidopa [Sinemet (S)], and 5-HTP

	Concentration (nmol/L)					
	L-DOPA	DA	NA	EPI	DHPG	DOPAC
<b>Plasma</b>						
Normal control (pediatric)	12.63 <sup>a</sup>	0.03 <sup>a</sup>	4.43 <sup>a</sup>		6.01 <sup>a</sup>	12.76 <sup>a</sup>
Lower range of normal	7.98 <sup>a</sup>	0.00 <sup>a</sup>	2.96 <sup>a</sup>		4.86 <sup>a</sup>	7.91 <sup>a</sup>
Normal control (adult)	9.08 <sup>a</sup>	0.17 <sup>a</sup>	1.49 <sup>a</sup>	0.26 <sup>b</sup>	7.60 <sup>a</sup>	14.21 <sup>a</sup>
Dietary Phe restriction	3.77	0.02	2.38	0.06	1.03	1.35
F (12.5)	7.14	0.03	4.63	0.10	2.09	1.94
F + S (4)	529	—	4.81	—	7.87	56.2
F + S (8)	1,001	—	4.27	—	6.76	69.2
F + S (10) + 5-HTP (25)	923	0.79	2.32	—	5.38	106
F + S (10) + 5-HTP (100)	396	—	1.80	—	3.50	25.8
<b>CSF</b>						
Normal control (adult)	3.81 <sup>a</sup>	0.02 <sup>a</sup>	0.50 <sup>a</sup>	≤0.01 <sup>a</sup>	9.68 <sup>a</sup>	2.28 <sup>a</sup>
Lower range of normal	2.82 <sup>a</sup>	0.00 <sup>a</sup>	0.35 <sup>a</sup>		7.48 <sup>a</sup>	0.98 <sup>a</sup>
Dietary Phe restriction	1.26	≤0.01	0.49	≤0.01	1.53	0.40
F (12.5)	1.32	≤0.01	0.44	≤0.01	1.86	0.33
F + S (4)	101	—	0.52	—	2.60	2.26
F + S (8)	135	—	0.34	—	3.09	2.23
F + S (10) + 5-HTP (25)	232	—	0.58	—	2.47	3.45
F + S (10) + 5-HTP (100)	253	—	1.30	—	4.87	5.10

	Concentration (nmol/L)			
	HVA	5-HIAA	5-HTP	3-MT
<b>CSF</b>				
Normal control (pediatric)	465 <sup>c</sup>	185 <sup>c</sup>		
Lower range of normal	154 <sup>c</sup>	89 <sup>c</sup>		
Normal control (adult)	236 <sup>d</sup>	95 <sup>d</sup>		
Lower range of normal	165 <sup>d</sup>	80 <sup>d</sup>		
Dietary Phe restriction	53	8.9		≤3
F (12.5)	34	5.2		≤3
F + S (8)	90	6.3	0.3	90.0
F + S (10) + 5-HTP (25)	103	17.3	23.9	170.1
F + S (10) + 5-HTP (100)	291	90.6	172.4	343.7

A dash indicates values below the detection limit. Numbers in parentheses represent drug doses (for F and S in mg/kg/day; for 5-HTP in mg/day).

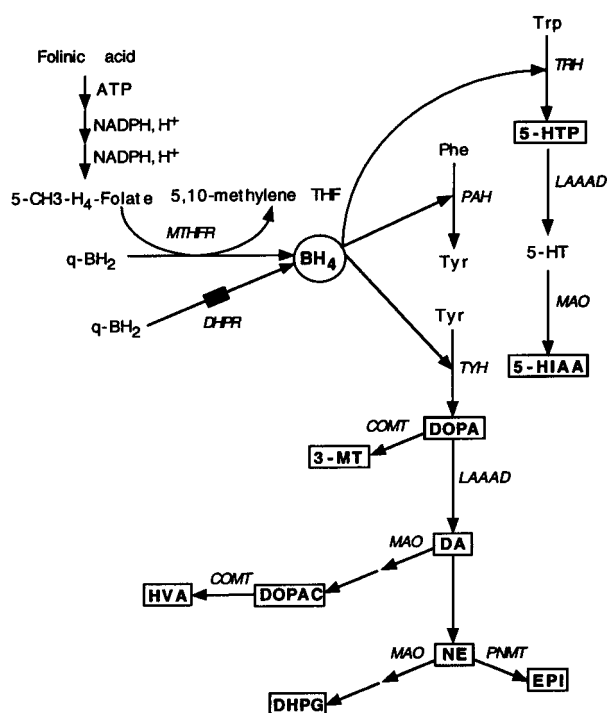
Normal pediatric values were obtained from the following: <sup>a</sup>Kaler et al. (1993); <sup>c</sup>Hyland et al. (1993). Normal adult values were obtained from the following: <sup>a</sup>Kaler et al. (1993); <sup>b</sup>Holmes et al. (1994); <sup>d</sup>Polinsky et al. (1988).

in the tetrahydro form, whereas normally >75% of the bioprotein is in the tetrahydro form (Milstien et al., 1980). These findings suggested DHPR deficiency, and enzymatic testing of erythrocytes (Narisawa et al., 1981) and cultured skin fibroblasts (Milstien et al., 1976) confirmed the absence of DHPR activity.

At the time of diagnosis and initiation of folinic acid treatment, the patient had a chronological age of 10 months but a developmental age of <6 months, with an inability to sit up or pull to stand.

As part of a clinical protocol approved by the appropriate institutional review committee, venous blood and lumbar CSF specimens were obtained from the patient under the following conditions: dietary Phe restriction alone (patient ~10 months old); after 10 weeks of folinic acid treatment (12.5 mg/kg/day; patient ~12 months old); after 1 week of Sinemet (1:4 carbidopa:L-DOPA, starting dose of 2 mg/

kg/day of L-DOPA, dose at the time of testing 4 mg/kg/day; patient ~13 months old), with folinic acid treatment continuing; after 3 weeks of Sinemet, when the dose was 8 mg/kg/day, with folinic acid treatment continuing (patient ~14 months old); after 7 weeks of Sinemet treatment, when the dose was 10 mg/kg/day and 3 days of treatment with 5-HTP at a dose of 25 mg/day (~2.5 mg/kg/day), with folinic acid treatment continuing (patient ~15 months old); and 11 days later, when the Sinemet dose was 10 mg/kg/day and the 5-HTP dose was 100 mg/day (~10 mg/kg/day), with folinic acid treatment continuing. Blood and CSF samples were obtained ~2 h after the last Sinemet dose. The samples were assayed in our laboratory by previously validated methods (Polinsky et al., 1988; Holmes et al., 1994). The limits of sensitivity per milliliter of assayed sample for L-DOPA, 3,4-dihydroxyphenylglycol (DHPG), norepinephrine [noradrenaline (NA)], and epinephrine



**FIG. 1.** Proposed biosynthetic cascade for  $BH_4$ , to explain detectable levels of catechols despite absence of DHPR and neurochemical evidence for increased catecholamine biosynthesis after treatment with folic acid. Boxes indicate compounds where plasma or CSF levels were measured. THF, tetrahydrofolate; MTHFR, 5,10-methylenetetrahydrofolate reductase; PAH, Phe hydroxylase; Tyr, tyrosine; TYH, tyrosine hydroxylase; Trp, tryptophan; TRH, tryptophan hydroxylase; LAAAC, L-aromatic amino acid decarboxylase; MAO, monoamine oxidase; COMT, catechol-O-methyltransferase; PNMT, phenylethanolamine-N-methyltransferase.

(EPI) are  $\sim 0.05$  pmol, for dopamine (DA)  $\sim 0.20$  pmol, and for 3,4-dihydroxyphenylacetic acid (DOPAC)  $\sim 0.50$  pmol.

## RESULTS

After initiation of folic acid treatment, the patient improved clinically. He became more active and alert, was able to roll over, and had increased truncal tone. After addition of Sinemet, the patient improved further, so that by 14 months of age he was able to sit unassisted and pull himself to stand with help. 5-HTP did not produce any clear added benefit.

Initial plasma NA levels were approximately normal (Table 1); L-DOPA levels were about one-third normal, and DHPG and DOPAC levels were very low—about 1/10th to 1/6th normal (Table 1). CSF levels of homovanillic acid (HVA), the *O*-methylated deaminated end product of metabolism of DA, and of 5-hydroxyindoleacetic acid (5-HIAA), the deaminated end product of metabolism of 5-HT, were also very low.

Folic acid treatment resulted in approximately a doubling of plasma levels of DOPA, NA, DHPG, and DOPAC. CSF levels of these compounds remained unchanged. Addi-

tion of Sinemet produced the expected marked increases in CSF DOPAC, HVA, and 3-methoxytyrosine (3-MT; 3-*O*-methylDOPA) levels, with no clear effects on NA levels in plasma or CSF and with only small increases in DHPG levels; plasma DOPAC levels also increased markedly. Addition of 5-HTP to this regimen produced the expected high CSF levels of 5-HTP and 5-HIAA, with variably decreased plasma and CSF levels of catechols.

## DISCUSSION

The finding of decreased but detectable plasma levels of endogenous L-DOPA and other catechols, with virtually normal levels of NA, in this patient with an absence of DHPR demonstrates that DHPR is not absolutely required for catecholamine biosynthesis in humans. The results therefore are consistent with a previous suggestion (Kaufman, 1991) that an alternative biochemical pathway independent of DHPR can generate  $BH_4$  from  $q-BH_2$  (Fig. 1).

Increased exocytotic release from sympathetic nerve terminals could explain normal plasma NA levels in this setting, with low DHPG levels revealing decreased overall NA turnover and low L-DOPA and DOPAC levels revealing decreased overall catecholamine biosynthesis in sympathetic nerves (Kvetnansky et al., 1992). L-DOPA in plasma derives at least partly but probably not exclusively from tyrosine hydroxylation in sympathetic nerves.

According to this alternative pathway hypothesis, folic acid would increase generation of  $BH_4$ , resulting in neurochemical and clinical improvement. Indeed, plasma levels of DHPG, DOPA, and DOPAC all increased during folic acid treatment, consistent with increased catecholamine synthesis and NA turnover in sympathetic nerves. The presence of an alternative metabolic pathway would not necessarily imply that a patient with DHPR deficiency should be clinically normal. CSF levels of catechols did not appear to change during folic acid treatment. One possible explanation for this lack of effect is that the alternative pathway may be more active in the periphery than in the brain, with the clinical benefit of folic acid independent of synthesis of monoamines in the CNS. Thus, CSF levels of HVA and 5-HIAA also did not appear to increase during treatment with folic acid. Full normalization even of peripheral catecholaminergic function, as reflected by plasma levels of catechols, seems to require additional treatment with L-DOPA.

High plasma levels of DOPAC during treatment with Sinemet probably reflect incomplete inhibition of L-aromatic amino acid decarboxylase by the L-DOPA/carbidopa combination outside the brain. Thus, despite the view that carbidopa prevents DA synthesis from L-DOPA outside the brain, the plasma DOPAC:DOPA ratio was about four times the CSF DOPAC:L-DOPA ratio during Sinemet treatment.

The combination of folic acid, Sinemet, and 5-HTP treatment led to approximately normal or even above-normal plasma and CSF levels of monoamine metabolites. If the monoaminergic abnormalities were responsible for the retarded neurobehavioral development in this patient, then one would hope that his clinical improvement will persist.

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