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RESEARCH LETTER

**Robert Steinfeld · Alfried Kohlschütter
Johannes Zschocke · Martin Lindner · Kurt Ullrich
Zoltan Lukacs**

Tetrahydrobiopterin monotherapy for phenylketonuria patients with common mild mutations

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The effect of tetrahydrobiopterin (BH_4) administration was studied in three infants with BH_4 responsive phenylalanine hydroxylase (PAH) deficiency by correlating different oral BH_4 doses with plasma phenylalanine levels under defined protein intake.

Primary hyperphenylalaninaemias are either caused by loss of activity of PAH (EC 1.14.16.1) or by lack of its cofactor (*6R*)-L-*erythro*-5,6,7,8-tetrahydrobiopterin (BH_4). PAH deficiency and disorders of BH_4 metabolism can be differentiated by a BH_4 loading test. Recently, however, several patients with BH_4 responsive PAH deficiency have been described [1, 4, 5, 6].

BH_4 responsiveness was initially demonstrated for our patients 1, 2 and 3, detected on neonatal screening, by oral BH_4 loading tests (data not shown). Cofactor deficiency was excluded by normal urinary pterin concentrations, normal activity of dihydropteridine reductase in erythrocytes and (for patient 2) by normal neurotransmitter concentrations in CSF (data not shown). PAH deficiency was confirmed by finding common PAH gene mutations in all patients: A104D + K320N in patient 1 and Y414C + Y414C in patient 2 suggested a mild PKU phenotype, whereas A403V + A395P in patient 3 suggested a mild hyperphenylalaninaemia phenotype not requiring dietary treatment [2].

Patients 1 and 2 were selected for the investigation of their BH_4 response in detail, to find the optimal BH_4 dose and to explore a possible long-term BH_4 treatment. For this purpose, plasma phenylalanine levels were correlated with different oral BH_4 doses under a protein intake corresponding to 100–150 mg phenylalanine/kg body weight and day (Fig. 1). In patient 1 (Fig. 1A), plasma phenylalanine levels fell remarkably within 12 h after application of 10 mg/kg BH_4. In the case of patient 2, the BH_4 response was slightly different (Fig. 1B) as a daily BH_4 dose of 5 mg/kg body weight was not sufficient to maintain phenylalanine values below 10 mg/dl. Both children with mild PKU (patients 1 and 2) were continued on oral BH_4 supplementation and were fed without protein restriction or special phenylalanine-free formulae. During the past 12 months, plasma phenylalanine concentrations of patients 1 and 2 remained within the desirable range at daily BH_4 doses between 5–10 and 10–20 mg/kg body weight respectively. Both infants have developed normally so far.

We have identified three children with PAH genotypes for which BH_4 responsive hyperphenylalaninaemia has not been previously reported. In addition, we report the first patient (patient 2) with BH_4 sensitivity who is homozygous for one mutation (Y414C). This indicates that PAH heterotetramers as well as PAH homotetramers are compatible with BH_4 responsiveness. Our findings question the concept of decreased cofactor affinity as a valid explanation for BH_4 responsiveness as no defined structural motif responsible for the perturbation of BH_4 affinity can be deduced from published mutations. Surprisingly, different individuals with an identical PAH genotype (R408W/Y414C) have shown divergent BH_4 responsiveness [5]. In fact, two large studies on genotype-phenotype correlation revealed several PAH alleles with inconsistent phenotypes [2,3]. Similarly, homozygosity for the Y414C mutation, the second most common PAH allele in Northern Europe, has not been linked to a BH_4 responsive phenotype before.

Several explanations for these phenomena are possible. Assuming that no enzyme other than PAH is involved in the hydroxylation of phenylalanine to tyrosine, one can consider two effects conferred by a high BH_4

R. Steinfeld · A. Kohlschütter · K. Ullrich · Z. Lukacs (✉)
Department of Pediatrics,
University of Hamburg,
Martinistrasse 52, 20246 Hamburg, Germany
E-mail: lukacs@uke.uni-hamburg.de
Tel.: +49-40-428033735
Fax: +49-40-42806717

J. Zschocke
Institute of Human Genetics,
University of Heidelberg, Heidelberg, Germany

J. Zschocke · M. Lindner
Department of Pediatrics,
University of Heidelberg, Heidelberg, Germany

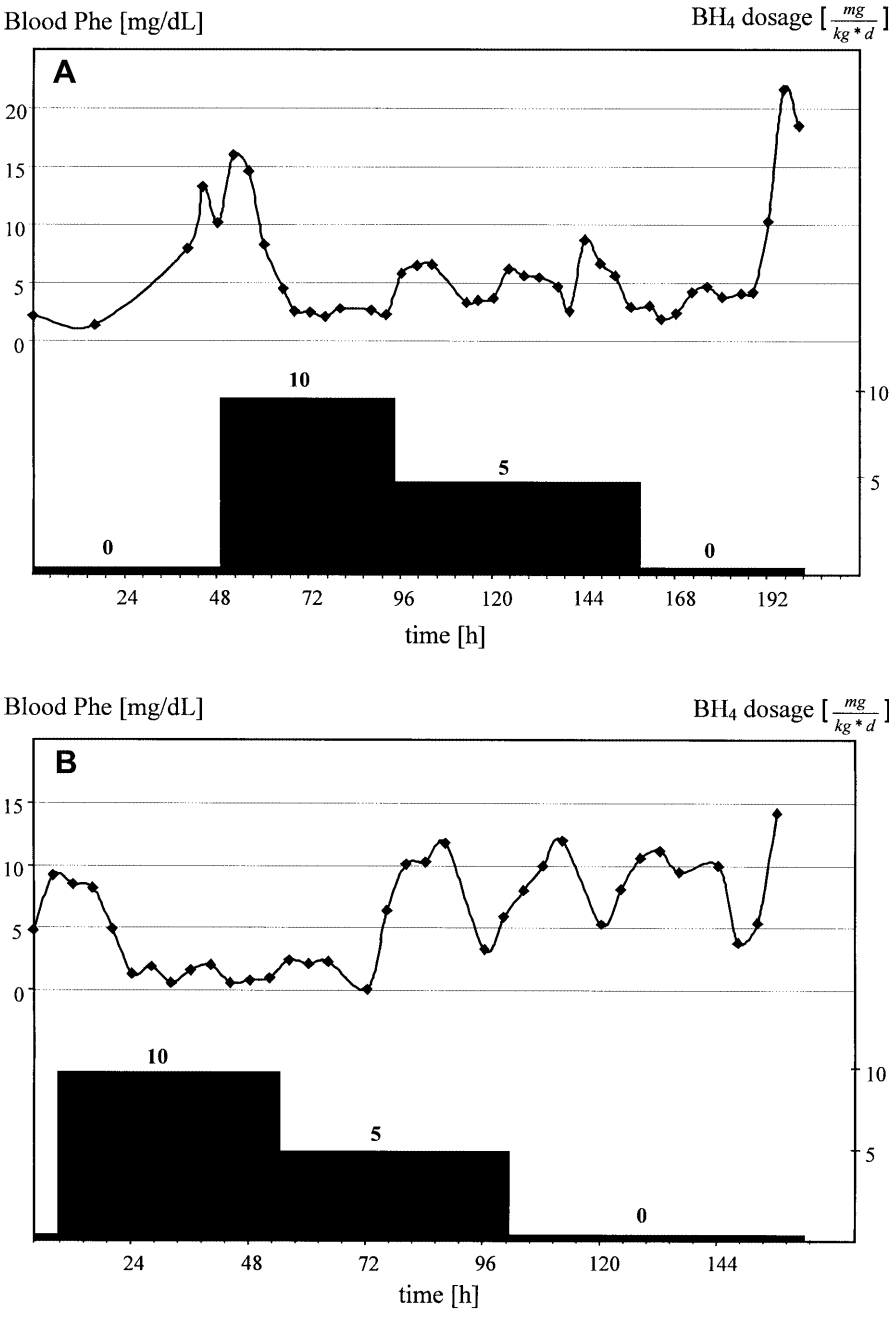

Fig. 1. Response of blood phenylalanine levels to oral BH_4 doses for patients 1 and 2. The daily protein intake was 100–150 mg/kg body weight for both patients. Samples were taken and analysed for phenylalanine content (*filled diamonds*) approximately every 4 h before the next dose of BH_4 was administered. **A** Patient 1 responded well to doses as low as 5 mg/kg per day of BH_4 (*black columns, right-sided scale*). **B** In contrast, patient 2 showed no response to a 5 mg/kg per day dose of BH_4 but had low and stable blood phenylalanine levels at a dose of 10 mg/kg per day

concentration. Either the total amount of PAH could be increased, thereby increasing the absolute amount of active enzyme or the total amount of PAH keeps constant but the overall activity of the enzyme is ameliorated. There is no experimental evidence for a transcriptional or translational increase in PAH synthesis or a direct inhibition of PAH degrading proteases. However, we consider it most likely that interindividual differences in cellular handling of PAH folding mutants will contribute to the observed phenotypic variability and may modulate the responsiveness to BH_4 activation.

Since one of our patients (patient 2) showed a rather moderate response to BH_4, we consider a 24 h phenylalanine determination after the first BH_4 administration helpful to detect slow-responding individuals. As a consequence of our observations, we recommend to determine individually the oral BH_4 dose necessary to maintain the blood phenylalanine in the desired range, as our patients required quite divergent BH_4 doses during treatment, ranging from 5 to 20 mg/kg and day. Our results indicate the feasibility of a BH_4 monotherapy in selected patients with phenylketonuria. Furthermore, evidence from our and previous studies substantiates the role of additional factors like chaperones in the phenotypic expression of genetic diseases.

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