Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency in Dutch neonates


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Summary  Four neonates with a positive phenylalanine screening test (Phe concentrations between 258 and 1250 μmol/L) were investigated further to differentiate between phenylalanine hydroxylase (PAH) deficiency and variant hyperphenylalaninaemia (HPA) forms. In patients 1 and 2 a tetrahydrobiopterin (BH4) load caused a significant decrease of the plasma Phe levels. A combined phenylalanine/BH4 loading test was performed in patients 2, 3 and 4. In the latter two patients, plasma Phe concentrations completely normalized within 8 h after the BH4 load (20 mg/kg). Basal urinary pterins were normal in all four patients. The activity of dihydropteridine reductase (DHPR) was normal in patients 1, 2 and 3 and 50% of control values in patient 4 (not in the range of DHPR-deficient patients). In patient 3 a subsequent phenylalanine loading test with concomitant analysis of plasma biopterins revealed a normal increase of biopterin, excluding a BH4 biosynthesis defect. Pterins and neurotransmitter metabolites in CSF of patients 1, 3 and 4 were normal. DNA mutations detected in the PAH gene of patients 1-4 were A313T, and L367fsinsC; V190A and R243X; A300S and A403V; R241C and A403V. The results are suggestive for mutant PAH enzymes with decreased affinity for the cofactor BH4.

Hyperphenylalaninaemia (HPA) is a disorder caused by a deficient or a decreased activity of phenylalanine-4-hydroxylase (PAH, EC 1.14.16.1) due either to a
mutated enzyme protein or to a deficiency of its obligatory cofactor tetrahydrobiopterin (BH4). The latter group comprises defects in the biosynthesis and in the regeneration of BH4 (Scriver et al 1995). Detection of HPA is included in the newborn mass screening programme. Differential diagnostic investigations are necessary, however to detect BH4 deficiencies even if phenylalanine (Phe) concentrations are only slightly elevated (Ponzone et al 1993). Screening for BH4 deficiency is performed by analysis of pterins in urine and measurement of dihydropteridine reductase (DHPR) activity in erythrocytes or skin fibroblasts (Blau and Blaskovics 1996). In the Netherlands as well as in several other countries, a BH4 loading test is included in the screening protocol of HPA. However, if the initial Phe concentration is below 400 μmol/L, a combined Phe/BH4 loading is performed (Ponzone et al 1993). Recently, Kure and colleagues (1999) reported that serum Phe concentrations in four patients with mild HPA decreased after a BH4 challenge. Urinary pterins in their patients and DHPR activities in blood appeared to be normal. In addition, mutations were detected in the PAH genes of those patients.

In the follow-up of a positive newborn PKU-screening test we found four children to be responsive after either a BH4 challenge or a combined Phe/BH4 loading test. DHPR deficiency was excluded and urinary pterins appeared to be normal. Mutations were found in the PAH genes of all these patients; thus this group of patients belongs to a new variant of BH4-responsive PAH deficiency.

PATIENTS AND METHODS

Patient 1, a girl, was born prematurely after a pregnancy of 28 weeks because of maternal complications. Her birth weight was 750 g, she was dysmature and suffered from severe neonatal complications. Patients 2, 3 and 4 were born at term after uncomplicated pregnancies. They were admitted to the academic hospital for evaluation of a positive newborn PKU screening test. Combined Phe/BH4 loading tests were performed according to Ponzone and colleagues (1993).

Amino acids were analysed by means of automated ion-exchange chromatography with postcolumn ninhydrin derivatization (Biochrom 20, Amersham Pharmacia Biotech). DHPR activity in erythrocytes was measured as described previously (Surplice et al 1990) or in cultured skin fibroblasts according to Bonafé and colleagues (2000). Urinary pterins were analysed by a HPLC procedure adapted from Fukushima and Nixon (1980) and Nixon and colleagues (1980). Neurotransmitter metabolites were analysed in CSF of patients 1, 3 and 4 as described (Blau et al 1999). Mutations in the PAH gene were detected by means of single-strand conformational analysis and subsequent sequence analysis (van der Sijs-Bos et al 1996).

RESULTS AND DISCUSSION

The positive PKU screening test in patient 1 was followed by a BH4 load of 20 mg/kg body weight. Figure 1 shows the response of plasma Phe to the BH4 load. Because of the rapid decrease of the plasma Phe concentration, treatment with BH4 (5 mg/kg per day) was continued during the next 8 days. From day 9 the treatment was stopped.
for 3 days, resulting in an increase of the Phe level. Subsequently, the treatment was instituted again. The initial Phe concentration in patient 2 was 450 μmol/L. A single BH₄ load of 20 mg/kg resulted in a decrease of Phe from 445 μmol/L before load to 251 μmol/L and 26 μmol/L, 8 and 33 h following load, respectively.

Because of the moderately increased Phe concentrations in patients 3 and 4, combined Phe/BH₄ loading tests were performed. Patient 2 was retested in the same way. Figure 2 shows the courses of the plasma Phe concentrations during the tests. Urinary pterins were normal in all four patients. DHPR activity in cultured fibroblasts of patient 1 and in erythrocytes of patients 2 and 3 were found normal, whereas in patient 4 DHPR activity was 46% of control (data not shown). Phenylalanine-induced biopterin synthesis was studied in patient 3 following an oral Phe load (100 mg/kg). The normal increase of biopterin (Figure 3) excluded a BH₄ biosynthesis defect. Analysis of neurotransmitter metabolites in CSF of patients 1, 3 and 4 did not reveal deficiencies of neurotransmitters (Table 1); CSF of patient 2 was not analysed. In addition, CSF pterins were found normal (data not shown).

These normal findings prompted us to analyse the PAH gene. Mutations in both alleles of the PAH gene were identified in all four patients (Table 2). Patients 3 and 4 share the same mutation (A403V) although they are not related. They showed a similar rapid response on BH₄ loading, resulting in normalization of plasma Phe.

Figure 1  Course of plasma phenylalanine and tyrosine concentrations in patient 1 after BH₄ supplementation: day 1, 20 mg/kg; days 2–9, 5 mg/kg per day; days 9–12 stop supplementation; from day 12 on continuation of treatment with BH₄ 5 mg/kg per day.
**BH₄-responsive phenylalanine hydroxylase deficiency**

Figure 2 Course of plasma phenylalanine concentrations during a combined Phe/BH₄ loading test in patients 2, 3 and 4

Figure 3 Course of plasma biopterin (---) and phenylalanine (bars) concentrations in patient 3 after a phenylalanine load (100 mg/kg)

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Table 1  Biogenic amines in CSF (nmol/L)

<table>
<thead>
<tr>
<th></th>
<th>5-HIAA</th>
<th>HVA</th>
<th>HVA/5-HIAA</th>
<th>DOPAC</th>
<th>MHPG</th>
<th>3-OMD</th>
<th>5-OH-Trp</th>
<th>L-Dopa</th>
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<tr>
<td>Patient 1</td>
<td>697</td>
<td>758</td>
<td>1.1</td>
<td>16.3</td>
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<td>335</td>
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<td>Patient 3</td>
<td>212</td>
<td>563</td>
<td>2.7</td>
<td></td>
<td></td>
<td>75.0</td>
<td>8.7</td>
<td>12.3</td>
</tr>
<tr>
<td>Patient 4</td>
<td>316</td>
<td>523</td>
<td>1.7</td>
<td>29.9</td>
<td>67.2</td>
<td>71.3</td>
<td>&lt;5</td>
<td>38.5</td>
</tr>
<tr>
<td>Controls&lt;sup&gt;a&lt;/sup&gt;</td>
<td>150–800</td>
<td>310–1100</td>
<td>1.5–3.5</td>
<td>8–18</td>
<td>98–168</td>
<td>&lt;300</td>
<td>&lt;10</td>
<td>&lt;25</td>
</tr>
</tbody>
</table>

<sup>a</sup> Age range: 0–0.5 years

5-HIAA, 5-hydroxyindoleacetic acid; HVA, homovanillie acid; DOPAC, 3,4-dihydroxyphenylacetic acid; MHPG, 3-methoxy-4-hydroxyphenylglycol; 3-OMD: 3-O-methyl-dopa; 5-OH-Trp, 5-hydroxytryptophan
within 8 h (Figure 2). The R241C mutation found in patient 4 was detected earlier in two other BH₄-responsive HPA patients. In contrast to our patient 4, plasma Phe concentrations in those two patients apparently did not decrease to normal values within 24 h after the BH₄ load. However, they received a lower dose of 10 mg/kg BH₄ (Kure et al 1999). The A300S and A403V mutations have been found in non-PKU HPA, though no BH₄-responsiveness was reported (Mallolas et al 1999). The A313T mutation due to a 937G>A transition, detected in patient 1, has not been reported before.

Normal human PAH protein is present as homopolymer produced by a single genetic locus (Scriver et al 1995). The HPA patients described in this study all bear two different allele mutations, leading to the presence of various heteropolymeric and homopolymeric PAH proteins. Increasing intracellular BH₄ concentrations by oral supplementation apparently increases residual PAH activity, possibly as result of a decreased affinity of the enzyme for this cofactor. In the patients who showed a fast normalization of plasma Phe within 8 h after BH₄ load, both heteropolymeric and homopolymeric PAH subunits may be involved in the restoration of enzymic activity. In patients who responded partially to BH₄ supplementation, only homopolymeric PAH molecules bearing one of the mutations may be activated by the cofactor, leading to a partially restored enzyme activity. So far, PAH mutations specifically affecting the BH₄ binding site of the enzyme are unknown.

The patients presented above belong to a group with a new variant of PAH deficiency, a BH₄-responsive one. Patient 1 had Phe and Tyr concentrations in the 120–150 μmol/L range on BH₄ monotherapy. In patients 2 and 4, plasma Phe levels remain well controlled (<350 μmol/L) on protein restriction and a PKU formula. Without protein restriction, Phe concentrations in patient 3 remain mostly below 250 μmol/L. In principle this form of PAH deficiency is treatable with BH₄. Institution of BH₄ supplementation in our patients is a point at issue. These novel BH₄-responsive subtypes of PAH deficiency can only be detected if a BH₄ loading test is included in the differential diagnostic investigations of HPA patients.

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