

BH₄-Sensitive Hyperphenylalaninemia: New Case and Review of Literature

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We report a patient with BH₄-sensitive phenylketonuria. In neonatal screening, phenylalanine levels above 10 mg/dl were detected. In the tetrahydrobiopterin-(BH₄) loading test, phenylalanine concentrations in serum fell significantly. Dihydropteridine reductase activity in blood, pterines, and neurotransmitters in cerebrospinal fluid, as well as pterines in urine were all normal. Mutation analysis revealed compound-heterozygosity for the mutations R408W and K320N. Under BH₄-supplementation without a specific phenylalanine-reduced diet, phenylalanine-concentrations are in the therapeutic range and our patient developed normally. © 2003 by Elsevier Inc. All rights reserved.

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Introduction

Hyperphenylalaninemia (HPAs) belong to the group of inborn metabolic disorders with multifactorial, multilocus, and complex traits. In Western countries the incidence of HPAs is about 1 in every 5000 newborns. Diagnosis can be made in neonatal screening programs on dry blood spot by measuring phenylalanine levels.

The underlying biochemical defect of HPA is a dysfunction of the phenylalanine-hydroxylase (PAH)-reaction. This reaction requires tetrahydrobiopterin (BH₄) as a co-factor. Not only the PAH-reaction is BH₄-dependent: tyrosine-3-hydroxylase and tryptophan-5-hydroxylase (two key enzymes in the biosynthesis of catecholamines and serotonin) need BH₄ as co-factor too [1].

The following diseases are collected under the umbrella of hyperphenylalaninaemia (HPA): (1) Classical phenylketonuria (PKU) with reduced activity of the apoenzyme PAH, which does not respond to BH₄ and requires a low-phenylalanine diet; (2) Defects of BH₄-metabolism (sometimes called *atypical PKU*), responding to BH₄ substitution; and (3) A mild variant of HPA (with phenylalanine levels < 10 mg/dl) that does not respond to BH₄ and does not require a diet (also known as *benign PKU*).

To differentiate between these forms of HPA a BH₄-loading test is performed [2]. Although patients with classical PKU usually do not demonstrate a significant reduction of blood phenylalanine concentration in the BH₄-loading test, patients with defects in BH₄-metabolism exhibit a decrease of phenylalanine levels after BH₄ application. Because of co-factor deficiency in patients with the latter form of PKU, reduced concentrations of neurotransmitters in cerebrospinal fluid (CSF) are found. Therapy of these patients consists of BH₄-supplementation and application of neurotransmitter precursors [3].

Recently, there have been a few reports on patients with classical PKU who respond to BH₄ [4-9]. In this subgroup, BH₄ metabolism is normal (as demonstrated by normal neurotransmitter concentrations, pterine levels, and dihydropteridine reductase activity in blood). It has been speculated that the underlying mutations cause a K_m-variant of PAH in which residual activity can be enhanced by supplementation of BH₄ [10]. A review of the published cases and the mutations found is given. Furthermore, a new patient with BH₄-sensitive PKU is presented.

Case Report

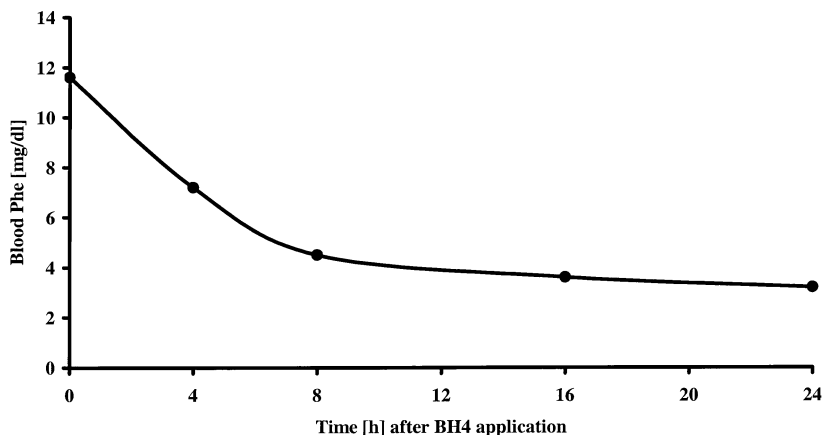
We report on a 2-month-old male infant with an unremarkable peripartum period and a normal neurologic status. He was the first child of an unrelated caucasian couple and had been delivered at 40 weeks of gestation with a birth weight of 3600 g.

In the neonatal screening performed at day 4 an elevated phenylalanine concentration was found. Before BH₄-loading, the blood phenylalanine concentration was 11.6 mg/dl (705 μmol/l). Eight hours after BH₄ application (20 mg/kg b.w.), phenylalanine concentration decreased to

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Figure 1. BH₄-test: Course of phenylalanine values.



4.5 mg/dl. A PKU resulting from a defect in BH₄ metabolism could be excluded since neurotransmitters and pterines in CSF and pterines in urine, as well as prolactin concentration in blood (which is inversely correlated to the CSF concentration of the neurotransmitter dopamine) were normal. Furthermore, activity of dihydropteridine reductase in blood was normal. Under continuous BH₄-supplementation (10 mg/kg per day), blood phenylalanine concentrations remained in the therapeutic range. Because of delivery problems (of the pharmacy), BH₄-therapy was interrupted for several days, resulting in a slight increase of blood phenylalanine-concentrations, despite mild dietary phenylalanine restriction. The time course of blood phenylalanine concentrations is presented in Figure 1. Molecular biology revealed compound-heterozygosity for the mutations R408W and K320N.

Discussion

Up to now, reports of 14 patients with BH₄-sensitive HPA not resulting from disturbed BH₄ metabolism have been published [4-10]. All had mild forms of “classical” PKU. As presented in Table 1 the underlying mutations of PAH are not uniform. A structural hypothesis for BH₄ responsiveness has been suggested by Erlandsen and Stevens who correlated genotype and residual activity of PAH in BH₄-responsive patients described by Kure, Spaapen, and Trefz [4-6,11]. Erlandsen and Stevens speculated that BH₄-responsiveness is caused by mutations located in the cofactor-binding regions of PAH or in regions that closely interact with the cofactor-binding regions, but they are not located at residues of the PAH molecule that are directly interacting with the cofactor [11]. Trefz postulated that the mutations in BH₄-sensitive patients cause a K_m variant of PAH with a lower binding affinity for BH₄, which can be overcome by BH₄ excess [10].

We report a new case of BH₄-sensitive HPA. Our patient is compound heterozygous for the mutations R408W and K320N. R408W is a known null mutation that causes a complete loss of phenylalanine hydroxylase activity, whereas K320N has not been described in the literature so far [12]. Interestingly, the second patient described in an abstract by Steinfeld [8] revealed the K320N mutation on one allele, whereas on the other allele

the mutation A104D, that usually causes a non-PKU HPA, could be found [8]. In our patient and Steinfeld’s, the K320N mutation may be responsible for the above mentioned K_m variant of PAH. However, apart from the mutation, additional factors seem to play a role in BH₄-sensitive HPA. Lindner published three HPA patients demonstrating all the mutations R408W/Y414C [7]. Only one of these patients was BH₄-sensitive. Therefore, mutation analysis alone does not help in diagnosing patients with a BH₄-sensitive form of PKU. The BH₄-loading test is the only diagnostic tool to detect BH₄-sensitivity in PKU-patients.

Conclusion

BH₄-sensitive PKU without a defect in BH₄ metabolism is a new form of inherited metabolic disease belonging to the group of HPAs. There seems to be no genotype-phenotype correlation. The BH₄-loading test is able to detect

Table 1. Mutations in the PAH gene in patients with BH₄-sensitive HPA

Kure et al. 1999		
1	P407S	R252 W
2	IVS4-IG→A	A373T
3	R413P	R241C
4	R413P	R241C
5	P407S	R111X
Spaapen et al. 2000		
6	V190A	R243X
7	A313T	L367fSinsC
8	R241C	A403V
9	A300S	A403V
Trefz et al. 2000/2001		
10	IVS10G→A	E390G
Steinfeld et al. 2001		
11	Y414C	Y414C
12	A104D	K320N
Lindner et al. 2001		
13	R408W	Y414C
Nuoffer et al. 2001		
14	del1194	Y414C
15	R408W	K320N

both the atypical PKU with disturbed BH₄ metabolism and the BH₄-sensitive PKU without defects in BH₄ metabolism.

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