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Canavan disease and the role of *N*-acetylaspartate in myelin synthesis

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Abstract

Canavan disease (CD) is an autosomal-recessive neurodegenerative disorder caused by inactivation of the enzyme aspartoacylase (ASPA, EC 3.5.1.15) due to mutations. ASPA releases acetate by deacetylation of *N*-acetylaspartate (NAA), a highly abundant amino acid derivative in the central nervous system. CD results in spongiform degeneration of the brain and severe psychomotor retardation, and the affected children usually die by the age of 10. The pathogenesis of CD remains a matter of inquiry. Our hypothesis is that ASPA actively participates in myelin synthesis by providing NAA-derived acetate for acetyl CoA synthesis, which in turn is used for synthesis of the lipid portion of myelin. Consequently, CD results from defective myelin synthesis due to a deficiency in the supply of the NAA-derived acetate. The demonstration of the selective localization of ASPA in oligodendrocytes in the central nervous system (CNS) is consistent with the acetate deficiency hypothesis of CD. We have tested this hypothesis by determining acetate levels and studying myelin lipid synthesis in the ASPA gene knockout model of CD, and the results provided the first direct evidence in support of this hypothesis. Acetate supplementation therapy is proposed as a simple and inexpensive therapeutic approach to this fatal disease, and progress in our preclinical efforts toward this goal is presented.
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Keywords: NAA; Aspartoacylase; Oligodendrocytes; Spongiform degeneration; Acetate hypothesis

Canavan disease was first reported by Myrtelle Canavan in 1931 and was recognized as a distinct disease by Van Bogaert and Betrand in 1949 (Adachi et al., 1973). The clinical symptoms of CD include poor head control, macrocephaly, marked developmental delay, optic atrophy, seizures, hypotonia and death in early childhood (Gascon et al., 1990; Matalon and Michals-Matalon, 2000). Three clinically distinct variants of CD are recognized: (1) the congenital form in which the disease is more severe and is recognizable in the first few weeks of life, (2) the infantile form, the most common form in which the disease is apparent by 6 months of age, and (3) the juvenile form in which the disease manifests only by age 4 or 5 (Adachi et al., 1973). The pathologies associated with CD include cortical and subcortical spongy degeneration, myelin defects, dysmyelination and hypertrophy and hyperplasia of astrocytes (Adachi et al., 1973; Matalon et al., 1995). Ultrastructural studies have demonstrated intramyelinic vacuolation, astrocyte hypertrophy and unusually elongated mitochondria within astrocytes (Adachi et al., 1973). Most of these pathological changes are detectable in the mouse model of CD (Matalon et al., 2000).

Initially, diagnosis of CD was confirmed by brain biopsy demonstrating spongy degeneration of the white matter with vacuoles within myelin sheaths, astrocyte swelling and deformed mitochondria. Biochemical analyses have shown that hypomyelination is a characteristic feature of Canavan disease (Matalon and Michals-Matalon, 2000). Patients with CD are found to excrete 10–100-fold higher amounts of NAA in their urine, and deficiency of the NAA degradative enzyme ASPA was demonstrated in their cultured skin fibroblasts (Hagenfeldt et al., 1987; Matalon et al., 1988). NAA levels are also elevated in the blood and cerebrospinal fluid of CD patients (Hagenfeldt et al., 1987; Hamaguchi et al., 1993; Jakobs et al., 1991), and proton nuclear magnetic resonance spectroscopy (MRS) of CD patients has revealed increased NAA levels in the brain (Barker et al., 1992; Wittsack et al., 1996). However, increased urinary NAA is currently the most reliable method for CD diagnosis, and can distinguish it from other leukodystrophies (Bartalini et al., 1992; Divry and Mathieu, 1989).

Cloning of the human ASPA gene has enabled molecular genetic studies of CD (Kaul et al., 1993; Namboodiri et al., 2000). Two mutations were found to be prevalent among Ashkenazi Jewish patients with CD (Kaul et al., 1994). A missense

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mutation in codon 285 causing substitution of glutamic acid to alanine accounts for 83.6% mutations identified in 104 alleles from 52 unrelated Ashkenazi Jewish patients. A nonsense mutation on codon 231, which converts tyrosine to a stop codon, was found in13.4% of the alleles from Jewish patients. Among non-Jewish patients, the mutations are different and more diverse (Kaul et al., 1996; Sistermans et al., 2000). The most common is in codon 305, a missense mutation substituting alanine for glutamic acid. This mutation was observed in 35.7% of the 70 alleles from 35 unrelated non-Jewish patients (Kaul et al., 1996). Fifteen other mutations were detected in 24 other CD patients. More recently, additional mutations, some with the children dying immediately after birth, have been reported (Zeng et al., 2002). The diverse mutations associated with CD limit the use of DNA analysis for prenatal diagnosis to couples who are both carriers, and whose mutations are among those that are known.

The pathogenesis of CD remains an unresolved issue. Our lack of understanding of the role of NAA in nervous system development and function is the primary reason for the uncertainty. NAA is synthesized by acetylation of aspartate by acetyl CoA. Aspartate *N*-acetyltransferase (Asp-NAT), the enzyme that catalyzes this reaction, is highly specific to aspartate, and is found only in the nervous system (Madhavarao et al., 2003; Truckenmiller et al., 1985). In contrast, ASPA, which degrades NAA to aspartate and acetate with high specificity, has a ubiquitous distribution (Kaul et al., 1991). In the brain, ASPA is distributed almost exclusively in oligodendrocytes (Kirmani et al., 2002; Madhavarao et al., 2004), but NAA is distributed primarily in neurons (Moffett and Namboodiri, 1995; Moffett et al., 1991). This suggests that export and uptake mechanisms exist in neurons and oligodendrocytes, respectively, which permit the transfer of NAA from the site of synthesis to the site of degradation.

1. NAA is an acetate carrier during CNS development

The connections between ASPA gene mutations and the loss of deacetylase activity are both well established in CD, and yet the specific connection between ASPA deficiency and the failure of proper CNS development and axonal myelination remains unclear (Matalon et al., 1995). In addition, the precise roles that NAA plays in the development of the CNS, and its proper functioning, remain a matter of study.

There are several hypotheses proposed to explain the role of NAA in the CNS. One of them states that NAA serves as a 'molecular water pump' to remove the metabolic water from neuronal mitochondria and neurons. This hypothesis draws strength from the fact that CD patients, who possess several-fold higher NAA than normal individuals, suffer from brain edema (Baslow, 2003). Scant experimental evidence supports an osmolyte function for NAA. Transport systems have not been demonstrated in neurons, and experimentally-induced NAA increases have not been shown to induce brain edema.

A recent report has shown that NAA is not toxic even at high concentrations (Tranberg et al., 2004). Three reports on gene therapy are available: in CD mice (Matalon et al., 2003) and in "tremor" rats (Klugmann et al., 2005; McPhee et al., 2005). No functional improvement was reported in CD mice although a decreased NAA level was reported consistent with the expression of introduced normal ASPA gene. Of the two reports on "tremor" rats, the most extensive study involving restoration of ASPA in CNS neurons demonstrated a lack of improvement in motor deficits and demyelination, although the seizure phenotype was rescued to some extent (Klugmann et al., 2005). Therefore, further research is required to definitely determine whether or not increased NAA plays a role in some component of CD pathogenesis.

One theory on the role of NAA for which there is mounting evidence is that NAA is essential for lipid synthesis and myelination in the CNS, especially during the period of peak, postnatal myelination that occurs in the CNS. In 1987, Hagenfeldt and colleagues proposed that the dismyelination observed in the brains of Canavan disease patients was due to the failure of NAA to act as an acetate carrier from mitochondria to the cytosol, thus impairing lipogenesis (Hagenfeldt et al., 1987). Some of the evidences for this view are detailed below.

1.1. Incorporation studies

The first report showing that NAA provides acetate groups for lipid synthesis during myelination was published by D'Adamo and Yatsu (1966). In a subsequent study, D'Adamo et al. (1968) showed that injection of acetyl labeled NAA into rats of various ages resulted in maximum incorporation into fatty acids just before and during myelination. Since that time, these observations have been confirmed and extended by at least three other research groups. In 1991, Burri et al. (1991) performed a detailed study on incorporation of acetyl labeled NAA into various lipids in the rat brain, and showed that NAA is a major source of acetyl groups for lipid synthesis during rat brain development. In similar incorporation studies using rat brain slices, free acetate and acetyl CoA were shown to be formed from the radiolabeled NAA under this condition, indicating acetyl CoA route for this NAA-lipid pathway (Mehta and Namboodiri, 1995). Finally, intra ocular injection studies have shown that neuronally-derived NAA supplies acetyl groups for myelin lipid synthesis in the optic nerve (Chakraborty et al., 2001). Taken together, these results provided strong evidence for the involvement of NAA-derived acetyl groups for lipid synthesis during myelination. However, the quantitative significance of this acetate source for myelin synthesis remained unclear until recently.

1.2. Cellular localization studies of ASPA

Early studies on the enzyme activity of ASPA had indicated that ASPA is enriched in the white matter in the CNS (D'Adamo et al., 1977). Two recent studies have reported cellular localization of ASPA using polyclonal antibodies against recombinant ASPA (Klugmann et al., 2003; Madhavarao et al., 2004). Both studies showed predominant localization of ASPA in white matter. The studies reported by our laboratory (Madhavarao et al., 2004) are detailed here. The polyclonal antibody preparation against ASPA showed a single band at 37 kD in Western blots

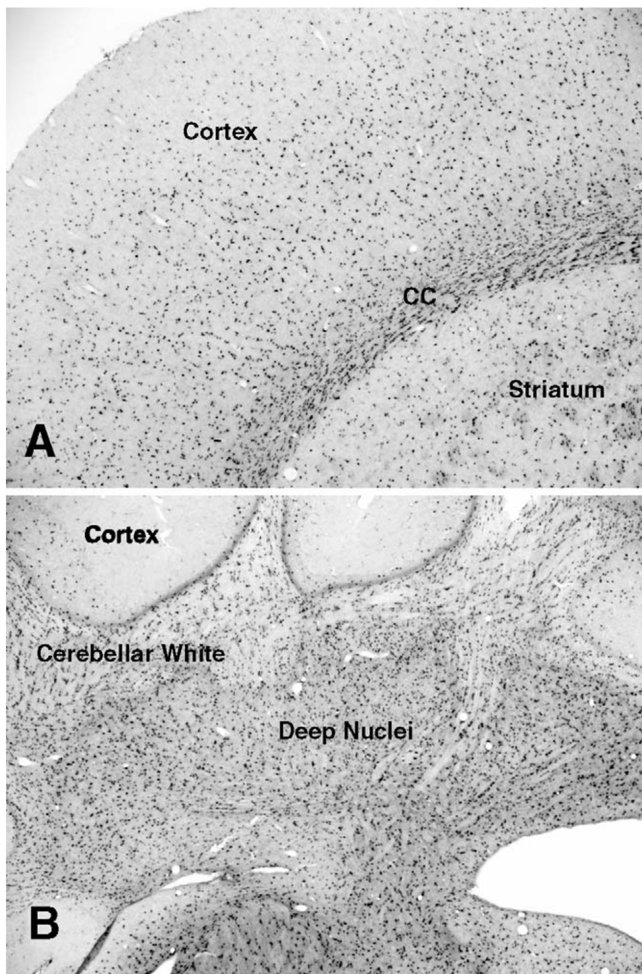

Fig. 1. ASPA immunoreactivity in the rat forebrain (A) and hindbrain (B). In forebrain, ASPA was present in oligodendrocytes throughout the corpus callosum (CC), and in fiber bundles in the striatum. Fewer ASPA-positive oligodendrocytes were observed in the superficial layers of cortex. In the hindbrain, ASPA-immunoreactive oligodendrocytes were present in great numbers throughout the cerebellar deep nuclei, white matter, and medulla, but were very sparse in the cerebellar cortex.

of brain homogenates. Immunohistochemical studies with the antibodies showed that ASPA is localized in the CNS predominantly in apparent oligodendrocytes (Fig. 1), and was found to be co-localized throughout the brain with CC1, a marker for oligodendrocytes. Many ASPA-positive cells were observed in white matter, including cells in the corpus callosum and cerebellar white matter. Relatively, fewer cells were labeled in gray matter, particularly in the superficial layers of cerebral cortex. ASPA staining was restricted to the cell body and proximal processes of oligodendrocytes. Interestingly, the ASPA antibodies labeled not only the cell bodies and proximal processes of oligodendrocytes, but also labeled their cell nuclei, indicating that ASPA is not restricted to the cytoplasm of these cells (Fig. 2). No astrocytes were labeled for ASPA, and neurons were unstained in the forebrain, although a small number of reticular and motor neurons were faintly to moderately stained in the brain stem and spinal cord. Finally, it should be mentioned that microglial cells were faintly stained throughout the brain.

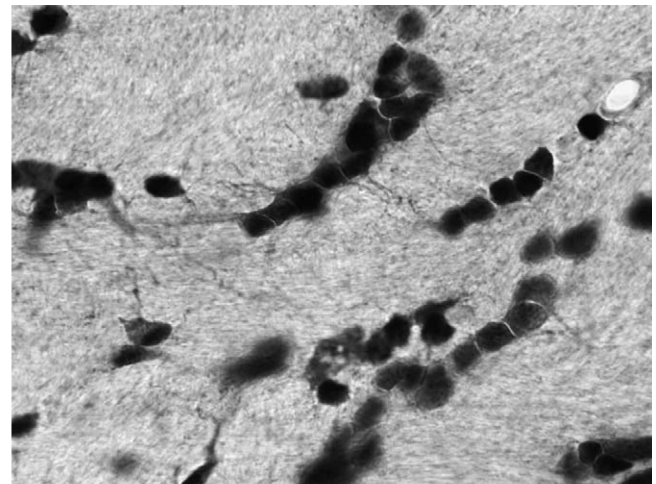

Fig. 2. ASPA immunoreactivity in the rat corpus callosum. Strongly stained oligodendrocytes are arranged in rows between myelinated axons, with immunoreactive processes visible on many of the stained cells. An unusual finding was that ASPA was present not only in the cytoplasm of oligodendrocytes, but was also strongly expressed in their nuclei.

The extensive localization of ASPA in oligodendrocytes strengthens the case for the NAA/ASPA system being required for myelination during development. However, it also raised additional questions because of the prominent localization of the substrate NAA in neurons.

1.3. Developmental increases in ASPA

Another line of evidence indicating a connection between ASPA and myelination is the parallelism between the developmental increases in ASPA activity and myelination, which was first studied by D'Adamo's group (D'Adamo et al., 1973). They showed that the increase of ASPA activity in the rat brain with development closely paralleled the pattern of myelination. In 2001, Bhakoo et al. (2001) performed a more detailed developmental study covering different rat brain regions, and have confirmed the similarity between the developmental increases in ASPA enzyme activity and myelination. More recently, we used in situ hybridization to study ASPA gene expression in the rat brain during development using a riboprobe based on a 400-bp cDNA fragment of murine ASPA (Kirmani et al., 2003). These studies also confirmed a temporal correlation between the developmental increase in ASPA and myelination.

2. Recent findings from ASPA (−/−) mice

Reports on incorporation of the acetyl moiety of NAA into acetyl CoA and lipids during myelination, and other evidence that ASPA is associated with myelination did not shed light on the relative importance of this pathway for myelination. It was not clear if this was a salvage pathway for lipid synthesis, or a primary pathway. In most cell types, such as hepatocytes, the enzyme ATP-citrate lyase provides the acetyl groups for fatty acid synthesis. The extent of contribution of NAA–ASPA system to myelin lipid synthesis was recently studied in the context of

the pathogenesis of CD by examining the rate of myelin lipid synthesis in ASPA knockout (*ASPA* −/−) mice (Madhavarao et al., 2005).

2.1. Deficiency of myelin lipid synthesis in CD mice

Comparison of the wild type (WT; *ASPA* +/+) and homozygous knockout (KO; *ASPA* −/−) mice showed that the two groups differed in only two measures; brain weight and ASPA activity. The brain weight of the KO mice was significantly greater than the WT (461 ± 17.8 mg versus 416 ± 22.3 mg, respectively; $p < 0.05$). In addition, ASPA activity was undetectable in KO mice, as compared with ASPA activity of 18.3 ± 5.6 nmol/h/mg protein in WT mice ($p < 0.001$).

Tritiated water incorporation was demonstrated to be a reliable method for determining the rate of lipid synthesis (Jungas, 1968), cholesterol synthesis (Dietschy and Spady, 1984) and myelin synthesis (Muse et al., 2001) and was adopted in our studies on ASPA KO and WT mice. The incorporation of tritium into lipids from liver, kidney and myelin was determined 5 h after intraperitoneal administration of 20 mCi $[^3H]_2O$ per animal. The specific activity of $[^3H]_2O$ in the serum samples from the WT and KO mice did not differ significantly ($p < 0.05$). Difference between the two groups with respect to lipids synthesized in liver was not significant, whereas total myelin lipids were decreased in KO mice by ∼30% ($p < 0.005$). Surprisingly, total lipid incorporation in kidney was increased by ∼18% in the KO group (Madhavarao et al., 2005).

Two-dimensional thin layer chromatography (2D-TLC) analyses of the myelin lipids showed that syntheses of both polar and non-polar lipid groups were affected in KO mice. Among non-polar lipids separated on the first dimension, significant decreases were noticed in KO mice samples in the spots corresponding to glycerol 1-fatty acids (decreased by ∼35%), cholesterol, (decreased by ∼22%), cholesterol fatty acids, (decreased by ∼35%), and glycerol tri fatty acids (trimyristin, tripalmitin, trilaurin, tristearin: decreased by ∼21%). Interestingly, glycerol 1,2 fatty acids (dimyristin, dipalmitin, dilaurin and distearin) did not show statistically significant change.

Polar lipids, separated on the second dimension, also showed significant reductions in the KO mice in the two lipid spots. One corresponded to phospholipids and sulfatides that was decreased by ∼38% (R_f 0.75; phosphatidylinositol, phosphatidyl choline, phosphatidyl glycerol, phosphatidic acid and cerebroside sulfate) and the second corresponded to R_f of 0.88 (phosphatidyl ethanolamine, galactocerebroside and hydroxy fatty acid ceramide), which was decreased by ∼35% (Madhavarao et al., 2005).

These data reveal that lipid synthesis in the brain, in part, requires an intact ASPA enzyme, providing the first direct evidence for deficiency of NAA-derived acetate as an etiological mechanism of CD. While earlier studies showed that acetate from neuronally-derived NAA is incorporated into myelin lipids in the CNS, the studies on ASPA KO mice established that the contribution of acetate from NAA for myelin synthesis is quantitatively sufficient to decrease myelin synthesis (Madhavarao et al., 2005).

2.2. Acetate deficiency in CD mice

Few metabolic pathways other than ASPA are known that can generate free acetate in mammals, and most serum acetate is thought to be derived from bacterial metabolism in the gut (Bach and Metais, 1970). We had proposed previously that NAA is a major source of acetate in the brain (Mehta and Namboodiri, 1995), and in order to confirm this we determined free acetate levels in the brains of KO mice using an enzymatic assay. The results showed that there was nearly an 80% reduction in free acetate levels in the brains of KO mice as compared with WT, but that the levels in kidney and liver were not reduced relative to WT (Madhavarao et al., 2005). The acetate data endorses the myelin lipid data that NAA is a major source of free acetate used in the myelin lipid synthesis in the brain via ASPA-mediated catalysis. A key question concerning the etiological role of reduced NAA-derived acetate in CD is the concentration of acetate in oligodendrocytes during the period of intense postnatal axonal myelination. Although the acetate levels reported pertain to whole brain homogenates of the KO mice, the predominant localization of ASPA in oligodendrocytes lends enough credence to infer that the acetate levels in the oligodendrocytes were substantially reduced in the KO animals.

2.3. A comprehensive model for NAA and CD pathogenesis

NAA and ASPA are expressed in different cellular compartments in the brain, with NAA being present predominantly in neurons and ASPA being expressed predominantly in oligodendrocytes. This differential expression pattern has been proposed as a mechanism for channeling NAA-associated acetate from neurons to oligodendrocytes (Chakraborty et al., 2001). Based on such data, and results of ongoing studies in our laboratory (Madhavarao et al., 2003), we have proposed an expanded model of NAA metabolism wherein NAA has multiple roles in the nervous system (Fig. 3) (Madhavarao et al., 2005). In neuronal mitochondria, the NAA biosynthetic enzyme Asp-NAT acts to remove excess aspartate from the matrix via acetylation, which would favor α-ketoglutarate formation from glutamate, and energy production via the citric acid cycle. Thus, the aspartate aminotransferase pathway in neuronal mitochondria aids in the production of ATP by facilitating the oxidation of glutamate (Madhavarao et al., 2003). The high steady-state levels of NAA in neuronal mitochondria reflect their metabolic condition because of the direct coupling of NAA production to α-ketoglutarate formation from glutamate. By preferentially using the aspartate aminotransferase reaction instead of the glutamate dehydrogenase reaction to generate α-ketoglutarate, neuronal mitochondria would prevent ammonia production associated with the latter reaction, and this might avoid additional metabolic stress on neurons. In this model, NAA synthesis is intimately associated with the proper functioning of neuronal energy metabolism via the aspartate aminotransferase reaction in neuronal mitochondria.

In its role as an acetate carrier, the NAA synthesized in neuronal mitochondria is transferred to oligodendrocyte cytosol by an as yet unknown mechanism, where ASPA liberates the acetate

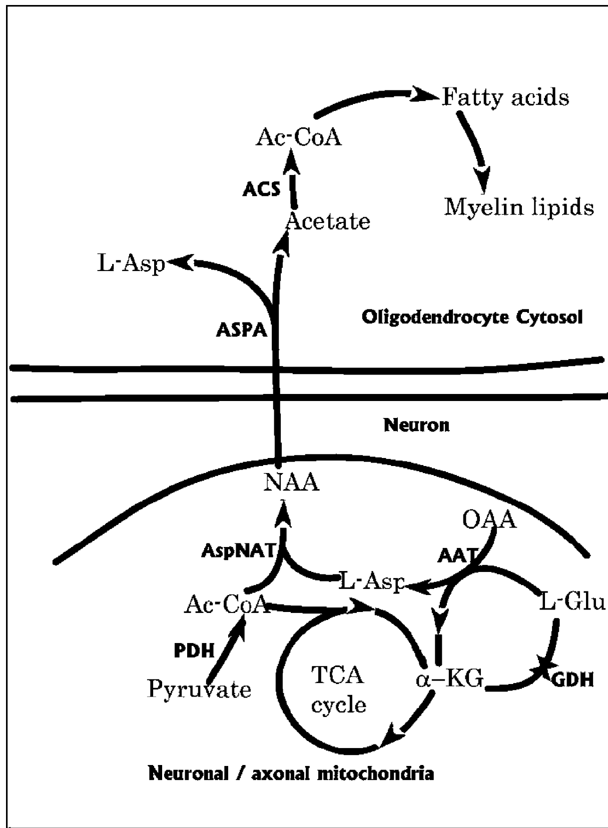

Fig. 3. Diagrammatic representation of NAA synthesis in neurons and degradation in oligodendrocytes. See text for details. OAA = oxaloacetic acid, AAT = aspartate aminotransferase, Asp-NAT = aspartate *N*-acetyltransferase, PDH = pyruvate dehydrogenase, ASPA = aspartoacylase, ACS = acetyl CoA synthetase, α-KG = alphaketoglutarate, GDH = glutamate dehydrogenase.

moiety. The free acetate is converted to acetyl CoA by acetyl CoA synthetase, which is used in myelin lipid synthesis. This hypothesis emphasizes the coupling of neuronal metabolism to oligodendrocyte myelin lipid synthesis, where axons provide a major biochemical precursor for the increased lipid synthesis required for myelination during early postnatal CNS development. Whether the excess aspartate that is liberated from NAA is utilized extensively in oligodendrocytes for metabolism and protein synthesis, or is instead recycled in bulk back to neurons for a new cycle of NAA synthesis, requires further study.

3. Preclinical efforts toward acetate supplementation therapy for CD

In view of the evidence presented above that brain acetate levels and myelin lipid synthesis are both significantly reduced in CD mice, then it follows that correcting the acetate deficit by acetate supplementation could provide a therapeutic approach for treating the dysmyelination in CD. In our preclinical efforts toward such a therapy for CD, we are currently examining glyceryl triacetate (GTA; Triacetin) and calcium acetate as potential exogenous acetate sources for delivering acetate to the brain. Glyceryl triacetate is a non-toxic glyceryl tri-ester of acetic acid that is used in external medicine and as a food additive, among other uses. Biochemical studies on glyceryl triacetate have shown that it is hydrolyzed in vivo by all tissues of mammals including the gastrointestinal tract (Bach and Metais, 1970). Calcium acetate is currently a Food and Drug Administration approved drug for the treatment of kidney disease to control high blood phosphate levels (Schiller et al., 1989).

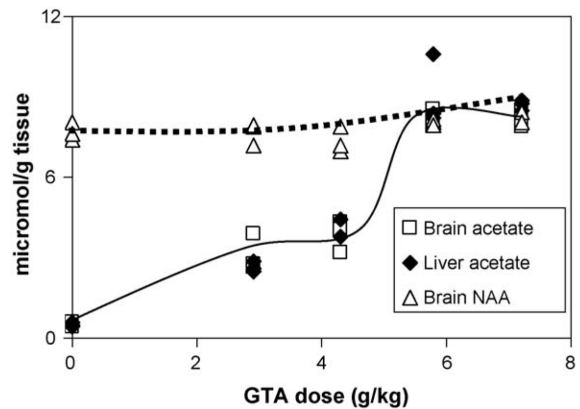

Fig. 4. Brain and liver acetate levels, and brain NAA levels 1 h after GTA administration in 20–21 day old mice of ∼8 g body weight. Values are mean ± S.D. of samples from three to five animals per group.

3.1. GTA increases brain acetate without affecting NAA levels

GTA was purchased from Sigma and was used without purification. It was administered to the mice intragastrically using a syringe/needle system that is used specially for intragastric administration. Fig. 4 shows that free acetate level in the brain increased by six-fold at a dose of 2.9 g/kg body weight of GTA and was above 10-fold on a doubling of GTA dose, but did not increase further at a higher dose of GTA (7.2 g/kg body weight). The liver acetate pattern was similar. Interestingly, the NAA levels in brain did not change significantly even at the highest concentration of administered GTA. This could be due to the saturation of the rate of NAA synthesis by the aspartate *N*-acetyltransferase, the biosynthetic enzyme of NAA at lower Acetyl CoA concentrations, due to its low (0.05 mM) K_m for acetyl CoA (Madhavarao et al., 2003).

Changes in the acetate levels monitored with time gave interesting results (Fig. 5). Free acetate levels in both liver and brain dramatically increased by the 1 h after GTA feeding but decreased at a lower rate with time. Brain acetate levels in treated animals remained higher than the control animals up to 8–12 h. However, NAA levels in the brain remained unchanged through the 12 h time course following GTA administration.

3.2. Comparison of GTA and calcium acetate

When equimolar concentrations of GTA and calcium acetate were administered intragastrically, acetate levels in the brain and liver were higher in GTA fed mice (Fig. 6). This may reflect the ability of GTA to penetrate cell membranes readily due to its hydrophobic nature.

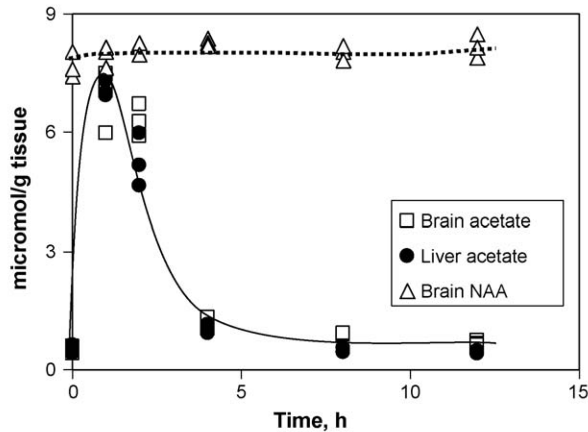

Fig. 5. Changes in the acetate and NAA levels with time following GTA (5.8 g/kg animal) administration in 20–21 day old mice. Animals were sacrificed at specific intervals following GTA feeding. Values are mean ± S.D. of samples from three to five animals per group.

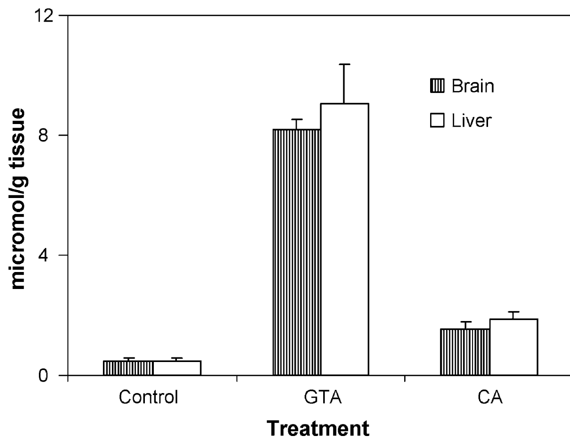

Fig. 6. Comparison of acetate levels for equimolar (26.5 mmol/kg) GTA and calcium acetate (CA) administration in 20–21 day old mice. Values (means ± S.D. of samples from three to five animals per group) represent 1 h after feeding. GTA administration increased brain and liver acetate levels significantly more than calcium acetate administration ($p < 0.01$; by Student's *t*-test).

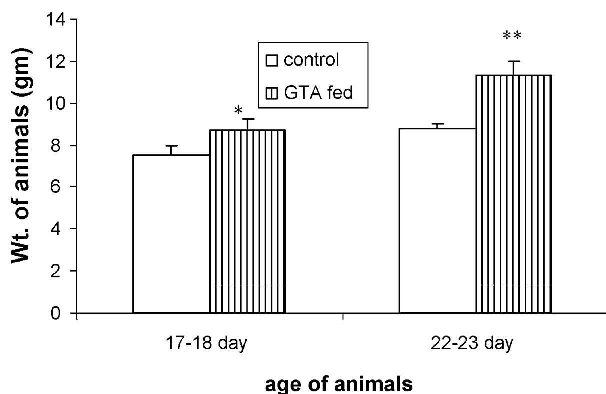

Fig. 7. Effect of GTA oral administration (7th day after birth to 23rd day after birth) on the body weight on mice. Values (mean ± S.D.) were from five animals per group. $^{*}p < 0.01$ and $^{**}p < 0.001$.

3.3. Chronic GTA administration does not cause significant toxicity

In a group of five 7-day old mice which received GTA twice a day orally for 18 days (2.9 g/kg animal weight until day 15, and after that 5.8 g/kg), a slight increase in their weight was apparent compared to their control group (Fig. 7) and they survived the experimental period in good health. They had normal appearance and behavior throughout and no diarrhea was observed. At termination, their plasma was clear, and the animals did not develop fatty livers. Autopsies revealed normal appearing stomach and intestine. The gain in weight could be attributed to the increased caloric intake (Mathew et al., 2005).

4. Final comments

Presently it remains a matter of controversy what pathological mechanisms are primary in CD, and which are less critical. It seems clear now that an acetate deficiency caused by dysfunction in a specific enzyme in oligodendrocytes has an etiological role in CD. It is also possible that osmotic dysregulation in the CNS is mediated by excessive extracellular NAA concentrations, and that high NAA concentrations lead to seizures, which further contribute to the pathogenesis. However, we should not neglect the fact that ASPA is extensively expressed in the kidney, and therefore it is possible that renal pathologies could also contribute to disease progression by as yet undetermined mechanisms. It is also not clear whether or not the primary substrate for ASPA in the kidney is NAA, because NAA levels there are very low. It is possible that ASPA may have additional catalytic functions not yet reported, other than deacetylation of NAA. If the function of ASPA in the kidney and other peripheral tissues is unrelated to deacetylation of NAA, which seems likely since NAA concentrations are too low in those tissues, then other approaches may need to be developed to deal with the pathological consequences of mutations to ASPA gene and loss of activity in those tissues.

One approach to testing if an acetate deficiency is a primary etiological mechanism of CD would be to determine whether myelin synthesis can be promoted in newborn CD infants by increasing brain acetate levels through dietary supplementation. CD pathogenesis develops predominantly after birth, and therefore this approach should be feasible. An orally administered form of acetate in a supplemented infant formula could provide the required substrate for the rapid myelination that takes place during early, postnatal neural development. Early diagnosis of CD by means of urinalysis to detect high NAA levels could be followed by immediate acetate supplementation of the diet. Our laboratory is currently working to test this hypothesis in *ASPA* −/− mice and the Tremor rat model of CD.

In conclusion, the findings presented above provide direct support for the proposed etiology of CD as a deficiency of NAA/ASPA-derived acetate, resulting in reduced lipid synthesis, and a failure of proper myelination during CNS development. The increase seen in the acetate level in liver and brain tissues in response to GTA administration would reflect the intracellular concentration of the respective tissues rather than the

increase in acetate concentration of the blood/serum per se for two reasons. First, GTA is a hydrophobic precursor of acetate and can readily be transported across the hydrophobic barriers and hence has a very good chance of crossing the blood brain and other cellular barriers. Second, we have found that at lower doses of GTA, the liver levels are considerably higher than that in the brain. Based upon these and other findings, early postnatal acetate supplementation trials in confirmed cases of CD are warranted.

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