Carbamoylphosphate Synthetase Deficiency in an Adult: Deterioration Due to Administration of Valproic Acid

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Summary: A 24-year-old patient had symptoms of lethargy, convulsions and hyperammonaemia during valproic acid therapy. Cessation of valproic acid treatment brought about an improvement both of the symptoms and of the hyperammonaemia. However, enzymatic analysis after the cessation of valproic acid therapy revealed a complete absence of carbamoylphosphate synthetase (CPS) activity in liver biopsy. A unique polypeptide band, corresponding to the control CPS protein in molecular weight ('CPS-like' protein), was found in normal amounts in the patient's liver on sodium dodecyl sulphate–polyacrylamide gel electrophoresis. This CPS-like protein seemed to be more labile than the control, because the polypeptide band became faint after freeze–thawing. Intravenous administration of L-alanine resulted in a significant increase of serum urea and a transient increase of blood ammonia concentrations. These results strongly suggest that the patient has a labile CPS protein with no activity in vitro but some activity in vivo. We consider that valproic acid may have disrupted some metabolic adaptation by reducing N-acetylglutamate in the liver, which in combination with CPS deficiency induced severe hyperammonaemia.

Valproic acid (2-n-propylpentanoic acid) has been used as an anticonvulsant drug with a broad spectrum of activity and relative safety, but some cases of hyperammonaemia not associated with hepatic failure have been reported (Gerber et al 1979; Suchy et al 1979; Ware and Millward-Sadler 1980). It is reported that valproic acid reduces the formation of N-acetylglutamate, which activates carbamoylphosphate synthetase (CPS), the first enzyme of the urea cycle (Coude et al 1983; Kamoun and Rabier 1987; Alonso et al 1989). Valproic acid administration may accelerate the clinical appearance of hyperammonaemia in inherited urea-cycle disorders. So far, three cases of ornithine transcarbamoylase (OTC) deficiency (McKusick 311250, Tripp et al 1981; Hjelm et al 1986; Kay et al 1986) and one case of CPS deficiency (McKusick 237300, Bourrier et al 1988) have been found in valproic acid-induced
hyperammonaemia. This report is the first biochemical demonstration of a patient with valproic acid-induced hyperammonaemia and concomitant abnormal CPS.

MATERIALS AND METHODS

Case report: The patient, a 24-year-old girl, was born in June 1965 after an uncomplicated full-term pregnancy. Delivery was normal and the parents were not consanguineous. She remained asymptomatic with normal growth through the infantile period, but she did not walk until 2 years and 8 months of age. She was mentally retarded with an IQ of 30, but did normal daily tasks by herself. She had tremor in her fingers from the age of 13 years. She received some anticonvulsant drugs, including valproic acid to control the tremor at the age of 21 years (as shown in Figure 1), but the tremor was not effectively controlled. She suffered from general convulsions and a tendency to sleep from that time. Biochemical examinations from April to June in 1988 revealed hyperammonaemia and a low concentration of arginine and citrulline in blood. Her protein intake was 70 g/day from a normal diet. There was no sign of metabolic acidosis. She was suspected of suffering from a urea-cycle disorder and her physician consulted our laboratory.

Clinical analyses: Blood ammonia and urea nitrogen were measured by routine methods. Valproic acid was measured by fluorescence polarization immunoassay (Dainabot Co., Japan) (McGregor et al 1978).

Enzyme studies: Urea-cycle enzyme activities were assayed according to published procedures (Saheki et al 1981). Liver biopsy specimens were stored at −80°C for 2 weeks (first biopsy) and 1 week (second biopsy) before assay. The specimens were disrupted with a Teflon homogenizer in 9 volumes of homogenizing buffer containing 20% (v/v) glycerol, 0.2% (w/v) cetyltrimethylammonium bromide, 2 mmol/L dithiothreitol and 0.02 mol/L Tris-HCl, pH 7.2. For sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE), the liver homogenate was mixed with the equal volume of 4.6% (w/v) SDS, 10% (v/v) 2-mercaptoethanol, 20% (v/v) glycerol and 0.125 mol/L Tris-HCl, pH 6.8. Immediately after mixing, the samples were treated at 95°C for 5 min. The heat-treated samples were subsequently subjected to SDS-PAGE. Liver homogenate was also stored at −20°C overnight. The next morning, thawed homogenates were treated with the same procedure and subjected to SDS-PAGE. Each lane in the SDS-PAGE had the same amount of liver protein (10 μg).

Alanine administration test: Alanine administration test was done according to a published method (Kekomäki et al 1967). The patient, who was not receiving any other drugs at the time, was intravenously administered 5% (w/v) alanine solution (5 mmol/kg body weight) over 150 min on 1 April 1989, after overnight fasting. Blood was taken before, during and after the administration. Blood ammonia was measured by flow injection analysis (Svensson and Anfält 1982), and amino acid analysis was performed with a Hitachi 835 Amino Acid Analyzer (Japan). This experiment was carried out with the agreement of the parents, and ethical approval was given.