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Metabolic changes associated with hyperammonemia in patients with propionic acidemia

Heather R. Filipowicz [a], Sharon L. Ernst [a], Carrie L. Ashurst [a], Marzia Pasquali [b], Nicola Longo [a,b,*]

[a] *Division of Medical Genetics, Department of Pediatrics, University of Utah Health Sciences Center, Salt Lake City, UT 84132, USA*
[b] *Department of Pathology and ARUP Laboratories, University of Utah Health Sciences Center, Salt Lake City, UT 84132, USA*

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Abstract

Propionic acidemia is an autosomal recessive disorder caused by deficiency of propionyl CoA carboxylase. Affected patients can develop severe hyperammonemia, whose causative mechanism is unknown. In this study, we monitored changes in metabolic parameters associated with hyperammonemia in patients with propionic acidemia. Levels of ammonia were correlated with plasma levels of individual amino acids and carnitine and with urinary organic acids. Significance of correlations was determined with analysis of variance. Hyperammonemia positively correlated with an increase in branched-chain amino acids (leucine and isoleucine) and a decrease in glutamine/glutamate and esterified carnitine. The urinary excretion of methylcitric acid, formed by the combination of propionic acid with oxaloacetate from the Krebs cycle, increased while that of citric acid decreased with hyperammonemia. These results suggest that in propionic acidemia, hyperammonemia is triggered by catabolism with the accumulation of propionic acid derivatives. The decrease of the plasma levels of glutamine/glutamate with hyperammonemia in patients with propionic acidemia indicates that the mechanism producing hyperammonemia differs from that in urea cycle defects. The increase in methylcitric acid and decline in citric acid urinary excretion suggest that hyperammonemia in propionic acidemia might be related to inability to maintain adequate levels of glutamine precursors through a dysfunctional Krebs cycle.

Keywords: Propionic acidemia; Hyperammonemia; Glutamine/glutamate; Ketoglutarate; Krebs cycle; Anaplerosis

Introduction

Propionic acidemia is an autosomal recessive disorder caused by deficiency of propionyl CoA carboxylase (EC 6.4.1.3), the enzyme that converts propionyl CoA to methymalonyl CoA with the help of the cofactor biotin [1]. This conversion, which occurs in mitochondria, is part of the pathway for degradation of the amino acids isoleucine, methionine, threonine, and valine, odd chain fatty acids, and cholesterol [1]. Propionic acid also originates from the catabolism of the nucleotides thymine and uracil and from bacterial production of propionate from pyruvate in the gut [1]. Propionyl CoA is eventually converted into succinyl CoA and enters the citric acid (Krebs) cycle for energy production.

Propionyl CoA carboxylase is composed of two distinct subunits: α and β, either of which can be defective in propionic acidemia [1]. As a result of defective propionyl CoA carboxylase, propionyl CoA accumulates and combines with oxaloacetate, another intermediate of the citric cycle, to form methylcitric acid, the diagnostic metabolite of propionic acidemia. Most cases of propionic acidemia present with lethargy progressing to coma from 16 h to weeks after birth, depending on the severity of the enzyme impairment caused by the genetic lesion [1]. Patients can have severe hyperammonemia associated or not with metabolic acidosis [2]. Hyperammonemia is an important contributor to

* Corresponding author. Fax: +1 801 587 7690.
E-mail address: Nicola.Longo@hsc.utah.edu (N. Longo).

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brain damage or death in these children. The mechanism producing hyperammonemia in patients with propionic acidemia and other organic acidemias is debated.

Ammonia accumulation in organic acidemias can be secondary to inhibition of the urea cycle enzyme carbamylphosphate synthase-1 (CPS-1) [1]. CPS-1 combines carbon dioxide with phosphorus (requiring 2 ATP molecules) and a molecule of ammonia to form carbamyl phosphate, the first step of the urea cycle [3]. Levels of *N*-acetylglutamate, an allosteric activator of CPS-1, are reduced in the liver of rats receiving propionic or methylmalonic acid [4]. This could be caused by competitive inhibition of *N*-acetylglutamate synthase, the enzyme responsible for the synthesis of *N*-acetylglutamate, by propionyl CoA [5]. Excess propionyl CoA favors the synthesis of *N*-propionylglutamate that cannot activate CPS-1 [5]. Possible deficiency of *N*-acetylglutamate in propionic acidemia is also suggested by the fact that *N*-carbamylglutamate, a precursor of *N*-acetylglutamate, reduces plasma ammonia levels in patients with propionic or methylmalonic acidemia [6,7].

The increase in plasma ammonia in propionic acidemia, however, differs from that of urea cycle defects. In fact, in urea cycle defects an increase in plasma ammonia is accompanied to an increase in glutamine [3], the amino acid that shuttles nitrogen groups to the liver for ammonia formation and detoxification. By contrast, patients with propionic acidemia had lower plasma levels of glutamine, alanine, and asparagine than patients with urea cycle disorders [2,8,9]. These results suggest that mechanisms other than impairment of a single urea cycle enzyme could cause hyperammonemia in these patients.

Low plasma carnitine levels have been associated with hyperammonemia [10–12]. In fact, carnitine supplementation improves hyperammonemia in patients with low carnitine levels due to treatment with valproic acid, an antiepileptic drug [12]. Further, the transcription of genes encoding urea cycle enzymes is suppressed in a mouse model of primary carnitine deficiency and can be normalized by treatment with carnitine [10].

In this paper, we evaluate changes in plasma amino acids, carnitine, and urine organic acids with hyperammonemia in patients with propionic acidemia. The results presented suggest that defective formation of glutamate/glutamine, rather than a block in the urea cycle, is the likely mechanism associated with hyperammonemia in patients with propionic acidemia.

Materials and methods

Subjects

This study was approved by the Institutional Review Board of the University of Utah (IRB # 00013784). Charts and laboratory data of three patients with propionic acidemia (age 0–6 years) attending the Metabolic Clinic at the University of Utah were retrospectively reviewed. Two of these patients presented before 3 days of life with severe hyperammonemia (up to 1400 μM) requiring dialysis or insulin therapy. The third patient presented at 3 weeks of age with failure to thrive and pancytopenia. He developed hyperammonemia resulting in coma when a feeding tube was placed to correct the failure to thrive. Diagnosis was confirmed in all cases by biochemical studies and enzyme assay on cultured fibroblasts. All patients were following a restricted protein diet (total proteins 2.0–2.7 g proteins/kg/day of which 45–60% deriving from medical foods depleted of propiogenic amino acids) and were taking carnitine supplements (100 mg/kg/day) in addition to their prescribed medical formula. The data reported were obtained either during routine visits or during hospital admissions for fever/dehydration/vomiting/pneumonia. All data available for which an ammonia level had been obtained together or within one hour from at least one other measurement were included in the analysis. Since not all laboratory studies were obtained at all times, the points are slightly different for each class of analytes.

Laboratory measurements

Plasma amino acids were measured by ion exchange chromatography with post-column ninhydrin detection (Biochrom 30 amino acid analyzer) [13]. Urinary organic acids were analyzed by gas chromatography-mass spectrometry (HP/Agilent) [14]. Plasma carnitine levels were determined by spectrophotometry [15]. All lab measurements were completed in our reference laboratory (Biochemical Genetics Laboratory of ARUP Laboratories at the University of Utah) and their reference ranges, established in an age-matched pediatric population, were used for comparison.

Statistical methods

The data collected were de-identified and placed in an Excel database spreadsheet for statistical analysis. Correlation coefficients were obtained by linear regression analysis. The significance of the regressions was established using ANOVA and considered statistically significant for $p < 0.05$.

Results

Changes in individual plasma amino acids with increasing plasma ammonia are shown in Figs. 1–3 and summarized in Table 1. Most amino acids did not change significantly with plasma ammonia (Fig. 1) including arginine (panel A), aspartate (panel B), cystine (panel C), citrulline (panel D), glycine (panel E), histidine (panel F), ornithine (panel G), proline (panel H), threonine (panel I), and valine (panel J). Glycine levels were elevated in all of our patients with propionic acidemia (panel E) but did not change significantly with plasma ammonia. On the other hand, valine levels were below the normal range reflecting dietary restriction. Threonine intake is also restricted in these patients. However, its levels were mostly in the normal range, probably reflecting interconversion from other sources of this non-essential amino acid.

Lysine levels were often above the normal range in patients with propionic acidemia and there was a positive correlation between the levels of plasma ammonia and the levels of lysine (Fig. 2C). Lysine has been shown to increase non-specifically with hyperammonemia [16] including in patients with organic acidemias [17,18]. Levels of isoleucine and methionine were often below the normal range, reflecting their dietary restriction (panels A and D). Hyperammonemia was associated with increased levels of the branched-chain amino acids isoleucine (panel A) and leucine (panel B), methionine (panel D), and the aromatic amino acids phenylalanine (panel E) and tyrosine (panel F). Hyperammonemia is usually triggered by infection