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Original Investigations

Atypical Phenylketonuria with Defective Biopterin Metabolism. Monotherapy with Tetrahydrobiopterin or Sepiapterin, Screening und Study of Biosynthesis in Man

A. Niederwieser[1]*, H.-Ch. Curtius[1], M. Wang[1]**, and D. Leupold[2]

[1] Department of Pediatrics, University of Zürich, Steinwiesstr. 75, CH-8032 Zürich, Switzerland
[2] Department of Pediatrics, University of Ulm/Donau, Federal Republic of Germany

Abstract. Administration of a single dose of tetrahydrobiopterin dihydrochloride, 10–20 mg/kg orally, to a patient with dihydrobiopterin deficiency led to disappearance of clinical symptoms for 4 days, normalization of urinary phenylalanine and serotonin and decrease of elevated neopterin for 2–3 days. A dose-dependent stimulation of serotonin production was observed. A similar effect was noted with even lower doses of L-sepiapterin. The patient is now under monotherapy with tetrahydrobiopterin · 2 HCl, 2.5 mg/kg daily. Other patients with this disease may not respond as well.

Results of screening for tetrahydrobiopterin deficiency in 228 cases with hyperphenylalaninemia, including 140 newborns, are reported.

There is evidence that biopterin biosynthesis in human kidney and liver proceeds via a dioxo compound and L-sepiapterin.

Key words: Atypical phenylketonuria – Dihydropteridine reductase deficiency – Hyperphenylalaninemia – Screening – Tetrahydrobiopterin – Therapy – Biopterin – Neopterin – Sepiapterin – Phenylalanine – Serotonin – Metabolism

The group of various forms of hyperphenylalaninemia includes rare and severe diseases caused by defects in biopterin metabolism [1–6]. The resulting neurological pathology seems to be due mainly to a secondary defect of catecholamine and serotonin neurotransmitter biosynthesis [3, 7, 8]. These findings have focused interest on a new area of research that may have significance for neuroscience and future treatment of affective disorders. In phenylalanine-4-hydroxylase deficiency the neurotransmitter deficiency is caused by competitive inhibition of tyrosine-3-hydroxylase and tryptophan-5-hydroxylase by the elevated phenylalanine concentration in tissue [7]. Here, the neurotransmitter deficit can be corrected by normalization of the elevated phenylalanine concentration by dietary treatment. All these 3 enzymes need tetrahydrobiopterin (BH_4) as a cofactor. In defects in biopterin metabolism, BH_4 is lacking either because of defective dihydrobiopterin (BH_2) biosynthesis [3, 5, 9, 10] or insufficient regeneration of BH_4 within the catalytic cycle (dihydropteridine reductase (DHPR) deficiency) [1, 2, 11]. We briefly report on advances in the treatment of patients with BH_2 deficiency found by screening for BH_4 deficiency in newborns with hyperphenylalaninemia, and on new aspects of biopterin biosynthesis in man.

* Corresponding author
** Present address: Department of Pediatrics, The 2nd Teaching Hospital, Wuhan Medical College, Wuhan, China

Monotherapy of Dihydrobiopterin-Deficient Patients with BH_4 or L-sepiapterin

Since 1978 we are treating patients suffering from BH_2 deficiency with BH_4 dihydrochloride under a normal phenylalanine unrestricted diet [5, 6, 12]. Because BH_4 was reported to enter the brain inadequately [13], continuation of the neurotransmitter replacement therapy [3, 14] consisting of L-Dopa, 5-hydroxytryptophan and a decarboxylase inhibitor, like Carbidopa, was thought to be necessary. By administration of still higher doses of BH_4, sufficient cofactor should penetrate the brain and correct the deficiency [13, 15]. Such a high-dose treatment has recently been tried for the first time by Kaufman [16]. Following this suggestion, we have investigated some of our patients.

Patient YZ. [17], a girl aged 5 years, had a crisis characterized by ptosis, sleepiness, ataxia, muscular hypotonia and symptoms resembling severe drug intoxication, 36 h after the neurotransmitter replacement therapy had been stopped. Then, 400 mg of BH_4-dihydrochloride (22 mg/kg) was administered orally. Within 3–4 h, all symptoms disappeared and the girl was running again and playing with other children. The effect of BH_4 lasted for 4 days. The clinical symptoms reappeared and were again removed by BH_4. Serum phenylalanine concentration, dopamine and serotonin excretion in urine were normalized and the elevated neopterin excretion, which is characteristic for such patients, decreased (Fig. 1). The concentrations of these compounds became abnormal again after 2 days. A single dose of 200 mg BH · 2 HCl (11 mg/kg) or even only 50 mg of L-sepiapterin (2.75 mg/kg) both had practically the same long-lasting effect. Unfortunately L-sepiapterin, a precursor of BH_2, is not yet available in greater amounts.

An important finding was the sharp rise in urinary serotonin to values above the normal range shortly after high-dose BH_4 administration, indicating effective stimulation of peripheral serotonin production by BH_4. From the disappearance of all clinical symptoms, it can be concluded that sufficient BH_4 entered the brain. The time lag between decrease of serotonin and reappearance of clinical symptoms (approx. 1 day) may approximate the time needed for exhausting the serotonin stores. For practical reasons, BH_4 was administered more frequently at lower doses, and a daily dose of 2.5 mg/kg was found to be sufficient. Under this monotherapy, the patient has been very well for 3 months.

Advantages of this treatment are: diet unrestricted in protein, administration of medication only once a day or less, avoidance of possible toxic decarboxylase inhibitor and

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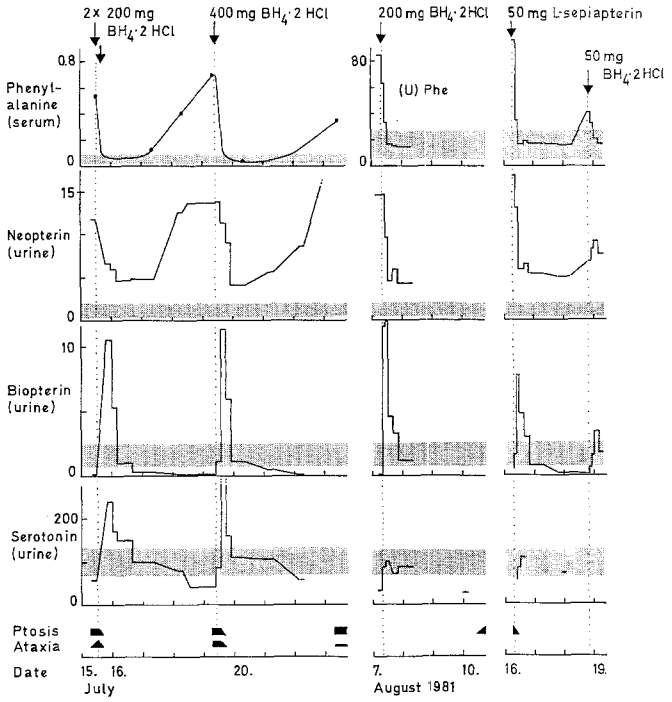


Fig. 1. Atypical PKU with dihydrobiopterin deficiency. Effect of tetrahydrobiopterin *(BH_4)* and L-sepiapterin, both administered orally, on serum and urine *(U)* phenylalanine *(Phe)*, urine neopterin, biopterin, and serotonin in the patient Y. Z. Despite the fact that neurotransmitter replacement therapy was omitted, the behaviour of the child was excellent, except during the short periods indicated. Serum Phe is shown in μmol/l, urine *Phe*, neopterin and biopterin in mmol/mol creatinine, and serotonin in μmol/mol creatinine. Serotonin was measured by mass fragmentography using deuterated serotonin as internal standard. The hatched area corresponds to the normal ranges

Table 1. Results of screening children with hyperphenylalaninemia for deficiency of BH_4, using BH_4 administration test and analysis of urinary pterins by HPLC[a]

Children investigated	Found
140 newborns with hyperphenylalaninemia	1 BH_2-deficient[a]
88 older, selected patients	15 BH_2-deficient 7 DHPR-deficient
205 phenylalanine-4-hydroxylase-deficient	No one responding to BH_4

Note: Two patients with BH_2 deficiency had normal or only slightly elevated blood phenylalanine concentrations in the newborn period. In two patients with DHPR deficiency, serum phenylalanine did not decrease after a single dose of $BH_2 \cdot 2$ HCl, 7.5 mg/kg

[a] Note added in proof. Present status: 3 BH_2-deficient patients found in 160 newborns with hyperphenylalaninemia

neurotransmitter replacement, and the possibility to control endogenous serotonin production by analysis of urinary serotonin.

Criteria for dosage are: disappearance of reversible neurological symptoms, serum phenylalanine within normal range ($<120\,\mu$mol/l), urine serotonin normal ($>70\,\mu$mol/mol creatinine), urine neopterin nearly normal (<5 mmol/mol creatinine).

Another patient (B. E.) did not respond as well to a high dose of BH_4 and the effect lasted for 1 day only. A third patient (M. K.) did not respond to BH_4 alone.

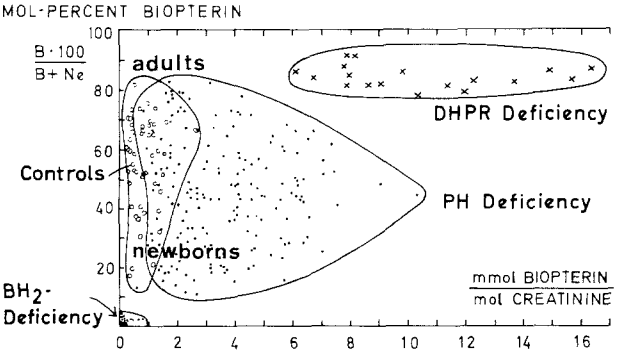


Fig. 2. Detection and differentiation of *BH_4*-deficient variants of hyperphenylalaninemia by urine biopterin *(B)*, neopterin *(Ne)* and creatinine. Urine samples were collected for 12 h at elevated serum phenylalanine concentrations, values which differed widely between patients (see also text)

Screening for BH_4 Deficiency in Newborns with Hyperphenylalaninemia

Early detection of BH_4 deficiency (including DHPR- and BH_2 deficiency) in newborns and early start of therapy are important in order to avoid irreversible brain damage in affected children. In 1978, we introduced tests of BH_4 deficiency by administration of BH_4 [6, 12, 19] in a single oral dose (7.5 mg $BH_4 \cdot 2$ HCl/kg presently recommended) while serum phenylalanine concentration was elevated with measurement of serum phenylalanine at 0, 4 and 8 h. A marked decrease of phenylalanine was observed in BH_2 deficiency, and slower in DHPR deficiency (in 2 patients with this disease phenylalanine remained constant). In addition, urinary pterins and creatinine were measured to confirm and differentiate BH_4-deficiency [5, 12, 18]. One year ago we have automated the previously used HPLC method [18], eliminating sample pretreatment, except oxidation by MnO_2. In this way, fast measurements of biopterin (B) and neopterin (Ne) became possible. The results of the screening are shown in Table 1. The newborn patient (B.E., female) who suffered from BH_2 deficiency, was detected in 1979 at the age of 6 weeks and was treated immediately with BH_4 and neurotransmitter precursor supplementation [20].

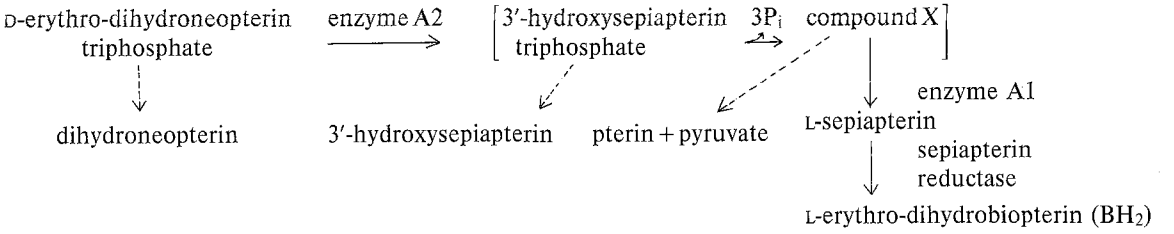
Variants with deficiency of BH_4 can best be distinguished [18] by using a two-dimensional plot of %B against B/C, with %B = 100 B/B + Ne and C = creatinine (Fig. 2). B/C can also be plotted in logarithmic scale. In patients (number n) with phenylalanine-4-hydroxylase deficiency, BH_2 deficiency and DHPR deficiency, the range of %B was 11-67 ($n=163$, one sample each, age 8d-7.7 m), 0.6-4.9 ($n=15$; 35 samples; age 46d-6 y) and 77-93 ($n=7$, 19 samples; 3 m-12 y) respectively.

Two of the BH_2 deficient patients (Y.Z. [17] and L.L. [21]) had normal or only slightly elevated serum phenylalanine concentrations in the newborn period, possibly because of sufficient BH_4 intake during breast-feeding. Consequently, the cut-off phenylalanine level for selected screening of BH_4 deficiency should be set as low as possible. Pediatric neurologists should be adequately informed and children with similar neurological symptoms should be tested for hyperphenylalaninemia and then for BH_4 deficiency.

New Aspects of Biopterin Biosynthesis in Man

BH_4 is synthesized from guanosine triphosphate in several enzymatic steps [22, 23]. The rate limiting enzyme in rat and

human brain was GTP cyclohydrolase [22], followed by D-erythro-7,8-dihydroneopterin triphosphate synthetase. Dihydroneopterin triphosphate was converted directly to quinonoid BH_2 by BH_2 synthetase, an enzyme not requiring pyridine nucleotides or other cofactors [22]. This is in contrast to other results [23], where 3 different protein fractions were required for that conversion in chicken kidney. A Mg^{++}-dependent enzyme A2 converted dihydroneopterin triphosphate to a compound X suggested to be 6-(1′,2′-dioxopropyl)-7,8-dihydropterin which could be degraded by dinitrophenylhydrazine to pterin and pyruvic acid dinitrophenylhydrazone. A heat labile NADPH-dependent enzyme A1 converted compound X to sepiapterin and this was reduced to BH_2 by sepiapterin reductase. We were also able to confirm these results from chicken kidney in human kidney and liver. This indicates that in human kidney and liver also biopterin biosynthesis might proceed via compound X and sepiapterin [24]:



In the patients with BH_2 deficiency, either enzyme A2 (triphosphate elimination step) or, less probably, the enzyme A1 is defective. This would explain the excessive urinary excretion of neopterin, dihydroneopterin [5, 6, 18] and 3′-hydroxysepiapterin [25] as well as the response of these patients to BH_4, BH_2 and even better, to L-sepiapterin [5] by normalization of the elevated serum phenylalanine concentration.

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References

1. Smith I, Clayton BE, Wolff OH (1975) New variant of phenylketonuria with progressive neurological illness unresponsive to phenylalanine restriction. Lancet I : 1108–1111
1a. Bartholomé K (1974) A new molecular defect in phenylketonuria. Lancet II : 1580
2. Kaufman S, Holtzman NA, Milstien S, Butler IJ, Krumholz A (1975) Phenylketonuria due to a deficiency of dihydropteridine reductase. New Eng J Med 293 : 785–790
3. Danks DM, Bartholomé K, Clayton BE, Curtius H-Ch, Gröbe H, Kaufman S, Leeming R, Pfleiderer W, Rembold H, Rey F (1978) Malignant hyperphenylalaninaemia–current status (June 1977). J Inher Metab Dis 1 : 49–53
4. Kaufman S, Berlow S, Summer GK, Milstien S, Schulman JD, Orloff S, Spielberg S, Pueschel S (1978) Hyperphenylalaninemia due to a deficiency of biopterin. A variant form of phenylketonuria. N Engl J Med 299 : 673–679
5. Niederwieser A, Curtius H-Ch, Bettoni O, Bieri J, Schircks B, Viscontini M, Schaub J (1979) Atypical phenylketonuria caused by 7,8-dihydrobiopterin synthetase deficiency. Lancet I : 131–133
6. Curtius H-Ch, Niederwieser A, Viscontini M, Otten A, Schaub J, Scheibenreiter S, Schmidt H (1979) Atypical phenylketonuria due to tetrahydrobiopterin deficiency. Diagnosis and treatment with tetrahydrobiopterin, dihydrobiopterin and sepiapterin. Clin Chim Acta 93 : 251–262
7. Curtius H-Ch, Baerlocher K, Völlmin JA (1972) Pathogenesis of phenylketonuria: Inhibition of dopa and catecholamine synthesis in patients with phenylketonuria. Clin Chim Acta 42 : 235–239
8. Butler IJ, Koslow SH, Krumholz A, Holtzman NA, Kaufman S (1978) A disorder of biogenic amines in dihydropteridine reductase deficiency. Ann Neurol 3 : 224
9. Rey F, Harpey JP, Leeming RJ, Blair JA, Aicardi J, Rey J (1977) Les hyperphénylalaninémies avec activité normale de la phénylalanine-hydroxylase. Arch Franc Péd 34, Suppl 2 : 109–120
10. Tanaka T, Aihara K, Iwai K, Kohashi M, Tomita K, Narisawa K, Arai N, Yoshida H, Usui T (1981) Hyperphenylalaninemia due to impaired dihydrobiopterin biosynthesis. Eur J Ped 136 : 275–280
11. Tada K, Narisawa K, Arai N, Ogasawava Y, Ishizawa S (1980) A sibling case of hyperphenylalaninemia due to a deficiency of dihydropteridine reductase: Biochemical and pathological findings. Tohoku J Exp Med 132 : 123–131
12. Niederwieser A, Curtius H-Ch, Viscontini M, Schaub J, Schmidt H (1979) Phenylketonuria variants. Lancet II : 550
13. Gál EM, Hanson G, Sherman A (1976) Biopterin I. Profile and quantitation in rat brain. Neurochem Res 1 : 511–523
14. Bartholomé K, Byrd DJ (1975) L-dopa and 5-hydroxytryptophan therapy in phenylketonuria with normal phenylalanine-hydroxylase activity. Lancet II : 1042–1043
15. Kapatos G, Kaufman S (1981) Peripherally administered reduced pterins do enter the brain. Science 212 : 955–956
16. Berlow S, Madison, USA, personal communication
17. Schaub J, Däumling S, Curtius H-Ch, Niederwieser A, Bartholomé K, Viscontini M, Schircks B, Bieri JH (1978) Tetrahydrobiopterin therapy of atypical phenylketonuria due to defective dihydrobiopterin biosynthesis. Arch Dis Child 53 : 674–676
18. Niederwieser A, Curtius H-Ch, Gitzelmann R, Otten A, Baerlocher K, Blehová B, Berlow S, Gröbe H, Rey F, Schaub J, Scheibenreiter S, Schmidt H, Viscontini M (1980) Excretion of pterins in phenylketonuria and phenylketonuria variants. Helv Paed Acta 35 : 335–342
19. Danks DM, Cotton RGH, Schlesinger P (1975) Tetrahydrobiopterin treatment of variant form of phenylketonuria. Lancet II : 1043
20. Endres W, Niederwieser A, Curtius H-Ch, Wang M, Ohrt B, Schaub J (1982) Helv Paed Acta, in press
21. Beck B and Güttler F, Copenhagen, personal communication
22. Gál EM, Nelson JM, Sherman AD (1978) Biopterin III: Purification and characterization of enzymes involved in the cerebral synthesis of 7,8-dihydrobiopterin. Neurochem Res 3 : 69–88
23. Tanaka K, Akino M, Hagi Y, Doi M, Shiota T (1981) The enzymatic synthesis of sepiapterin by chicken kidney preparations. J Biol Chem 256 : 2963–2972
24. Häusermann M, Ghisla S, Niederwieser A, Curtius H-Ch (1981) New aspects of bopterin biosynthesis in man. FEBS Letters 131 : 275–278
25. Niederwieser A, Matasović A, Curtius H-Ch, Endres W, Schaub J (1980) 3′-Hydroxysepiapterin in patients with dihydrobiopterin deficiency. FEBS Letters 118 : 299–302

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