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Research Article

We have identified a generalized deficiency of monoamine neurotransmitters in a patient with a defect in biopterin synthesis. Neurotransmitter precursors (L-3,4-dihydroxyphenylalanine [L-dopa]; 5-hydroxytryptophan [5-HTP] and a tetrahydropterin [6-methyltetrahydropterin (6MPH4)] were investigated for their ability to normalize monoamine neurotransmitter metabolism. Before treatment, the concentrations of dopamine (DA), norepinephrine, epinephrine, and six monoamine metabolites were very low or undetectable in plasma, cerebrospinal fluid, or urine. L-Dopa and 5-HTP replacement was begun at age 7 mo. This therapy generally corrected the deficiency of monoamines and their metabolites, and improved neurological development until the age of 25 mo. Despite these benefits, the intermittent administration of L-dopa could not produce a stable improvement of acute neurological function or DA metabolism. In the 3 h after L-dopa administration, plasma DA and the motor activity and alertness of the patient rose and fell in parallel. Doses of L-dopa that were clinically optimal produced normal plasma levels of norepinephrine and epinephrine, but excessive concentrations of DA and its metabolites. Furthermore, the clinical and biochemical effects of L-dopa were inhibited by phenylalanine and 5-HTP, respectively, demonstrating that these amino acids have antagonistic pharmacological effects. Physiological correction of the monoamine deficit and the hyperphenylalaninemia of this disorder was attempted at age 35 mo using high doses (8-38 mg/kg per d) of 6MPH4. 6MPH4, a synthetic analogue of tetrahydrobiopterin, controlled the […]

Biopterin Synthesis Defect

Treatment with L-Dopa and 5-Hydroxytryptophan Compared with Therapy with a Tetrahydropterin

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Abstract. We have identified a generalized deficiency of monoamine neurotransmitters in a patient with a defect in biopterin synthesis. Neurotransmitter precursors (L-3,4-dihydroxyphenylalanine [L-dopa]; 5-hydroxytryptophan [5-HTP] and a tetrahydropterin [6-methyltetrahydropterin {6MPH$_4$}] were investigated for their ability to normalize monoamine neurotransmitter metabolism. Before treatment, the concentrations of dopamine (DA), norepinephrine, epinephrine, and six monoamine metabolites were very low or undetectable in plasma, cerebrospinal fluid, or urine. L-Dopa and 5-HTP replacement was begun at age 7 mo. This therapy generally corrected the deficiency of monoamines and their metabolites, and improved neurological development until the age of 25 mo. Despite these benefits, the intermittent administration of L-dopa could not produce a stable improvement of acute neurological function or DA metabolism. In the 3 h after L-dopa administration, plasma DA and the motor activity and alertness of the patient rose and fell in parallel. Doses of L-dopa that were clinically optimal produced normal plasma levels of norepinephrine and epinephrine, but excessive concentrations of DA and its metabolites. Furthermore, the clinical and biochemical effects of L-dopa were inhibited by phenylalanine and 5-HTP, respectively, demonstrating that these amino acids have antagonistic pharmacological effects. Physiological correction of the monoamine deficit and the hyperphenylalaninemia of this disorder was attempted at age 35 mo using high doses (8–38 mg/kg per d) of 6MPH$_4$. 6MPH$_4$, a synthetic analogue of tetrahydrobiopterin, controlled the hyperphenylalaninemia. Significant concentrations of 6MPH$_4$ were obtained in the cerebrospinal fluid; no neurological improvement or stimulation of monoamine synthesis in the central nervous system was detected. These findings indicate the complexity in replacement therapy with L-dopa and 5-HTP, but suggest that this treatment may be partially effective in biopterin-deficient patients who are unresponsive to high doses of tetrahydropterins.

Introduction

Approximately 1–3% of patients with persistent hyperphenylalaninemia have a deficiency of tetrahydrobiopterin (BH$_4$),[1] the cofactor of phenylalanine, tyrosine, and tryptophan hydroxylases (1, 2). BH$_4$ deficiency may result from inadequate

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1. *Abbreviations used in this paper:* BH$_4$, tetrahydrobiopterin; CSF, cerebrospinal fluid; DA, dopamine; DHPG, 3,4-dihydroxyphenylethyleneglycol; DHPR, dihydropteridine reductase; DOPAC, dihydroxyphenylacetic acid; E, epinephrine; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HTP, 5-hydroxytryptophan; HVA, 4-hydroxy-3-methoxyphenylacetic acid or homovanillic acid; L-dopa, L-3,4-dihydroxyphenylalanine; MHPG, 3-methoxy-4-hydroxyphenylethyleneglycol; 6MPH$_4$, 6-methyltetrahydropterin; NE, norepinephrine; PKU, phenylketonuria; VMA, 3-methoxy-4-hydroxymandelic acid or vanillylmandelic acid.

biopterin synthesis (Fig. 1 *A*) (3), or decreased regeneration by dihydropteridine reductase (DHPR) (4). In either case, the impaired hydroxylation of tyrosine and tryptophan would be expected to reduce the formation of the catecholamines and serotonin (Fig. 1 *B*), which may account for the developmental delay and seizures of these patients. In only one patient, however, has the defect in catecholamine metabolism been studied in any detail (5, 6). Although dopamine (DA) and epinephrine (E) levels were severely depressed, norepinephrine (NE) and its metabolites were normal. Other DHPR-deficient patients, in contrast, have had evidence of decreased NE synthesis (7). Documentation of the monoamine deficit in additional patients has been generally limited to one or two measurements of 5-hydroxyindoleacetic acid (5-HIAA) and 4-hydroxy-3-methoxyphenylacetic acid (HVA) in cerebrospinal fluid (CSF) or urine (8–13). As a result, the precise nature of the catecholamine defect in BH_4 deficiency remains largely unknown.

Treatment of the purported defects in monoamine synthesis has been attempted by the administration of L-3,4-dihydroxyphenylalanine (L-dopa) and 5-hydroxytryptophan (5-HTP) to bypass the defective hydroxylation reactions (6–9, 11, 14–16). In some subjects, the clinical course of the disease has been favorably altered. Biochemical evaluation of this therapy, however, has been limited (6, 7, 13, 16). In this study, we have delineated the extent of the monoamine abnormalities in a patient with a deficiency of BH_4 due to a severe defect in biopterin synthesis. We measured the levels of the catecholamines and six major monoamine metabolites before L-dopa and 5-HTP administration, and then evaluated the ability of these neurotransmitter precursors to normalize monoamine metabolism during 28 mo of treatment.

In the course of this work, it became clear that serious limitations existed in the ability of L-dopa and 5-HTP to provide a physiological correction of the defects in monoamine neu-

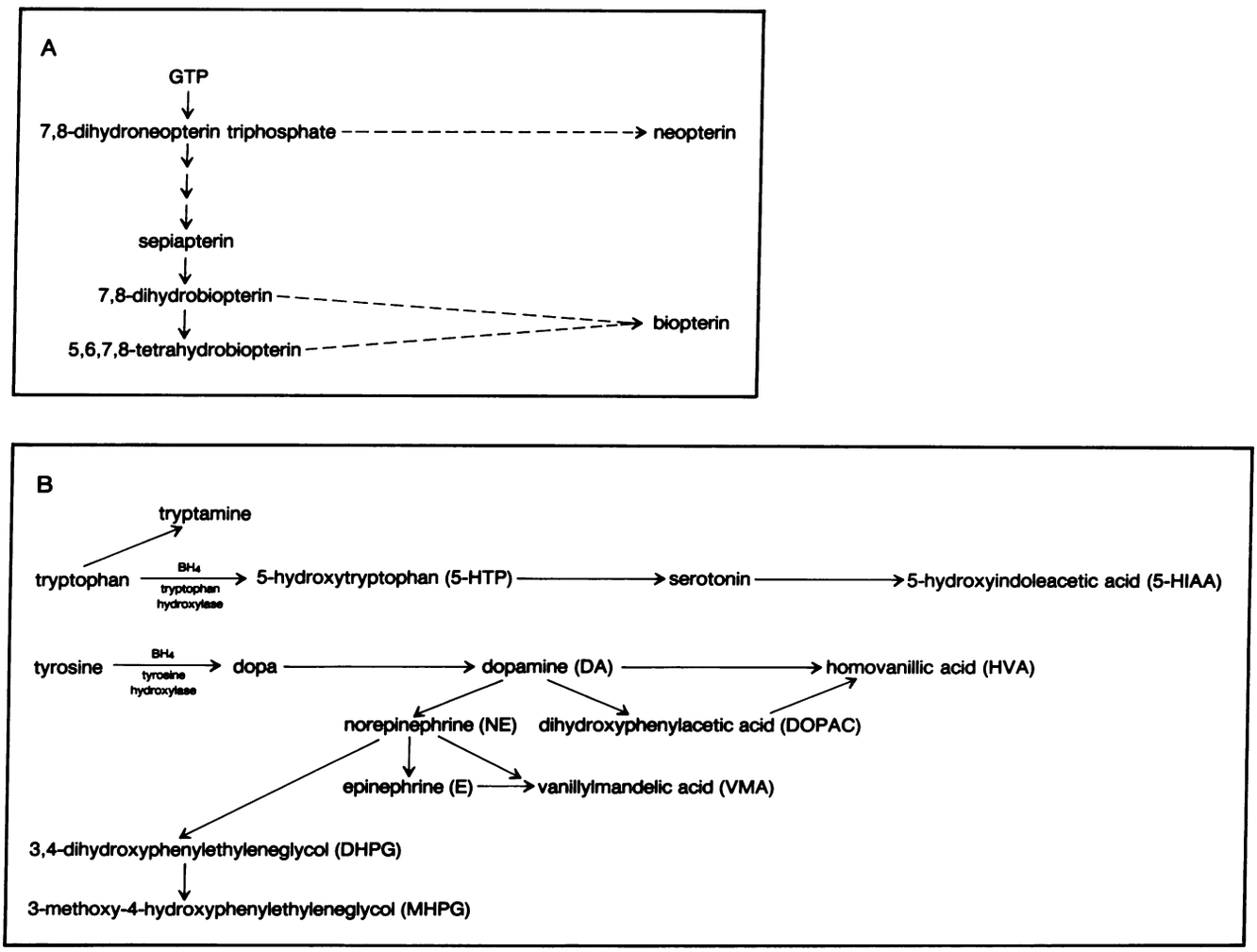

Figure 1. (*A*) The BH_4 synthesis pathway. (*B*) Pathways of serotonin and catecholamine synthesis and of monoamine metabolite formation.

rotransmitters. Consequently, we attempted a more direct therapy with 6-methyltetrahydropterin ($6MPH_4$), a lipophilic analogue of BH_4, which is less expensive and more readily available. $6MPH_4$ has been shown to enter rodent brain 10 times more readily than BH_4 (17) and to cross the blood-brain barrier of our patient (18). In addition, it is comparable to BH_4 in efficacy as a cofactor for brain tyrosine and tryptophan hydroxylases in vitro (19, 20); it is an excellent cofactor for rat liver phenylalanine hydroxylase (21); and it increases the activity of rat phenylalanine hydroxylase in vivo (22). During a period of withdrawal of the L-dopa, 5-HTP, and carbidopa therapy, we examined the effect of $6MPH_4$ on the synthesis of the monoamines and their metabolites in both the brain and the periphery of this patient. The effect of $6MPH_4$ on phenylalanine hydroxylation was also evaluated.

Methods

Subject. The patient was born to first-cousin parents after a 35-wk-gestation (body wt 1,910 g). Hyperphenylalaninemia (2.5 mM) was detected at 4 d of age, and led to a diagnosis of classical phenylketonuria (PKU). Physical examination was normal. Good dietary control of blood phenylalanine (<0.8 mM) was obtained. She was well until 3 mo of age, when the parents noted occasional episodes of deviation of her eyes to the right and increased tone. These episodes increased in frequency until 5 mo of age, when they were controlled with anticonvulsants.

Physical examination. At birth, her height, weight, and head circumference (30 cm) had been on the 10th, 10th, and 3rd percentiles, respectively, for corrected age. By 7 mo, she was clearly microcephalic (40 cm), and since 24 mo of age, her head circumference has remained at 43.5 cm. Her systolic blood pressure ranged from 70 to 90 mmHg. On examination at 5 mo of age, she had a continuous mucopurulent nasal discharge. Developmental assessment showed her level of function to be at ~2–4 wk. She had no purposeful movements. She smiled, but not in response to any particular social stimulus. On gross motor assessment she was found to have generalized hypotonia and poor head control. She clenched her fists continually. Plasma prolactin was elevated (67 ng/ml, normal for age <10 ng/ml) at 7 mo of age, before treatment.

Human studies approval. All studies described in this paper were carried out within the guidelines of the Human Experimentation Committee of The Hospital for Sick Children, Toronto.

Administration of BH_4 and $6MPH_4$. Immediately before their intravenous administration, BH_4 or $6MPH_4$ were dissolved in a citrate buffer (23) with 0.83 mg of L-lactic acid and 1.0 mg of ascorbic acid/mg of pterin, and passed through a 0.22-μm filter (Millipore Continental Water Systems, Bedford, MA). $6MPH_4$ for gastric administration was prepared similarly, but without the lactic acid. BH_4 was purchased from Dr. B. Schircks, Zurich, Switzerland, and $6MPH_4$ from Sigma Chemical Co., St. Louis, MO.

Biochemical measurements. Amino acids were measured by automated ion-exchange column chromatography. All samples for pterin analysis were collected in 1 mg/ml of ascorbic acid and frozen immediately until they were assayed. Neopterin, $6MPH_4$, and total biopterin (total biopterin = dihydro plus tetrahydro forms) were determined by the high-pressure liquid chromatographic method of Nixon et al. (24), except in the earliest studies, in which the concentration of unconjugated pterins was assayed microbiologically, as described previously (3). All blood and CSF samples for catecholamine assay were collected in tubes on ice. Blood (1 ml) or CSF (the first milliliter to be collected) for catecholamine assay was transferred to tubes containing 2 mg dithiothreitol and 10 μl EGTA solution (100 mg/ml) and inverted gently several times. Within 90 min, blood specimens were centrifuged at 4°C, and the plasma was separated. All samples were stored at −70°C until assay. Plasma and CSF catecholamines were quantitated with a radioenzymatic method (25), plasma HVA and dihydroxyphenylacetic acid (DOPAC) by a modification (26) of a gas chromatographic method (27), and plasma L-dopa by high-pressure liquid chromatography using electrochemical detection (28). Specimens of lumbar CSF for 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG), DHPG, HVA, 3-methoxyl-4-hydroxymandelic acid (VMA), and 5-HIAA quantitation were the initial 2–3 ml of fluid to be collected, and were dripped into tubes containing ascorbic acid (1 mg/ml), and then frozen at −20°C until assay by a gas chromatography-mass spectrometric method (29–32). Specimens of urine for MHPG, tryptamine, DHPG, HVA, VMA, and 5-HIAA were collected into brown bottles on ice, and then frozen at −20°C until assay by gas chromatography-mass spectrometry (29–32), or for 5-HIAA by fluorometric assay (33). Urine specimens for dopamine (DA) quantitation were collected in acid-containing bottles and frozen at −20°C until analysis. DA was measured in random urine samples by a high-pressure liquid chromatographic method using electrochemical detection (34).

Results

Diagnosis of biopterin synthesis defect

The clinical presentation suggested that the patient had a deficiency of BH_4. This was confirmed, and shown to be due to a defect in biopterin synthesis, by the following studies.

Intravenous BH_4 infusions: effect on plasma phenylalanine and tyrosine. In BH_4 deficiency, the administration of BH_4 reduces the plasma phenylalanine concentration to normal levels (23). BH_4 (2.3 mg/kg) infused intravenously into our patient at 8 and 24 mo of age normalized the plasma phenylalanine, an effect that lasted up to 19 h (Fig. 2 *A*). Plasma tyrosine increased threefold in the hours after each BH_4 injection (Fig. 2 *A*).

Neopterin and biopterin concentrations in extracellular fluids. Neopterin accumulation is characteristic of patients with defective biopterin synthesis (24). The urine neopterin/biopterin ratio of our patient was 655, 300-fold greater than normal (35). The serum neopterin/biopterin ratio of 40 was also increased (normal for age: 2.4±0.8, mean±SD [35]). Similarly, the CSF neopterin (69.3 ng/ml) was elevated, while the concentration of biopterin (0.81 ng/ml) was greatly decreased (adult controls: 12.2±8.4 ng/ml and 18.6±12.7 ng/ml, respectively, $n = 5$). In addition to increasing the serum biopterin (Fig. 2 *B*), the intravenous infusion of BH_4 decreased the serum neopterin by 80%. No increase was detected in the biopterin concentration of CSF sampled 3 h after the intravenous injection of 2.5 mg/kg BH_4 (data not shown).

In one patient with a biopterin synthesis defect, the expected increase in serum biopterin did not occur following phenylalanine loading (3). Similarly, no increase occurred in our patient. The serum biopterin was <1.0 ng/ml before the test (100 mg/kg phenylalanine) and remained at this level from 1 to 4 h after phenylalanine administration. Using ring-tritiated phenylalanine (3 μCi/kg) (3, 36), in vivo phenylalanine hydroxylation was also measured during this phenylalanine load, and found to be only

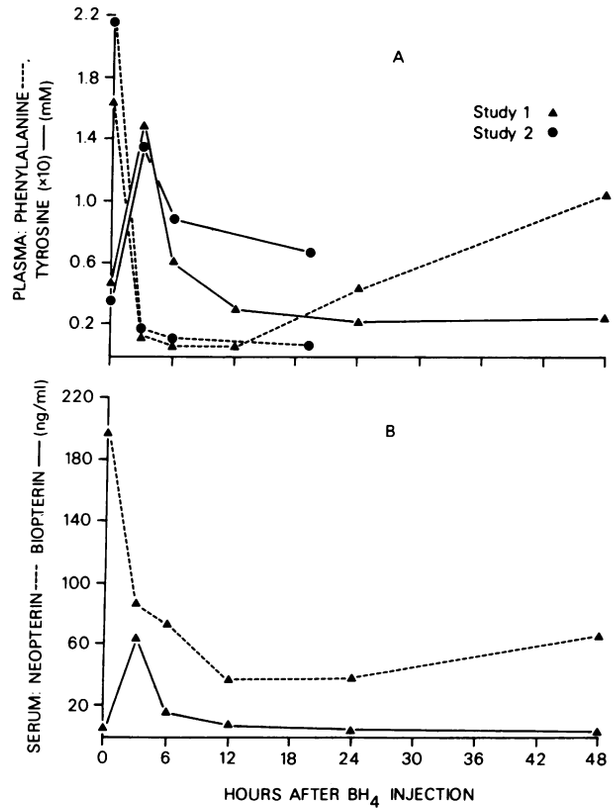

Figure 2. (*A*) Intravenous BH_4 (2.3 mg/kg): effect on plasma phenylalanine and tyrosine. An abrupt fall in plasma phenylalanine and a rise in plasma tyrosine occurred within 3 h after the BH_4 injection. The effect of the BH_4 on plasma phenylalanine lasted 12–19 h. (*B*) Intravenous BH_4 (2.3 mg/kg): effect on serum neopterin and biopterin from study 2 of Fig. 2 *A*. The rise in the serum biopterin concentration is associated with a fall in serum neopterin that lasted ~24 h. The plasma phenylalanine (Fig. 2 *A*) remained normal as long as the serum biopterin exceeded its preinjection level.

0.6% of normal (1.6 nmol/ml body water per h). This defect in phenylalanine hydroxylation was not due to an abnormality in hepatic DHPR. By the method of Cheema et al. (37), DHPR activity was 0.35 U/mg protein (three controls: 0.25, 0.27, 0.49 U/mg protein) and by the method of Kaufman et al. (4), the activity was 56.8 nmol NADPH oxidized/min per mg protein (control mean: 82.1, range 70.2–91.9, $n = 4$).

Variation in serum neopterin and biopterin in BH_4 deficiency: effect of phenylalanine and L-dopa-carbidopa. We observed that increase of the plasma phenylalanine concentration resulted in parallel changes in serum neopterin (~0.98, $n = 9$, $P < 0.01$) (data not shown). No relationship was apparent between serum biopterin and phenylalanine. Variation in serum pterins was also produced by the L-dopa, 5-HTP, and carbidopa therapy. As shown in Fig. 3, biopterin fell and neopterin rose in the hours following administration of L-dopa (0.6 mg/kg) and carbidopa (0.8 mg/kg).

Delineation of the monoamine deficiency and the effects of therapy with L-dopa, 5-HTP, and carbidopa

PLASMA CATECHOLAMINES AND THEIR METABOLITES. *Basal concentrations and response to chronic treatment.* Plasma DA, NE, and E were undetectable before therapy, and only low concentrations were detectable 8 d after withdrawing treatment at 36 mo of age (Table I). The administration of L-dopa and carbidopa normalized plasma NE and E (Table I). Their precursor DA, on the other hand, attained supranormal levels (Table I). This increase in DA was accompanied by a striking elevation of one of its major metabolites, DOPAC, which rose from <0.04 ng/ml before treatment to 1,118 ng/ml (adult controls: 4.7–30.4 ng/ml, $n = 5$) (Van Loon, G. R., C. Kim, and G. M. Brown, manuscript in preparation) after L-dopa was begun at 7 mo of age. The plasma level of HVA, another DA metabolite, was unaffected by L-dopa: 11.3 ng/ml before L-dopa and 15.3 ng/ml during L-dopa administration (adult controls: 5.1–14.6 ng/ml, $n = 5$) (Van Loon, G. R., C. Kim, and G. M. Brown, manuscript in preparation).

Effect of 5-HTP on the conversion of dopa to DA. We observed that L-dopa-induced vomiting in our patient was eliminated by increasing the dose of 5-HTP. To determine whether this effect was mediated by an inhibition of the conversion of dopa to DA by 5-HTP, we administered L-dopa and carbidopa with and without 5-HTP and measured plasma dopa and catecholamines (Fig. 4). After L-dopa and carbidopa alone, plasma dopa and DA rose acutely and were maximal at 30 min (study 1, Fig. 4). 4 h after drug administration, plasma DA but not dopa was still elevated. Co-administration of 5-HTP, however, produced a greater rise in plasma dopa and a smaller decrease in its product, DA (study 2, Fig. 4). The threefold increase in plasma dopa in study 2 compared with study 1 was obtained despite the administration of a smaller dose of L-dopa in study 2 (4.8 mg vs. 7.3 mg). In contrast to these abrupt changes, NE and E levels remained stable during each study (data not shown).

CSF MONOAMINES AND THEIR METABOLITES. Removal of the L-dopa-carbidopa therapy for 8 d (before $6MPH_4$ admin-

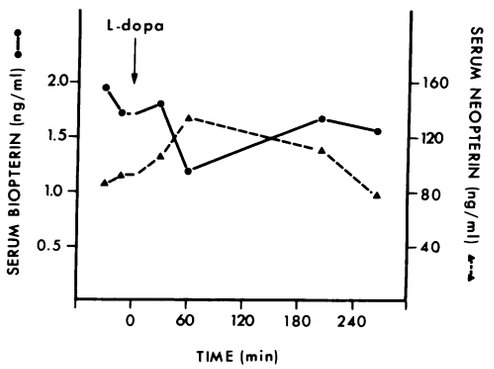

Figure 3. Effect of L-dopa (0.6 mg/kg) and carbidopa (0.8 mg/kg) on serum neopterin and biopterin. The serum biopterin fell and the serum neopterin rose in the 2–3 h after L-dopa and carbidopa administration.

*Table I. Plasma Catecholamines before and during Therapy with L-Dopa, Carbidopa, and 5-HTP or 6MPH$_4$**

	L-Dopa	Carbidopa	5-HTP	DA	NE	E
	mg/kg/d	*mg/kg/d*	*mg/kg/d*	*pg/ml*	*pg/ml*	*pg/ml*
Hyperphenylalaninemia controls	None	None	None			
<1 yr age‡				79±33	1,045±116	296±44
Range				31–226	723–1,362	196–439
Hyperphenylalaninemia controls	None	None	None			
3–5 yr age§				78±25	261±44	82±24
Range				27–161	142–472	49–210
Adult controls‖	None	None	None	57±5	237±6	78±5
Patient						
Treatment / Age						
None / 7 mo	0	0	0	<10	<20	<25
L-Dopa, carbidopa, 5-HTP / 7.3 mo	11.4	4.0	4.1	531	435	69
11 mo	9.2	3.2	3.0	1,829	798	291
24 mo	5.3	2.5	2.3	82	354	94
34 mo	2.3	2.5	2.3	406	291	94
6MPH$_4$ study at 35 mo on L-dopa,	2.3	2.5	2.5	—	374	243
carbidopa, 5-HTP alone	2.3	2.5	2.5	—	387	344
No treatment for 8 d	0	0	0	39	49	42
6MPH$_4$ alone for 10 d	0	0	0	32	46	30
11 d	0	0	0	32	41	28

* Control data are expressed as mean±SEM; all blood samplings in the patient were taken >2 h after the last doses of L-dopa, carbidopa, and 5-HTP. ‡ PKU or benign hyperphenylalaninemia patients with plasma phenylalanine <0.8 mM. Age: 5.2±1.1 mo, range 1–8 mo, n = 7. § PKU or benign hyperphenylalaninemia patients with plasma phenylalanine <0.8 mM. Age: 4.5±0.5 yr, range 3.3–5.8 yr, n = 6. ‖ Combined data of G.R. Van Loon et al. from three studies (56) using a total of 20 normal adult volunteers.

istration at 35 mo) reduced CSF DA and E to barely detectable levels (Table II). NE was only 20% of adult controls, whose mean value was similar to that obtained in a 6-mo-old patient with classical PKU (Table II). The L-dopa, 5-HTP, and carbidopa therapy normalized the DA and E concentrations, and increased NE two to threefold.

The monoamine metabolites MHPG, DHPG, HVA, and 5-HIAA could not be detected in CSF before commencing the L-dopa, 5-HTP, and carbidopa treatment (Table II). After 11 d of therapy, MHPG, HVA, and 5-HIAA each increased to the normal range (Table II). When the doses of the drugs were later reduced according to clinical criteria (see below), HVA was only one-tenth that of controls at 35 mo, while 5-HIAA was one-half of controls (Table II).

NEUROTRANSMITTER METABOLITES IN URINE. The urinary excretion of MHPG, HVA, and VMA was very low or undetectable before treatment (Table III), while all three metabolites reached or exceeded the control range during L-dopa, 5-HTP, and carbidopa administration. The relatively high excretion of HVA observed before therapy was also apparent during therapy (Table III). HVA excretion increased to levels up to 10-fold above the mean of controls, whereas MHPG and VMA excretion rose to levels ranging from only 25 to 100% of the mean of controls. The excretion of 5-HIAA was <5% of PKU controls before treatment, but increased to levels up to sixfold above the range of the controls during therapy. On the other hand, tryptamine excretion (0.71, 0.57, and 1.16 μg/kg per 24 h) was within the normal adult range (1–2 μg kg/24 h [38]) before treatment, but decreased by an order of magnitude (0.01 and 0.05 μg/kg per 24 h) when L-dopa, 5-HTP, and carbidopa were given.

CLINICAL RESPONSES TO L-DOPA, 5-HTP, AND CARBIDOPA. *Neurological function.* At 7 mo of age and within hours of initiating treatment, the patient had a dramatic improvement in alertness, tone, and spontaneous movement. The maximal clinical response to these drugs regularly occurred 90 min after the peak in plasma DA (120 min after L-dopa administration, Fig. 4). She continued to improve neurologically until 2.5 yr of age, but has shown little subsequent progression. Her neurological status after 1 mo and after 4 yr of L-dopa, 5-HTP, and carbidopa therapy is given in Table IV.

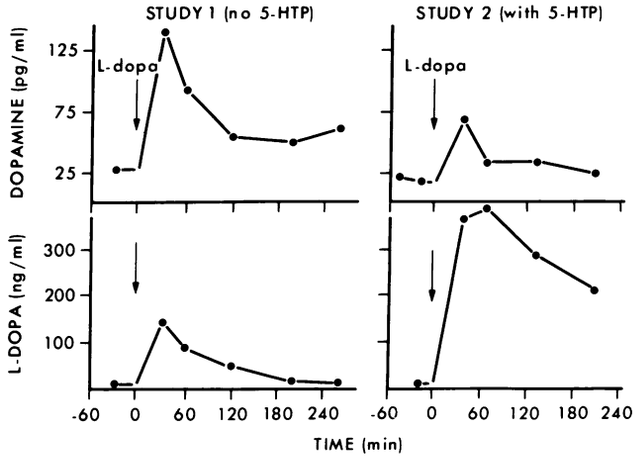

Figure 4. Study 1. Plasma concentrations of L-dopa and DA after administration by gastrostomy of 7.3 mg of L-dopa and 10 mg of carbidopa. The maximal increase in both compounds was at 30 min, but the plasma DA concentration remained elevated at 4 h, whereas the L-dopa level had returned to base line. Study 2. Plasma concentrations of L-dopa and DA after administration of 4.8 mg of L-dopa and 10 mg carbidopa, plus 9.8 mg of 5-HTP. The co-administration of 5-HTP is associated with a greater rise in plasma L-dopa than in study 1, despite a larger dose of L-dopa having been given in study 1. The rise in plasma DA is less than in study 1.

Dosage of L-dopa, 5-HTP, and carbidopa. The doses of L-dopa (11.5 mg/kg per d), 5-HTP (4.1 mg/kg per d), and carbidopa (1 mg/kg per d), which we used initially were based on reports in the literature (1), although the rationale for these dosages is not given. The dose of L-dopa has been gradually decreased, to control hyperactivity and insomnia, to 2 mg/kg per d (in 4 doses). The only clinical effect of 5-HTP we observed is that doubling the dose to 5 mg/kg per d eliminated L-dopa-induced vomiting at 3 yr of age. The dose of 5-HTP at age 5 yr is 3 mg/kg per d, with 40% of the total dose being given in the evening. Carbidopa doses as high as 4.1 mg/kg per d, which have been used in adults (39), were initially required to control vomiting. The current dose of carbidopa at 5 yr of age is 2 mg/kg per d.

Blunting of the clinical response to L-dopa by phenylalanine loading. We repeatedly observed that phenylalanine loading tests dramatically suppressed the beneficial effect of L-dopa on neurological function. In six studies over a 3-yr period, a normal diet raised the plasma phenylalanine to 1–3 mM. Despite continued L-dopa-5-HTP-carbidopa therapy on each occasion, the patient was incapable of many of the motor functions she readily performed only 24 h previously, and which she recovered within hours of reducing the phenylalanine.

Effects of 6MPH$_4$ administration

The studies with 6MPH$_4$ assessed whether this reduced pterin could: (*a*) increase the activity of phenylalanine hydroxylase; (*b*) be absorbed adequately by the gut to allow oral administration; (*c*) cross the blood-brain barrier in the reduced state; and (*d*) increase the activity of peripheral and/or central tyrosine and tryptophan hydroxylases in this patient.

6MPH$_4$: effect on phenylalanine concentration in plasma. With the patient on a normal phenylalanine diet (130 mg/kg per d), intravenous 6MPH$_4$ (5 mg/kg) reduced the plasma phenylalanine by 30% in 3 h (Fig. 5 *A*). A higher dose (10 mg/kg) was more effective, decreasing the phenylalanine from 1.0 mM to 0.2–0.3 mM for 18 h (Fig. 5 *A*). Plasma tyrosine rose as the phenylalanine fell (Fig. 5 *A*), as it had done following the injection of BH$_4$.

6MPH$_4$, given by gastrostomy tube in increasing doses (2–8 mg/kg per d), began to reduce the phenylalanine at a dose of 4 mg/kg per d (Fig. 5 *B*). At 8 mg/kg per d, the phenylalanine was 0.6 mM, one-half its pretreatment level. In addition, after 8 d of gastrically administered 6MPH$_4$ (20 mg/kg per d) at age 36 mo, the plasma phenylalanine was 0.43 mM with the patient on a normal diet. When the dose was increased to 38.6 mg/kg per d for three more days, the phenylalanine fell further, to 0.19 mM.

6MPH$_4$: entry into CSF and effect on tyrosine and tryptophan hydroxylase. The effects of 6MPH$_4$ on tyrosine and tryptophan hydroxylase were assessed by comparing monoamines and metabolite levels after 8 d without any treatment, with the levels found after 12 d of treatment with 6MPH$_4$ alone (10 mg/kg per 12 h for 9 d, then 19.3 mg/kg per 12 h for 3 d).

After 12 d of 6MPH$_4$, the CSF concentration of this reduced pterin was 60.5 ng/ml when the serum level was 88.5 ng/ml. The entry of 6MPH$_4$ into the CSF of this patient has been discussed in more detail elsewhere (18). 6MPH$_4$ reduced the neopterin concentration in CSF from 66.5 to 47.9 ng/ml and in serum from 130 ng/ml to 70.7 ng/ml.

Withdrawal of L-dopa, 5-HTP, and carbidopa reduced the concentrations of the monoamines or their metabolites in plasma, CSF, and urine to subnormal levels (Tables I–III, bottom rows). 6MPH$_4$ increased the urinary excretion of HVA and 5-HIAA (Table III) as well as DA. On L-dopa therapy, DA excretion (851 and 1,432 μg/g creatinine) was in the normal range (222–3,020 μg/g creatinine, n = 37, infants aged 24–48 mo). When L-dopa was withdrawn, DA excretion (209, 216, and 236 μg/g creatinine) fell below normal and increased modestly (315 and 346 μg/g creatinine) during 6MPH$_4$ administration. In contrast to these effects, 6MPH$_4$ did not increase the CSF or plasma concentrations of the monoamines or their metabolites. Furthermore, the patient showed no clinical response to the 6MPH$_4$ trial. She remained listless and hypotonic until L-dopa, 5-HTP, and carbidopa were recommenced.

On the last day of 6MPH$_4$ administration, we looked for evidence of hepatic, renal, or bone marrow toxicity. The serum glutamic oxaloacetic transaminase was slightly elevated at 40 U/liter (normal < 30 U/liter). Alkaline phosphatase, total and direct bilirubin, blood urea nitrogen, creatinine, and a complete blood count were all normal.

Discussion

This research has identified a global and virtually complete deficiency in the formation of the catecholamines and their

Table II. Lumbar CSF Catecholamines and Neurotransmitter Metabolite Levels before and during Therapy with L-Dopa, Carbidopa, and 5-HTP, or 6MPH$_4$

		L-Dopa	Carbidopa	5-HTP	HVA	MHPG	DHPG	5-HIAA	DA	NE	E
		mg/kg/d	*mg/kg/d*	*mg/kg/d*	*ng/ml*	*ng/ml*	*ng/ml*	*ng/ml*	*pg/ml*	*pg/ml*	*pg/ml*
Controls*											
This study					71±15‡	—	—	31±8‡	23§	209§	36§
Other studies:	0–1 yr‖				120±12	14±2		43±6	—	—	—
	2–4 yr‖				132±10	13±1		40±5			
	Adult¶				—	—	—	—	—	284±39	—
	Adult**				—	—	—	—	33±4	191±25	39±6
Patient											
Treatment	Age										
None	5.3 mo	0	0	0	7.3	—	—	<1	—	—	—
None	5.3 mo	0	0	0	<1.0	<1.0	<0.5	<1	—	—	—
L-Dopa, etc.,	7.3 mo	11.5	4.1	4.1	126	13.5	2.0	21.2	—	—	—
– pre BH$_4$	24 mo‡‡	5.3	2.5	2.3	—	—	—	—	43	110	24
– post BH$_4$	24 mo‡‡	5.3	2.5	2.3	—	—	—		42	148	38
6MPH$_4$ study at 35 mo											
On L-dopa, etc. alone§§		2.3	2.5	2.5	9.9	—	—	24.4	42	85	35
No treatment for 8 d		0	0	0	<1.0	—	—	1.8	20	49	30
									18‖‖	37	22
6MPH$_4$ alone for 11 d		0	0	0	1.0	—	—	0.7	17	44	21
									21‖‖	44	24

* Data are expressed as mean±SEM. ‡ Six children, age 2.8±0.5 yr, with neurological disorders not known to affect the neurotransmitters measured. § 6 mo female with hyperphenylalaninemia and mild developmental delay not due to BH$_4$ deficiency, on low phenylalanine diet. ‖ Seifert et al. (54). ¶ Lake et al. (55). 20 adults. ** Hugenholtz, H., and G. R. Van Loon, unpublished observations: lumbar CSF from 12 adults, anesthetized for clipping of a cerebral aneurysm. ‡‡ CSF taken before and after intravenous BH$_4$ at 24 mo age. §§ L-Dopa, etc. = L-dopa, carbidopa, and 5-HTP. ‖‖ The first row of catecholamine results were obtained in the 1st ml of CSF. The second row is from the 4th ml.

major metabolites, as well as 5-HIAA (the principal catabolite of serotonin) (Fig. 1 *B*) in a patient with a severe defect in biopterin synthesis. Treatment of this monoamine deficiency with L-dopa and 5-HTP resulted, acutely, in a dramatic restoration of alertness, muscle tone, and motor activity, and, during the first 18 mo of therapy, in a rapid acceleration of development. The provision of L-dopa and 5-HTP also corrected the deficiency of the monoamines and their metabolites in plasma, urine, and CSF. That the effect of L-dopa and 5-HTP on monoamine metabolism is far from physiological, however, is shown by the findings of abnormally high levels of plasma DA and its metabolites, the close dependence of plasma DA on the time after the administration of L-dopa, the brevity of the period during which the clinical response to L-dopa is optimal, the presence of normal levels of a metabolite in one body fluid concomitant with its excess in another (e.g., 5-HIAA in CSF vs. urine), and the inhibition by 5-HTP (and probably also phenylalanine) of the conversion of L-dopa to DA. Since a more physiological restoration of the defective hydroxylation reactions might be expected to result from the administration of a tetrahydropterin, we evaluated the effect of 6MPH$_4$ on phenylalanine and monoamine metabolism. Despite the ability of 6MPH$_4$ to be absorbed by the gut, control the hyperphenylalaninemia, and cross the blood-brain barrier, this pterin demonstrated no neurological effect and no ability to increase monoamine synthesis in the brain. Consequently, for BH$_4$-deficient patients who do not respond to tetrahydropterin therapy with an increase in monoamine synthesis by the brain, L-dopa, 5-HTP, and carbidopa still represent the best available treatment. Considerable research is required in the use of these drugs in BH$_4$-deficient patients, however, before the most beneficial results can be expected.

The studies of pterin metabolism in our patient corroborate and extend the findings reported for the first case of a biopterin synthesis defect (3). The rapid reduction in plasma phenylalanine by BH$_4$ confirms the diagnostic value of this test (16, 23, 40) in patients with defects in biopterin synthesis. The increase in plasma tyrosine, which occurred following BH$_4$ or 6MPH$_4$ administration, has not been previously reported, but undoubtedly reflects increased tyrosine formation from phenylalanine.

The BH$_4$- and 6MPH$_4$-induced depression of serum neop-

Table III. Urinary Neurotransmitter Metabolites before and during Therapy with L-Dopa, Carbidopa, and 5-HTP or 6MPH$_4$

		L-Dopa	Carbidopa	5-HTP	HVA	VMA	MHPG	DHPG	5-HIAA
		mg/kg/d	*mg/kg/d*	*mg/kg/d*	*μg/24 h*	*μg/24 h*	*μg/24 h*	*μg/24 h*	*μg/24 h*
Controls*	Age								
	0.3–15 mo‡	—	—	—	—	—	431±79 (223–667)	68±28 (22–158)	554±100 (229±834)
	4–24 mo§	—	—	—	1,160±120 (200; 3,260)	950±80 (200; 1,880)	—	—	—
	24–48 mo‖	—	—	—	2,240±230 (700; 2,200)	1,680±140 (800; 1,100)	—	—	—
Patient									
Treatment	Age								
	5.3 mo¶	0	0	0	66	<100	8	—	16
	6.3 mo	0	0	0	160	<100	4	2	30
	7.0 mo¶	0	0	0	200	<100	18	—	16
L-Dopa, carbidopa,	7.3 mo	11.5	4.1	4.1	4650	600	160	—	1,340
5-HTP	7.3 mo¶	11.5	4.1	4.1	13,220	1,220	460	—	1,080
	9.0 mo	11.5	4.1	4.1	11,000	980	232	46	4,940
	9.0 mo¶	11.5	4.1	4.1	4,020	210	132	30	3,060
	25 mo	5.3	2.5	2.8	6,210	960	146	42	4,110
	25 mo	5.3	2.5	2.8	4,660	720	126	33	3,210
6MPH$_4$ at 35 mo									
On L-dopa, carbodopa, 5- HTP alone	day 1¶	2.3	2.5	2.5	1,512	88	76	12	322
	day 2¶	2.3	2.5	2.5	712	52	24	8	156
No treatment for:									
	8 d¶	0	0	0	14	10	22	10	58
	9 d¶	0	0	0	50	30	38	12	64
6MPH$_4$ alone for:									
	10 d¶	0	0	0	314	88	36	4	100
	11 d¶	0	0	0	138	22	58	16	226

* Control data: expressed as mean±SEM (range) except HVA and VMA where mean±SEM (5th percentile; 95th percentile confidence limits) are given. ‡ Four patients with classical PKU, one with atypical PKU, plasma phenylalanines all <0.9 mM. Age (mean±SEM): 4.7±3.1 mo. § 24-h urine collections from 62 patients investigated for, but not found to have, neural crest tumors. ‖ 24-h urine collections from 34 patients investigated for, but not found to have, neural crest tumors. ¶ Urine collections on these dates were for 12 h only (p.m. to a.m.), but data are expressed per 24 h (i.e., doubled) for convenience. The diet was unchanged during the 6MPH$_4$ study, except for administration of a normal phenylalanine intake (140 mg/kg per d).

terin may result from a direct inhibition of the biopterin synthesis pathway (24). Alternatively, these pterins may decrease serum neopterin by first decreasing phenylalanine, as suggested by the linear relationship that we observed between serum neopterin and phenylalanine. We have also demonstrated that both serum biopterin and neopterin are influenced by the administration of L-dopa and 5-HTP. A direct inhibition of sepiapterin reductase by monoamines has been recently described (41), and could account for the rise in neopterin and fall of biopterin induced by L-dopa and 5-HTP in our patient. These results suggest that care should be taken to perform diagnostic studies on the levels of biopterin and neopterin before L-dopa and 5-HTP are initiated, and only after good control of the hyperphenylalaninemia has been obtained.

In agreement with Danks et al. (10), we observed that 2.3 mg/kg of intravenous BH_4 did not elevate the CSF concentration

Table IV. Summary of Neurological Status before and during L-Dopa, 5-HTP, and Carbidopa Therapy

Age	5–7 mo	8 mo	35 mo
Therapy	None	L-Dopa, 5-HTP, carbidopa for 1 mo	L-Dopa, 5-HTP, carbidopa for 28 mo
Neurological exam	Hypotonic, fisting	Hypertonic, irritable, hyperreflexic	Hypertonic, choreoathetotic
Developmental exam: overall	Newborn–4 wk	2–3 mo	6–12 mo*
Age-equivalent	2–4 wk	2–3 mo	6–9 mo
Gross-motor	Newborn	2–3 mo	6 mo
Vision/fine motor	4 wk	2 mo	4 mo*
Speech	—	2 mo	8 mo
Social-adaptive	2 wk	2 mo	6–9 mo

* By history, function was up to the 12-mo level.

of BH_4. Since other studies by us (18, 42) indicate that higher doses (12–20 mg/kg) of tetrahydropterins are required to augment CSF pterin levels, we are unable to explain the observation of Niederwieser et al. (14) that 2.5 mg/kg per d of BH_4 dramatically improved neurological function in a biopterin-deficient patient.

In the studies using $6MPH_4$, we have shown that this tetrahydropterin can be administered orally to control the hyperphenylalaninemia of BH_4 deficiency. The more crucial question, however, is why $6MPH_4$ did not stimulate monoamine synthesis in the brain of our patient. The increases in the urinary excretion of DA, HVA, and 5-HIAA produced by $6MPH_4$ presumably reflect the stimulation of tyrosine and tryptophan hydroxylase in peripheral tissue(s). Since identical doses of $6MPH_4$ increased the activity of tyrosine and tryptophan hydroxylase in the brain of a patient with a less severe defect in biopterin synthesis (42), the unresponsiveness of our patient is not representative, nor is it easily explained. One possibility is that the absence of monoaminergic activity during brain development has led to the underdevelopment of monoaminergic target areas (43, 44) and to greatly decreased numbers of monoaminergic neurons (45). Our patient may therefore have insufficient numbers of monoaminergic neurons to respond to $6MPH_4$, but the exposure of this reduced number of neurons to an excess of the precursor L-dopa, as in Parkinson's disease, is able to normalize catecholamine concentrations in CSF.

In contrast to the DHPR-deficient subject of Koslow and Butler (5, 6), who had normal NE metabolism, the biopterin

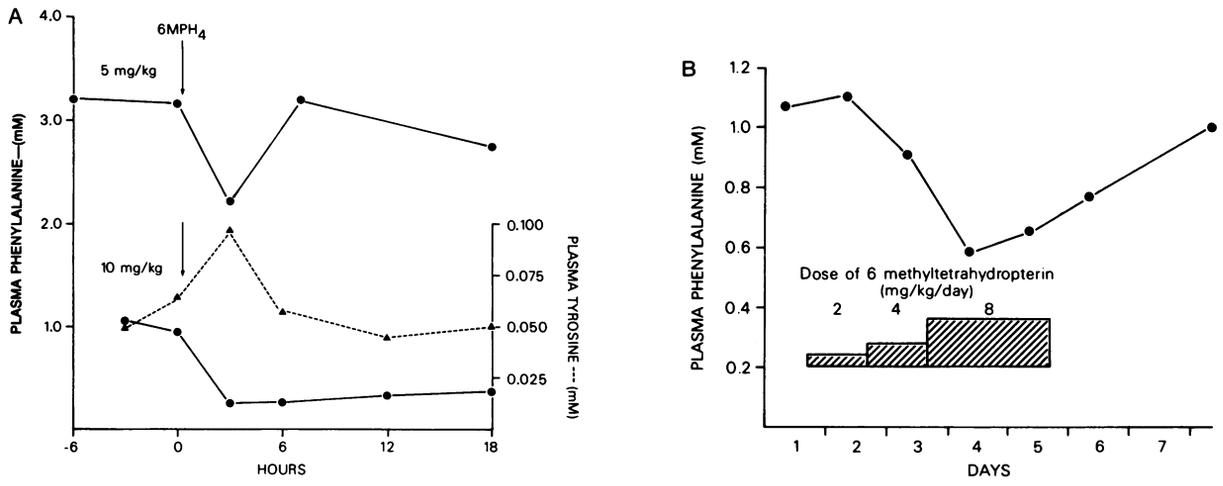

Figure 5. (*A*) Effect of intravenous $6MPH_4$ administration on plasma phenylalanine and tyrosine. The top curve shows the response to 5 mg/kg $6MPH_4$, the bottom curve to 10 mg/kg $6MPH_4$. The maximal decrease in plasma phenylalanine occurred at 3 h in each case, but the decrease was sustained only with the higher dose. Plasma tyrosine rose in response to the higher dose. (*B*) Effect of $6MPH_4$, administered by gastrostomy, on plasma phenylalanine. $6MPH_4$ was given in increasing doses (2–8 mg/kg per d) in a 5-d period (normal diet). The 8-mg/kg per d dose effected therapeutically significant control of plasma phenylalanine.

defect in our patient severely depressed the synthesis of NE as well as DA and E. Since other DHPR-deficient patients have had evidence of impaired NE synthesis (7), the patient of Koslow and Butler may be atypical. Our results, in combination with reports of decreased 5-HIAA, HVA, or VMA in other BH_4-deficient subjects (8–13), indicate that the defect in monoamine synthesis is very similar in DHPR-deficient and biopterin-deficient patients. Finally, since DA is an established inhibitor of prolactin secretion (46), the hyperprolactinemia of our patient also indicates that a hypothalamic DA deficiency was present before treatment. A patient with a less severe defect in biopterin synthesis, in contrast, had normal prolactin levels (42).

The striking fall in plasma NE and E levels with age in our control hyperphenylalaninemic population highlights the importance of using age-matched controls in the diagnosis and treatment of monoamine deficiency. The increase with age in the urinary excretion of HVA and VMA, which we observed, has also been reported by others (47).

Inspection of the biochemical effects of L-dopa and 5-HTP leads to two major conclusions. First, normalization of the levels of a monoamine or a metabolite in one extracellular fluid is not necessarily associated with normalization of its concentrations throughout the body. The dose of L-dopa that corrected the CSF HVA at 7.3 mo of age, for example, produced supranormal urinary HVA excretion. Similar observations have been made during the treatment of parkinsonism (48, 49), and indicate that HVA formation may take place via pathways not requiring sequential DA and DOPAC formation (50). Second, both strikingly elevated and depressed levels of specific monoamines or metabolites can result from the treatment. The elevation of plasma DA, in the presence of normal NE and E, implies that the conversion of DA to NE becomes rate-limiting during L-dopa replacement in this disease. The pronounced depression of tryptamine excretion, on the other hand, is best explained by the competitive inhibition of aromatic amino acid decarboxylase by 5-HTP and L-dopa (51).

Some of the biochemical abnormalities we observed with L-dopa and 5-HTP replacement, for example the elevation of plasma DOPAC, may be of no clinical importance. Others, such as the reduced conversion of dopa to DA by 5-HTP, are of therapeutic significance. Consequently, the dose of 5-HTP cannot be changed without affecting the pharmacology of L-dopa, and vice-versa. This 5-HTP-L-dopa interaction is likely to be at the level of transport (52) or aromatic amino acid decarboxylase, and reflects the structural similarity of these amino acids. Similarly, the effect of phenylalanine on the clinical response to L-dopa also probably reflects transport inhibition (52), or an inhibition by phenylalanine of monoamine synthesis from L-dopa, as has been demonstrated in PKU (7, 53). Treatment of the hyperphenylalaninemia with low doses (2 mg/kg per d) of tetrahydropterins would avoid the fluctuations in plasma phenylalanine that accompany dietary therapy, and perhaps optimize the response to L-dopa replacement.

Our preliminary data (18, 42) and those of Niederwieser et al. (14) show that therapy with a tetrahydropterin may be effective in treating the defect in monoamine synthesis in some patients with BH_4-deficiency. These findings imply that in all newly diagnosed hyperphenylalaninemic patients who have a defect in brain monoamine synthesis, a trial of high dose BH_4 should be given. However, clinical trials using high doses of BH_4 or $6MPH_4$ must be monitored with great care, since virtually nothing is known about the toxicity of the tetrahydropterins. The results presented in this paper and others (14, 16) indicate that certain patients will not be neurologically responsive to high dose pterin treatment; as we have shown, such patients may still respond, at least partially, to L-dopa and 5-HTP (8, 9, 13, 16). The limited response of our patient may be related to the premature removal of maternally derived BH_4 due to the 35-wk-gestation, the late age at which treatment was begun (7 mo), and/or to the severity of her defect. The most important question, whether infants unresponsive to tetrahydropterins can achieve normal development if treated early with L-dopa and 5-HTP, remains to be answered.

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