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N-Acetylglutamate Synthetase Deficiency: Clinical and Laboratory Observations

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Summary: Two male siblings presented in the first 6 weeks of life with emesis, diarrhoea, metabolic acidosis and lethargy. A male sibling had previously died at 14 months of age from liver failure of unknown aetiology. Both of the current cases had mild hyperammonaemia with normal orotic acid, organic acid and argininosuccinic acid levels. Citrulline and arginine levels were normal or mildly decreased. One of the brothers was biopsied and had no detectable *N*-acetylglutamate synthetase activity and normal values for other enzymes of the urea cycle in liver. Treatment with a low-protein diet and sodium benzoate/sodium phenylacetate resulted in near normal blood ammonia levels, except during viral illness. Subsequent neurological development has been normal to mildly delayed. These patients differ from those previously decribed with *N*-acetylglutamate synthetase deficiency in that their presentation and subsequent course were relatively benign.

Ammonia detoxification in mammals via the urea cycle is biochemically well characterized and the metabolic consequences of a deficiency of each of the enzymes in the urea cycle have been described in man (Brusilow *et al.*, 1989). Biosynthesis of urea occurs almost exclusively in the liver and requires six enzymatic reactions; the first three are mitochondrial and the remainder occur in the cytosol (Figure 1). The *in vivo* activity of carbamyl phosphate synthetase (CPS), the first step of the urea cycle, is regulated by the concentration of *N*-acetylglutamate, which is produced from acetyl-CoA and glutamate by *N*-acetylglutamate synthetase (NAGS; EC 2.3.1.1). This enzyme has been extensively studied in rodents (Shigesada and Tatibana, 1971, 1972; Uchiyama *et al.*, 1981) and reference values have been established in normal human liver (Colombo *et al.*, 1982).

In 1981, the first case of NAGS deficiency (McKusick 23731) was reported (Bachmann *et al.*, 1981). Owing to a previous history of neonatal death in two siblings, the patient was carefully monitored from birth. Hyperammonaemia (160–240 μmol/L) was noted on the third day prior to onset of clinical symptoms. The plasma amino

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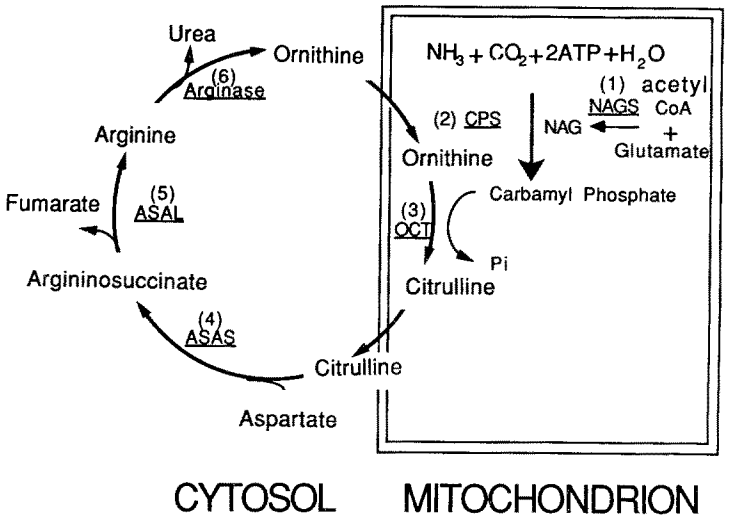

Figure 1 The urea cycle and enzymatic activity found in patient 1 compared with normal controls. Activities are designated by numbers in parentheses with the following abbreviations. (1) *N*-acetylglutamate synthetase (NAGS): not detectable (normal 6–35 nmol min^{-1}(g.w.w.)$^{-1}$ method as by Colombo *et al.*, 1982). (2) Carbamyl phosphate synthetase (CPS): 3.3 μmol min^{-1}(g.w.w.)$^{-1}$ (normal 2.5–6.9, method as by Nuzum *et al.*, 1976). (3) Ornithine carbamyltransferase (OCT): 36.2 μmol min^{-1}(g.w.w.)$^{-1}$ (normal 58–162, method as by McLaren and Ng; 1977). (4) Arginosuccinic acid synthetase (ASAS): 0.69 nmol min^{-1} (mg protein)$^{-1}$ (control 0.99, method as by Su *et al.*, 1981). (5) Arginosuccinic acid lyase (ASAL): 2.05 nmol min^{-1} (mg protein)$^{-1}$ (control 2.06, method as by O'Brien and Barr, 1981). (6) Arginase: 209 nmol (30-min)$^{-1}$ (mg Hb)$^{-1}$ (control 209, method as by Spector *et al.*, 1980).

acid pattern was non-specific and urinary excretion of orotic and organic acids was normal. Enzyme assay revealed no activity of NAGS in a liver biopsy specimen.

Recently a male child with a similar disorder was reported (Bachmann *et al.*, 1988). He presented at 6 days of age with lethargy, hypotonia, poor feeding, emesis and tachypnoea. Ammonia was elevated (711 μmol/L), and orotic and organic acid excretions were normal. Despite aggressive treatment, the child died at 8 days of age. NAGS activity was not detectable in liver at necropsy.

We report two brothers with NAGS deficiency who presented to us in early infancy.

RESULTS

Patient 1: He was born at term to a gravida 2 para 1 mother after an uncomplicated pregnancy, labour and delivery. He weighed 3.2 kg at birth. At 4 days of age he vomited; this was not bilious or bloody. During the first 4 weeks of life he was constipated on a diet containing 90–140 g of regular Enfamil formula every 3 h. At the age of 5 weeks he was hospitalized with diarrhoea, dehydration and lethargy. Laboratory evaluation for sepsis was negative. Initial bicarbonate was 15 mmol/L, which improved with hydration. The discharge diagnosis was viral gastroenteritis.

Four days after discharge, he developed 1–3 episodes per day of left upward lateral conjugate gaze with extension of arms and legs, and tonic-clonic movements of the left arm lasting 2–4 min. On admission he was vigorous, alert and had no focal neurological deficit. There was no evidence of organomegaly.

Family history is positive for petit mal seizures in a maternal aunt and maternal grandfather and for migraine headaches in the mother. The patient's brother died at 14 months of age after developing seizures and liver failure.

White blood cell count was 16 600/mm^3, Hb 12.1 g/100 ml, and blood sugar and calcium were normal. Bicarbonate was 13 mmol/L, lactate 6.1 mmol/L (normal 0.6–2.0 mmol/L) and plasma ammonia was 215 μmol/L (normal 18–47 μmol/L). Plasma amino acid analysis revealed no argininosuccinic acid, low arginine and citrulline and an elevated glutamine. Urinary organic acids and orotic acid excretions were normal on many occasions. An open liver biopsy was performed and sent for light and electron microscopy, which were reported normal. Enzymatic analysis (Figure 1) indicates normal levels of CPS and OCT, but NAGS activity was undetectable. Assay for ASAS and ASAL activities in cultured skin fibroblasts and arginase estimation in erythrocytes were all normal.

Hyperammonaemia decreased on intravenous fluids and remained low on a protein-restricted diet of 0.9 g kg^{-1} day^{-1}. Oral sodium benzoate (50 mg kg^{-1} day^{-1}) was started 1 month later, which maintained ammonia levels between 18 and 30 μmol/L.

At 18 months he has normal growth and development. Neurologically, he has intermittent episodes of disconjugate gaze without associated seizure activity. EEG pattern does not show epileptic foci. Currently he is on Ucephan (sodium benzoate/sodium phenylacetate) 250 mg kg^{-1} day^{-1}) and tolerates 1.5 g protein kg^{-1} day^{-1}. His ammonia levels have been maintained between 24 and 47 μmol/L on the above regimen.

Patient 2: The younger brother of patient 1 was born at 38 weeks gestation by a caesarean section following an uncomplicated pregnancy. Birth weight was 3.1 kg. He was carefully monitored at birth with serial blood ammonia levels. There were no neonatal problems and blood ammonia was normal. A few days after discharge he began emesis and diarrhoea, with a frequency of 5–6 watery, yellowish stools per day. Two days prior to his admission he had 10 liquid stools, was irritable and had an increased respiratory rate. Physical examination revealed a malnourished and dehydrated 26-day-old infant who weighed 2.96 kg ($<$ 5th centile). He was tachypnoeic and lethargic, with poor response to stimuli. Craniofacial examination was unremarkable. There was no evidence of organomegaly. Tone was increased and deep tendon reflexes were normal.

There was no evidence of sepsis. Bicarbonate was low (8 mmol/L) and ammonia elevated (185 μmol/L). Plasma amino acid analysis revealed elevated glutamine and alanine but citrulline and arginine concentrations were normal. An EEG was suggestive of an irritative encephalopathic process.

Treatment included intravenous fluids, sodium benzoate and arginine. Ammonia levels decreased to 28 μmol/L in 2 days, and he was started on oral Ucephan at

250 mg kg^{-1} day^{-1}. Oral feeds were started after 3 days and the protein intake was gradually increased from 0.5 g kg^{-1} day^{-1} to 0.9 g kg^{-1} day^{-1}. The protein was provided through infant formula and commercial essential amino acid supplements.

Currently, he is 6 months old and is below the 5th centile for weight; head circumference is at the 10th centile and his development is at approximately 4 months. Neurological examination is significant for mild appendicular spasticity.

Both brothers have had several viral illnesses, especially involving the gastrointestinal tract, which were well tolerated with mild ammonia elevation (59–71 μmol/L). These episodes were managed by adequate hydration and hospitalization when necessary.

DISCUSSION

Intramitochondrial *N*-acetylglutamine concentration has been shown to play an important role in the short-term control of flux through the urea cycle, under different nutritional and hormonal conditions. *N*-acetylglutamate synthetase is the enzyme required for its synthesis. We report a child with congenital NAGS deficiency, his clinical course, and treatment administered. His younger sibling is also probably affected, though not confirmed by enzyme assay. Two children with a similar enzyme deficiency have been described (Bachmann *et al.*, 1981, 1988).

The clinical presentation of our cases was mild, characterized by emesis, diarrhoea, lethargy and metabolic acidosis. The older brother had focal seizures, without residual neurological deficit. The younger sibling had normal plasma ammonia during the neonatal period, unlike the child described by Bachmann (1988), who had elevated ammonia on day 3 prior to onset of clinical symptoms. In our patients the highest ammonia concentrations (Figure 2) were 215 μmol/L (patient 1) and 185 μmol/L (Patient 2) on initial presentation. Urinary orotic acid excretion, measured during the hyperammonaemic phase and subsequently, was normal. Their clinical presentation and laboratory data indicated a urea-cycle disorder, and thus enzyme assay on liver tissue was pursued in patient 1.

Hyperammonaemia improved with intravenous fluids, and remained low on a diet consisting initially of 0.9 g kg^{-1} day^{-1} protein. Oral sodium benzoate helped in maintaining normal ammonia levels in patient 1. His younger sibling required intravenous sodium benzoate, which was later changed to oral Ucephan. Maintenance therapy with Ucephan has been without untoward effects so far. The use of carbamyl glutamate as advocated previously (Bachmann *et al.*, 1981, 1988) was not employed owing to the good biochemical control in our patients. Bachmann *et al.* (1981, 1988) have described adverse reaction to carbamyl glutamate characterized by sympathomimetic reactions such as tachycardia, profuse sweating, increased bronchial secretions, hyperthermia and persistent crying.

Of the two patients reported in the literature, one died aged 6 days despite aggressive management and the other is slightly retarded, ataxic and spastic. Of the two brothers reported here, the older one is developmentally normal and doing well. The younger brother is mildly spastic and has a developmental level of 4 months at the age of 6 months.

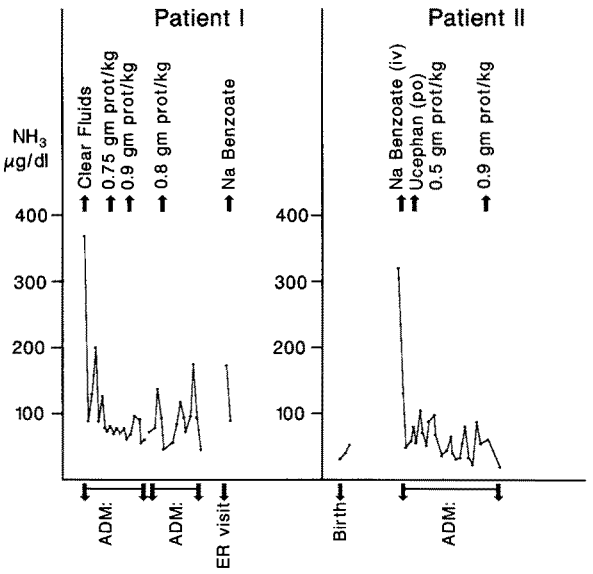

Figure 2 Ammonia levels with time for the brothers patients 1 and 2. ADM indicates admission. Mean features of their treatment/diet are indicated above the relevant times.

Thus to date, four cases of congenital NAGS deficiency have been described, including the two previously reported (Bachmann *et al.*, 1981, 1988). They all presented by the age of 6 weeks with emesis, lethargy, refusal of feeds and hyperammonaemia. The brothers described in this review had significant history of diarrhoea in addition to the above. Their presentation and subsequent clinical course have been relatively mild. Long-term management with Ucephan has maintained plasma ammonia in the range of 18–47 μmol/L and enabled them to tolerate a protein intake between 0.9 and 1.5 g kg^{-1} day^{-1}.

Although there is a paucity of cases, the wide range of variability in presentation and clinical course may reflect marked heterogeneity in this disorder.

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