Differential Diagnosis of Tetrahydrobiopterin Deficiency

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Six hundred and seventy-three children (483 newborns and 190 older selected children) were screened for tetrahydrobiopterin (BH4) deficiency by HPLC of urine pterins and BH4 load test. One patient with GTP cyclohydrolase I deficiency, 36 patients with dihydrobiopterin synthetase (DHBS) deficiency (of which six were in the newborn and 30 in the older children) and 14 with dihydropteridine reductase deficiency (DHPR) were found. All 37 patients with defective BH4 biosynthesis responded to a BH4 load by lowering of the elevated serum phenylalanine concentration but four of 14 patients with DHPR deficiency did not. Measurement of DHPR activity in blood spots on Guthrie cards is recommended. Since subvariants of patients with BH4 deficiency exist, homovanillic acid, 5-hydroxyindole acetic acid, pterins, phenylalanine, and tyrosine in cerebrospinal fluid should be measured for diagnosis and the control of therapy. The activity of the phosphate-eliminating enzyme (a key enzyme in BH4 biosynthesis and part of “DHBS”) was measured in human liver and activities of approx. 1 nU (mg protein)^{-1} were found. In the liver biopsy of a patient with DHBS deficiency no activity (less than 3% of controls) was demonstrated.

Ten years ago the first patients with tetrahydrobiopterin (BH4) deficiency were described independently by Bartholomé (1974) and Smith et al. (1974, 1975). At that time it became obvious that BH4 was essential for human well-being and normal function of the central nervous system. Because tetrahydrobiopterin deficiency is a severe disease with progressive neurological symptoms (Danks et al., 1978) and is treatable (Bartholomé and Byrd, 1975; Danks et al., 1978), it became necessary to develop screening tests for the early detection of BH4 deficiency in infancy. Every newborn with even only slight but persistent hyperphenylalaninaemia should be tested for BH4 deficiency. Such tests have been introduced in many developed nations, but even today older children are detected because of the appearance of clinical symptoms, such as muscular hypotonia of the trunk, hypertonia of the extremities and often myoclonic epilepsy, which are unresponsive to a low phenylalanine diet.

Today we recognize four metabolic defects causing hyperphenylalaninaemia including the classical form with a defect in phenylalanine 4-hydroxylase (EC 1.4.16.1) (McKusick 26160) (Figure 1). The three variants are rare disorders, with an estimated incidence of 1% in newborns with hyperphenylalaninaemia, and lead to BH4 deficiency either by a defect in regeneration of this cofactor or by one of two defects in biosynthesis. GTP cyclohydrolase I (EC 3.5.4.16) deficiency has been detected only recently (Niederwieser et al., 1982c, 1984a) and four patients have been reported. This defect blocks BH4 biosynthesis at the very first step of conversion of GTP to dihydropteridine triphosphate and the patient is practically unable to form any pterins. Therefore, this disease may be a worthwhile model for investigating the potential involvement of pterins in other biological systems, in addition to the known cofactor role of BH4 for phenylalanine, tyrosine, and tryptophan hydroxylases.

“Dihydrobiopterin synthetase” is a provisional name for an enzyme system described by Gál et al. (1978) converting dihydropteridine triphosphate to BH4. The system consists of two or three enzymes which have not yet been characterized in detail (Curtius, 1985). Most patients with BH4 deficiency suffer from a defect in “dihydrobiopterin synthetase” (McKusick 26164, 26169). The first patient with this variant was described by Rey et al. (1977). The patients excrete huge amounts of dihydropterin and neopterin in urine (Niederwieser et al., 1979; Kaufman et al., 1979) as well as 3'-hydroxy-D-sepiapterin (Niederwieser et al., 1980b) as degradation products of the accumulated dihydropterin triphosphate (Figure 1), and low concentrations only of bioperinos.

Dihydropteridine reductase (DHPR) (EC 1.6.99.7) (McKusick 26163) deficiency was detected by Kaufman et al. (1975). Because of the absence of feedback inhibition of GTP cyclohydrolase I due to the lack of BH4, bioperin biosynthesis is activated and leads to the

![Figure 1 Possible metabolic defects in hyperphenylalaninaemia. BH4 deficient variants: defects 1–3; classical phenylketonuria: defect 4](Image)
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acumulation and excretion of the inactive 7,8-dihydrobiopterin and biopterin. The incidence is less than that of "dihydrobiopterin synthetase" deficiency and an increased frequency has been found in Sicily.

METHODS FOR DETECTION AND DIFFERENTIAL DIAGNOSIS OF BH₄ DEFICIENCY

A number of different methods are available. We use and recommend the following (Niederwieser et al., 1982b):

1. Analysis of total pterins and creatinine in urine collected at elevated serum phenylalanine concentration (not on PKU diet).
2. Administration of a BH₄ load, 7.5 mg kg⁻¹ orally, and measurement of serum/plasma phenylalanine and tyrosine at zero, 4 and 8 h after the load.
3. DHPR activity should be measured in blood or dried blood spots (Guthrie cards) according to the method of Arai et al. (1982). In patients with BH₄ deficiency, we measure homovanillic acid and 5-hydroxyindole acetic acid in CSF by HPLC with electrochemical detection (Niederwieser et al., 1984a), as well as pterins, phenylalanine, tyrosine, and tryptophan. Lumbar CSF should be taken between 10 and 12 a.m. (in newborns, discard the first 0.2 ml, collect the next 0.5–0.6 ml in an EDTA tube and freeze immediately).

For the analysis of pterins we have developed a routine method with a high degree of automation, selective for pterins and designed to detect other as yet unknown pterins which may differ widely in polarity (Niederwieser et al., 1982d, 1984b). Other HPLC methods are available (Fukushima and Nixon, 1980; Lunte and Kissinger, 1983). BH₄ can be determined by differential oxidation in alkaline and acidic medium (Fukushima and Nixon, 1980) but can also be measured directly by HPLC with electrochemical detection (Niederwieser et al., 1982d, 1984b; Lunte and Kissinger, 1983; Brüutigam et al., 1984). Total biopterins can also be measured microbiologically in blood and urine (Leeming et al., 1984).

Enzyme measurements are now possible for all three variants: GTP cyclohydrase I in liver biopsies (Blau and Niederwieser, 1983; Niederwieser et al., 1984a), DHPR in dried blood spots (Arai et al., 1982) and the phosphate-eliminating enzyme (PEE) in liver biopsies from patients with "dihydrobiopterin synthetase" deficiency.

For measurement of the phosphate-eliminating enzyme activity, approx. 10 mg of liver tissue was homogenized in 100 µl of 10 mmol l⁻¹ potassium phosphate pH 6.8 and centrifuged at 12 000 g for 20 min. The supernatant was applied onto 500 µl of Sephadex G-25 in a Pasteur pipette and the brown zone eluted and collected. The volume and the protein concentration were determined; an aliquot of 150 µl was heated at 65 °C for 1 min and then centrifuged at 12 000 g for 20 min. Of the supernatant, 50 µl was incubated at 37 °C for 2 h at pH 7.4 in a total volume of 125 µl containing 24 mmol l⁻¹ Tris·HCl, 24 mmol l⁻¹ dihydroxypteridine triphosphate, 8 mmol l⁻¹ magnesium chloride, 1 mmol l⁻¹ NADPH, 5 mmol l⁻¹ dithioerythritol, and approx. 1 mU of human liver seNapterin reductase. The reaction was stopped by addition of 25 µl of 200 mmol l⁻¹ EDTA (final concentration 33 mmol l⁻¹), and the formation of BH₄ was measured immediately by HPLC with electrochemical detection (Niederwieser et al., 1982, 1984b).

RESULTS AND DISCUSSION

Detection and differential diagnosis of BH₄ deficiency may often be possible directly from characteristic pattern of urinary pterins HPLC chromatograms (Niederwieser et al., 1984a). In GTP cyclohydrase deficiency only trace amounts of pterins are detected, while in "dihydrobiopterin synthetase" deficiency, the excessive concentrations of neopterin and monapterin, which is a neopterin isomer, are impressive. It should be emphasized, however, that small but significant peaks of biopterin can be detected, particularly in older children with this disease. We do not know the origin of this biopterin but it does not appear to result from a significant residual activity of biopterin biosynthesis in one of the children tested. A large peak of biopterin dominates the chromatograms from samples from patients with dihydropteridine reductase deficiency. Patients with classical PKU excrete increased amounts of pterins depending on the phenylalanine concentration in blood and tissue. On a low phenylalanine diet the excretion of pterins is nearly normal and, most important, patients with DHPR deficiency would not be diagnosed under such conditions. The differential diagnosis is straightforward when biopterin is expressed as a fraction of the sum of biopterin and neopterin (%B) and plotted versus the biopterin to creatinine ratio. The values then fall into characteristic areas for the different diseases and controls (Niederwieser et al., 1980a, 1982a).

The results of our screening for BH₄ deficiency in children with hyperphenylalaninaemia is shown in Table 1. In a total of 673 cases (483 newborn, 190 older

Table 1. Results of screening children with hyperphenylalaninaemia for BH₄ deficiency caused by defects in GTP cyclohydrase I (GTPCH), "dihydrobiopterin synthetase" (DHBS), or dihydropteridine reductase (DHPR)

<table>
<thead>
<tr>
<th>Test used</th>
<th>Children investigated</th>
<th>Deficiencies found</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>GTPCH</td>
</tr>
<tr>
<td>HPLC of urinary pterins</td>
<td>483 newborns</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>190 older, selected</td>
<td>1</td>
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<tr>
<td></td>
<td>673 total</td>
<td>1</td>
</tr>
<tr>
<td>BH₄ test</td>
<td>total 440, responded</td>
<td>1</td>
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