CONFIRMATION OF THE ENZYME DEFECT IN THE FIRST CASE OF ß-UREIDOPROPIONASE DEFICIENCY

ß-alanine deficiency

ANDRE B.P. VAN KUILENBURG*, HENK VAN LENTHE*, GUDRUN GOHLICH RATMANN#, BIRGIT ASSMANN$, GEORGE F. HOFFMANN$, CHRISTA BRAUTIGAM$, AND ALBERT H. VAN GENNIP*

*Academic Medical Center, University of Amsterdam, Emma Children’s Hospital and Department of Clinical Chemistry, Laboratory Genetic Metabolic Diseases, PO Box 22700, 1100 DE Amsterdam, The Netherlands.; #University Hospital Essen, Germany. $University Children’s Hospital Heidelberg, Germany.

1. INTRODUCTION

In man, the pyrimidine bases uracil and thymine are degraded via a three-step pathway leading to the synthesis of ß-alanine and ß-aminoisobutyric acid, respectively. A number of patients have been described with defects of dihydropyrimidine dehydrogenase (DPD) and dihydropyrimidinase, the first two enzymes of the pyrimidine degradation pathway, presenting with a considerable variable clinical phenotype including neurological problems. The third enzyme of the catabolic pathway ß–ureidopropionase (EC 3.5.1.6) catalyses the conversion of N-carbamyl-ß-alanine or N-carbamyl-ß-aminoisobutyric acid into ß-alanine or ß-aminoisobutyric acid, ammonia and CO₂. Very recently, the first patient with the putative defect of ß-ureidopropionase, the third enzyme of the pathway has been reported. Direct measurement of the activity of ß-ureidopropionase has been hampered by the fact that the enzyme is almost exclusively expressed in liver tissue. In this study we report the analysis of all three enzymes of the pyrimidine degradation pathway in a liver biopsy of this patient and provide
unambiguous evidence for a complete deficiency of β-ureidopropionase activity.

2. MATERIALS AND METHODS

2.1 DPD, dihydropyrimidinase and β-ureidopropionase activity

The DPD activity of human liver was determined in a reaction mixture containing 35 mM potassium phosphate (pH 7.4), 2.5 mM MgCl₂, 1 mM dithiothreitol, 2.5 mM NADPH and 40 µM [4-¹⁴C]thymine. Separation of radiolabeled thymine and the radiolabeled reaction products dihydrothymine, N-carbamyl-β-aminoisobutyric acid and β-aminoisobutyric acid was performed isocratically (50 mM NaH₂PO₄ (pH 4.5) at a flow rate of 2 ml/min) by reversed-phase HPLC on an Alltima C18 column (250 x 4.6 mm, 5 µm particle size) with on-line detection of the radioactivity.

The activity of dihydropyrimidinase was determined in an assay mixture containing 100 mM Tris-HCl (pH 8.0), 1 mM dithiothreitol and 500 µM [2-¹⁴C]dihydouracil. Separation of radiolabeled dihydouracil from N-carbamyl-β-alanine was performed isocratically (50 mM NaH₂PO₄ (pH 4.5) at a flow rate of 1 ml/min) by reversed-phase HPLC on a Supelcosil LC-18-S column (250 x 4.6 mm, 5 µm particle size) with on-line detection of radioactivity combined with detection of ¹⁴CO₂ by liquid scintillation counting.

The activity of β-ureidopropionase was determined in a standard assay mixture containing 200 mM Mops (pH 7.4), 1 mM dithiothreitol and 500 µM [¹⁴C]-N-carbamyl-β-alanine. After termination of the reaction by perchloric acid, the reaction product ¹⁴CO₂ was quantitated by liquid scintillation counting.

3. RESULTS AND DISCUSSION

A 17 month old girl presented with muscular hypotonia, dystonic movements, scoliosis, microcephaly and severe developmental delay. Urinary analysis revealed strongly elevated levels of N-carbamyl-β-alanine (691 µmol/mmol creatinine) and N-carbamyl-β-aminoisobutyric acid (572 µmol/mmol creatinine) when compared to the range of N-carbamyl-β-amino acids observed in controls (N-carbamyl-β-alanine, 6-37 µmol/mmol creatinine; N-carbamyl-β-aminoisobutyric acid, 2-9 µmol/mmol creatinine).