

Short Communication

Variant of dihydropteridine reductase deficiency without hyperphenylalaninaemia: Effect of oral phenylalanine loading

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Dihydropteridine reductase (DHPR; EC 1.6.99.7) deficiency (McKusick 261630), the second most common form of tetrahydrobiopterin (BH₄) deficiency, presents phenotypically with hyperphenylalaninaemia (HPA) and neurotransmitter deficiency (Blau et al 2000). The clinical course of the illness is similar to that seen in severe forms of GTP cyclohydrolase I and 6-pyruvoyltetrahydropterin synthase deficiencies. Common but variable symptoms are mental retardation, convulsions, disturbance of tone and posture, abnormal movements, hypersalivation and swallowing difficulties. In addition, extensive neuronal loss, calcification and abnormal vascular proliferation have been noted in the central cortex, white matter, basal ganglia, and thalamus. So far, more than 110 patients have been detected through newborn phenylketonuria screening and by selective screening of urinary pterins and DHPR activity on Guthrie cards. However, absence of HPA does not exclude BH₄ deficiency. Recently, a new variant of DHPR deficiency affecting only the central nervous system that does not lead to HPA was described (Blau et al 1998). A similar metabolic phenotype was described for the dominant form of GTP cyclohydrolase I deficiency (dopa-responsive dystonia) (Hyland et al 1996). Although presenting without HPA, these patients show an abnormal phenylalanine-to-tyrosine ratio after oral phenylalanine challenge, indicating abnormal hepatic phenylalanine turnover (Hyland et al 1997).

Here we describe similar observations in two patients with a newly recognized

variant of DHPR deficiency, one of which has been reported previously (Blau et al 1998). We investigated plasma phenylalanine, tyrosine, neopterin and biopterin concentrations after oral administration of phenylalanine (100 mg/kg) with and without BH₄ (20 mg/kg).

MATERIALS AND METHODS

The patients reported here are registered in the International BIODÉF Database (<http://www.unizh.ch/~blau/biodef1.html>).

The first patient (BIODÉF ID360), a 10-year-old boy of consanguineous Turkish parents, has been described in more detail elsewhere (Blau et al 1998). The second patient (BIODÉF ID229), a 5-year-old boy of unrelated Turkish parents, was diagnosed by pterins and neurotransmitter analysis of cerebrospinal fluid (CSF). Plasma phenylalanine, urinary and plasma total neopterin and biopterin, and red cell DHPR activity were all in the normal range; however, the CSF profile of pterins was typical for DHPR deficiency (neopterin 24/14 nmol/L, normal 9–20; biopterin 77/53 nmol/L, normal 10–30) with normal levels of BH₄ (16 nmol/L, normal 10–55) but increased levels of 7,8-dihydrobiopterin (42 nmol/L, normal < 14) and oxidized biopterin (5.7 nmol/L, normal < 2). 5-Hydroxyindoleacetic acid and homovanillic acid were very low: 4 nmol/L (normal 105–299) and 49 nmol/L (normal 211–871), respectively.

Phenylalanine was obtained from Merck, Darmstadt, Germany. Tetrahydrobiopterin (BH₄) was obtained from Dr Schircks Laboratories (Jona, Switzerland).

Phenylalanine was dissolved in orange juice and administered at a dose of 100 mg/kg body weight. A combined loading test with BH₄ (20 mg/kg body weight) was performed 1 h prior (ID229) or 3 h after (ID360) phenylalanine administration.

High-performance liquid chromatography (HPLC) of urinary and CSF pterins was performed as described previously (Curtius et al 1991), except that isocratic separation was performed on a C8 Spherysorb, 5 µm precolumn (4.6 × 40 mm) and ODS-1 Spherysorb, 5 µm analytical column (4.6 × 250 mm) (both from Phase Separation, London, UK), using 1 mmol/L KH₂PO₄ buffer, pH 4.6, with 5% (v/v) methanol at a flow rate of 1.2 ml/min. Urine and CSF samples were oxidized with manganese dioxide at pH 1.0–1.5 prior to HPLC.

Neurotransmitter metabolites in CSF were measured by HPLC with electrochemical detection. Separation was achieved on a YMC Pack-Pro C18 (4.6 × 250 mm) column (YMC, Inc., Wilmington, NC, USA), using a 50 mmol/L sodium phosphate buffer, pH 2.0, containing 5 mmol/L octanesulphonic acid, 0.05 mmol/L EDTA, and 25% (v/v) ethanol as the mobile phase. The flow rate was 1.1 ml/min and the analytical cell (Model 5011, ESA, Bedford, MA, USA) was adjusted to + 0.45 V (ESA Coulochem Model 5100A, ESA) with a response time of 2 s.

Amino acids in plasma were measured using the ion exchange analyser System 6300 (Beckman, Fullerton, CA, USA).

RESULTS AND DISCUSSION

Previously it has been shown that with the oral phenylalanine loading test (100 mg/kg) the time course of change of serum phenylalanine was similar in PKU and in DHPR-deficient patients, and the same phenylalanine profile can be obtained in patients with other forms of BH₄ deficiency (Ponzzone et al 1993a,b). The administration of BH₄ (20 mg/kg) 1 h before or 3 h after the phenylalanine challenge results in unchanged plasma phenylalanine in PKU patients, whereas in patients with BH₄ deficiency it almost completely normalizes within 4–8 h. Thus, a simple phenylalanine loading test can be used to detect disorders of BH₄ metabolism presenting without HPA. In patients with dopa-responsive dystonia, the dominant form of GTP cyclohydrolase I deficiency can be differentiated from the tyrosine hydroxylase deficiency by this test (Hyland et al 1997).

In two patients with the new variant of DHPR deficiency without HPA, plasma phenylalanine concentrations remained elevated up to 6 h after oral administration of phenylalanine (100 mg/kg), indicating impaired hydroxylation in the liver (Figure 1A). A similar plasma phenylalanine profile was obtained in a patient with the dominant form of dopa-responsive dystonia, while in controls phenylalanine normalizes within 6 h after the loading test. Simultaneously, and in contrast to controls, in both patients with the variant of DHPR deficiency as well as in a patient with dopa-responsive dystonia, plasma tyrosine remained almost unchanged during the test (Figure 1B). The ratio of phenylalanine to tyrosine clearly separates patients with defective BH₄ homeostasis from controls (Figure 1C), with peak values 1–2 h after a phenylalanine challenge. Further discrimination between BH₄-deficient patients and controls was obtained by measuring plasma total biopterin. There was only a slow increase in plasma biopterin in both patients with the variant of DHPR deficiency as well as in a patient with dopa-responsive dystonia 1, 2, 4, and 6 h after loading. In controls and in PKU patients, plasma biopterin concentrations usually increase by a factor of 5–6 one hour after phenylalanine loading (Figure 1D). This is thought to be due to activation of GTP cyclohydrolase I by high phenylalanine concentrations via GTP cyclohydrolase I feedback regulatory protein (GFRP). While phenylalanine activates GTP cyclohydrolase I, BH₄ acts as an inhibitor. Therefore, in a patient with the dominant form of dopa-responsive dystonia (GTP cyclohydrolase I deficiency), exogenous phenylalanine cannot stimulate BH₄ production. In DHPR deficiency, however, biosynthesis of BH₄ is intact. Instead, its regeneration is defective, resulting in a high level of 7,8-dihydrobiopterin and of fully oxidized biopterin. It is still not clear why these patients do not accumulate biopterin during a phenylalanine challenge. In order to prove that there is a marginal deficiency of BH₄ in the periphery, one patient (ID360) was loaded with BH₄ (20 mg/kg) 3 h after phenylalanine administration and the second one (ID229) was loaded with the same amount of BH₄ 1 h before the phenylalanine challenge. In both patients, plasma phenylalanine concentrations as well as phenylalanine/tyrosine ratio normalized within a few hours (Figure 1E).

These data demonstrate that the phenylalanine loading test (100 mg/kg) is a useful additional diagnostic tool for detecting secondary disorders of biogenic amine

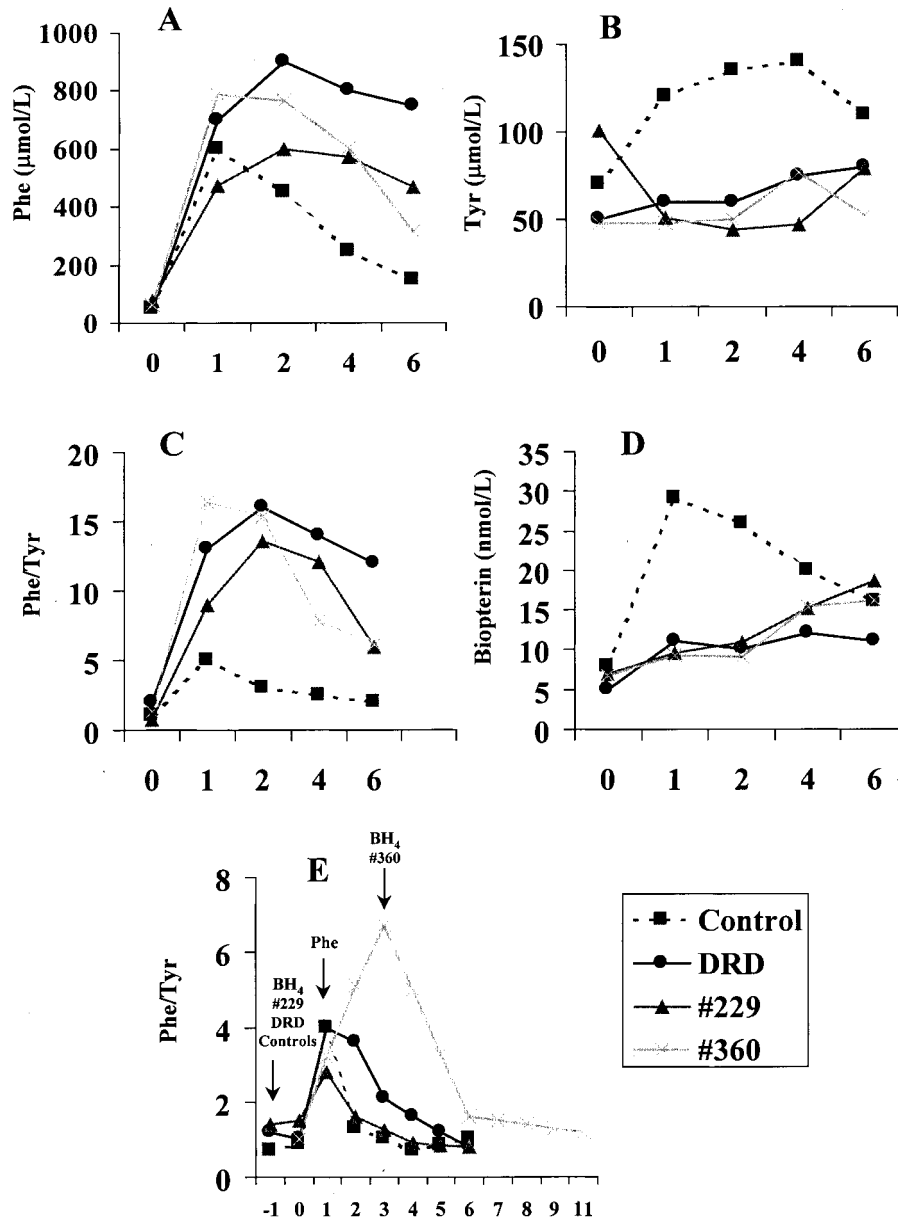


Figure 1 Phenylalanine (A), tyrosine (B), phenylalanine-to-tyrosine ratio (C), and bipterin (D) in response to oral phenylalanine loading (100 mg/kg) in two patients with DHPR deficiency, one patient with GTP cyclohydrolase I deficiency/dopa-responsive dystonia (DRD), and control. (E) Phenylalanine-to-tyrosine ratio before and after the combined BH₄-phenylalanine (ID229) and phenylalanine-BH₄ (ID360) loading test

neurotransmitters caused by defects in BH₄ metabolism. Not only patients with the dominant form of GTP cyclohydrolase I deficiency, as demonstrated previously, and two patients with DHPR deficiency described in this report, but also children with other forms of BH₄ deficiencies may present without hyperphenylalaninaemia. They may not be detected by a newborn phenylketonuria screening and even during selective investigations in urine and plasma later on. Therefore, careful investigation of CSF neurotransmitter metabolites and pterins is essential for the diagnosis.

NOTE ADDED TO PROOF:

DHPR activity in the cultured fibroblasts of one patient (ID229) was significantly reduced (10.4 mU/mg protein; normal 21–55), indicating insufficient regeneration of BH₄.

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