Molecular Genetics and Metabolism 95 (2008) 127–132

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Technical and biochemical factors affecting cerebrospinal fluid 5-MTHF, biopterin and neopterin concentrations

M.M. Verbeek [a,b,*], A.M. Blom [a,c], R.A. Wevers [a,b], A.J. Lagerwerf [a], J. van de Geer [a], M.A.A.P. Willemsen [c]

[a] Department of Laboratory of Pediatrics, Radboud University Nijmegen Medical Centre, Donders Centre for Neuroscience, The Netherlands
[b] Department of Neurology, 830 LKN, Radboud University Nijmegen Medical Centre, Donders Centre for Neuroscience, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands
[c] Department Pediatric Neurology, Radboud University Nijmegen Medical Centre, Donders Centre for Neuroscience, The Netherlands

ARTICLE INFO

Article history:
Received 2 June 2008
Received in revised form 8 July 2008
Accepted 8 July 2008
Available online 22 August 2008

Keywords:
Cerebrospinal fluid
Biopterin
Neopterin
Folate
5-MTHF

ABSTRACT

Background: The diagnosis of pediatric neurologic disorders with a deficiency in the biosynthesis of either the neurotransmitters serotonin and dopamine, or the co-factor tetrahydrobiopterin or a cerebral 5-methyltetrahydrofolate (5-MTHF) deficiency, strongly relies on a robust analysis of neurotransmitter metabolites, pterins and 5-MTHF in the cerebrospinal fluid (CSF). The aim of this study was to investigate which technical and biochemical factors affect the CSF concentration of 5-MTHF, neopterin and biopterin in a pediatric population.

Methods: We studied effects of the ventriculo-spinal gradient, total protein concentration, pretreatment with ascorbic acid (in case of 5-MTHF analysis), pretreatment of CSF with trichloro acetic acid (TCA)/dithiotreitol (DTE) and oxidation with either iodine or manganese oxide (in case of pterin analysis), storage time and age of the patients. We included CSF samples from children until the age of 18 years and analysed 5-MTHF, neopterin, biopterin, homovanillic acid (HVA), 5-hydroxy-indoleacetic acid (5-HIAA) and total protein.

Results: The major findings of our study are: (1) CSF 5-MTHF, neopterin and biopterin concentrations are not affected by the ventriculo-spinal gradient; (2) pretreatment of CSF with ascorbic acid has negligible effects on 5-MTHF concentrations; (3) pretreatment of CSF with TCA/DTE and oxidation with iodine results in the most accurate determination of neopterin and biopterin; (4) when adjusted for age and total protein, CSF 5-MTHF correlated with 5-HIAA, but not with HVA; (5) the reference value of 5-MTHF in CSF in childhood is age-dependent ($r = -0.634$; $p \leqslant 0.001$); (6) we did not observe an age-dependency for neopterin and biopterin in CSF.

Conclusion: 5-MTHF, neopterin and biopterin can be analysed in any volume of CSF that is collected. For correct analysis of pterins, CSF will have to be pretreated to stabilize the concentrations and stored properly, whereas such pretreatment is not necessary for 5-MTHF.

Introduction

Several pediatric neurological disorders are characterized by a decreased production of the neurotransmitters serotonin, dopamine and (nor)epinephrin (Fig. 1). Some of these disorders are caused by a defect in the biosynthesis of these neurotransmitters, e.g. deficiencies in tyrosine hydroxylase (TH) [1] or aromatic aminoacid decarboxylase (AADC) [2]. These disorders are characterized by strongly reduced cerebrospinal fluid (CSF) concentrations of homovanillic acid (HVA) in case of TH deficiency or of HVA and 5-hydroxy-indoleacetic acid (5-HIAA) in case of AADC deficiency. Other disorders are caused by a defect in the biosynthesis of an essential co-factor, tetrahydrobiopterin (BH_4). BH_4 is, amongst others, a co-factor for TH, tryptophan hydroxylase (TPH) and phenylalanine hydroxylase (PAH). A deficiency in BH_4 synthesis leads to reduced activity of these enzymes or the accumulation of inhibitors of the enzymes, such as dihydrobiopterin (BH_2). BH_4 is synthesized from guanosine triphosphate (GTP) by the subsequent action of various enzymes (Fig. 2). Mutations in several of these enzymes (including GTP cyclohydrolase (GTPCH), 6-pyruvoyl-tetrahydropterin synthase (PTPS), sepiapterin reductase (SR) and dihydropteridine reductase (DHPR)) may each lead to an aberrant synthesis of BH_4 and each of these disorders is characterized by a specific pattern of neopterin, biopterin and sepiapterin in the CSF [3].

In the central nervous system, folate is predominantly present as 5-methyltetra-hydrofolate (5-MTHF). A few years ago, a neurological syndrome designated as cerebral folate deficiency (CFD) has been described. CFD is biochemically characterized by low CSF

* Corresponding author. Address: Department of Neurology, 830 LKN, Radboud University Nijmegen Medical Centre, Donders Centre for Neuroscience, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands. Fax: +31 24 3668754.
E-mail address: m.verbeek@cukz.umcn.nl (M.M. Verbeek).

1096-7192/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ymgme.2008.07.004

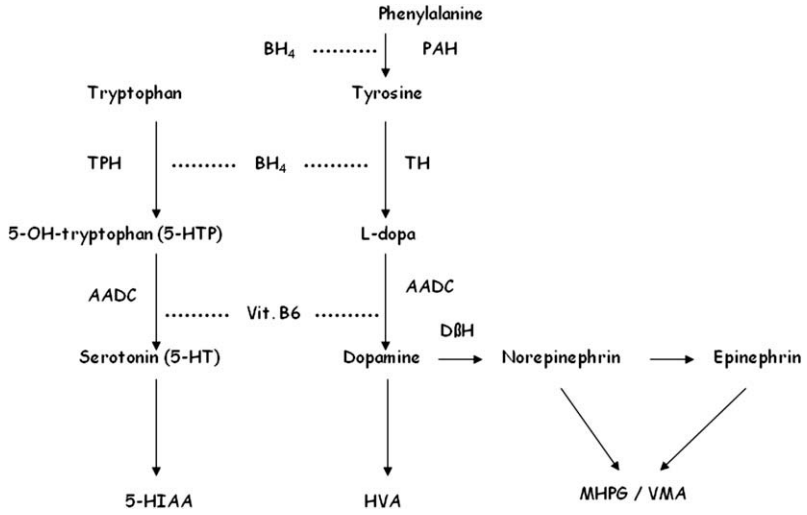

Fig. 1. Overview of the biosynthesis of serotonin and dopamine. *Abbreviations:* TH, tyrosine hydroxylase; TPH, tryptophan hydroxylase; PAH, phenylalanine hydroxylase; AADC, aromatic L-amino acid decarboxylase; DβH, dopamine β hydroxylase; BH_4, tetrahydrobiopterin; vit. B6: vitamin B6; HVA, homovanillic acid, MHPG, 3-methoxy-4-hydroxyphenylglycol; 5-HIAA, 5-hydroxy indole acetic acid; VMA, vanylmandelic acid.

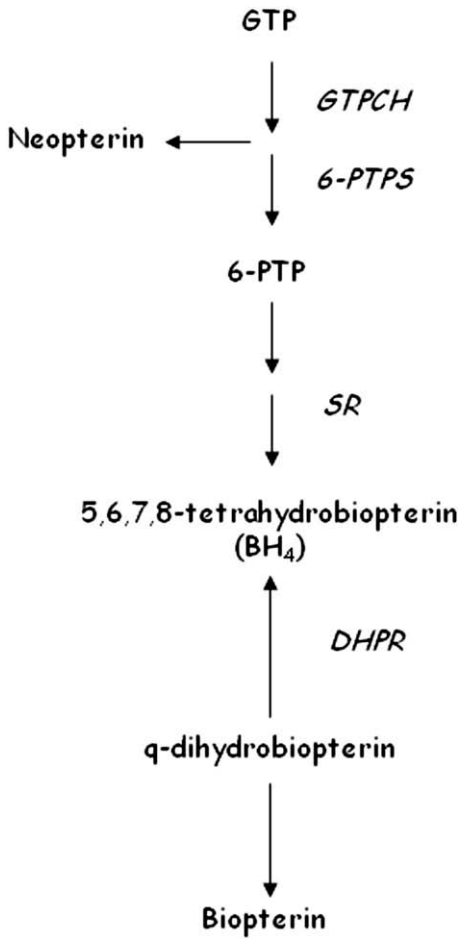

Fig. 2. Overview of the biosynthesis of tetrahydrobiopterin (BH_4). *Abbreviations:* GTP(CH), guanosine triphosphate (cyclohydrolase); 6-PTP(S), 6-pyruvoyl-triphosphate (synthase); SR, sepiapterin reductase; DHPR, dihydropteridine reductase.

5-MTHF concentrations with normal concentrations in plasma [4]. In addition to (primary) CFD, a number of disorders are associated with a secondary decrease in cerebral folate, such as Rett syndrome, Aicardi–Goutières syndrome and Kearns–Sayre syndrome [5–8].

The diagnosis of the above-mentioned neurological disorders have in common that they rely on the analysis of CSF [9]. In general, the analysis of HVA, 5-HIAA, neopterin, biopterin, sepiapterin and 5-MTHF will provide a characteristic biochemical profile that leads to the correct diagnosis. The importance of a correct and robust analysis of CSF is substantiated by the fact that some of these disorders can be very well treated, such as L-dopa treatment in TH, SR and GTPCH deficiencies, and folinic acid treatment in CFD. In a number of previous publications, the technical requirements for a correct analysis of HVA and 5-HIAA have been described, including the need for collection of a standardized volume of CSF and the application of age-dependent reference values [1]. In contrast, however, the pre-analytical and analytical requirements for a robust analysis of neopterin, biopterin and 5-MTHF have not been thoroughly studied.

The aim of this study was to investigate some of the technical and biochemical factors that may affect the CSF concentration of 5-MTHF, neopterin and biopterin, such as sample pretreatment and the effect of the ventriculo-spinal gradient, as well as to define the age-dependency of reference values for these compounds in a pediatric population.

Methods

Patients

This study was approved by the local medical ethical committee. We included CSF samples from children until the age of 18 years, who underwent a lumbar puncture mainly for evaluation of developmental delay, seizures or suspected intracranial infection. Patients did not suffer from an inborn error of folate metabolism, tetrahydrobiopterin biosynthesis, neurotransmitter metabolism or any other known inborn error of metabolism. Lumbar punctures were performed at the Department of Pediatric Neurology of the Radboud University Nijmegen Medical Centre after informed consent was obtained from the patient's legal representative. CSF from all participants was collected in polypropylene tubes, within 30 min transported to the adjacent laboratory at room temperature and centrifuged after routine investigations.