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Canavan Disease

Analysis of the Nature of the Metabolic Lesions Responsible for Development of the Observed Clinical Symptoms

Morris H. Baslow* and Tracy R. Resnik

Nathan S. Kline Institute for Psychiatric Research, Center for Neurochemistry, Orangeburg, NY 10962

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Abstract

Canavan disease (CD), a rare recessive autosomal genetic disorder, is characterized by early onset and a progressive spongy degeneration of the brain involving loss of the axon's myelin sheath. After a relatively normal birth, homozygous individuals generally develop clinical symptoms within months, and usually die within several years of the onset of the disease. A biochemical defect associated with this disease results in reduced activity of the enzyme *N*-acetyl-L-aspartate amidohydrolase (aspartoacylase) and affected individuals have less ability to hydrolyze *N*-acetyl-L-aspartate (NAA) in brain and other tissues. As a result of aspartoacylase deficiency, NAA builds up in extracellular fluids (ECF) and is excreted in urine. From an analysis of the NAA biochemical cycle in various tissues of many vertebrate species, evidence is presented that there may be two distinct NAA circulation patterns related to aspartoacylase activity. These include near-field circulations in the brain and the eye, and a far-field systemic circulation involving the liver and kidney, the purpose of which in each case is apparently to regenerate aspartate (Asp) in order for it to be recycled into NAA as part of the still unknown function of the NAA cycle. Based on the authors' analysis, they have also identified several metabolic outcomes of the genetic biochemical aspartoacylase lesion. First, there is a daily induced Asp deficit in the central nervous system (CNS) that is at least six times the static level of available free Asp. Second, there is up to a 50-fold drop in the intercompartmental NAA gradient, and third, the ability of the brain to perform its normal intercompartmental cycling of NAA to Asp is terminated, and as a result, the only remaining long-term source of Asp for NAA synthesis is via nutritional supplementation of Asp or its metabolic precursors. Finally, the authors identify a potential maternal–fetal interaction that may be responsible for observed normal fetal development in utero, and that provides a rationale for, and suggests how, CD might respond to far-field nutritional, transplantation, or genetic engineering techniques to alter the course of the disease.

Index Entries: *N*-acetylaspartate; *N*-acetylaspartic aciduria; *N*-acetylaspartic acidemia; N-acetylhistidine; Canavan disease; hypoaspartia.

*Author to whom all correspondence and reprint requests should be addressed.

Introduction

Canavan disease (CD), a rare autosomal recessive genetic disorder, is recognized by early onset and a characteristic and progressive degeneration of the brain. It has been classified as an infantile encephalitis periaxialis diffusa in which there is degeneration of the axons' medullary sheaths, but the axons themselves remain intact (Canavan, 1931). The degeneration of the central nervous system (CNS) is further characterized by elevated cerebrospinal fluid (CSF) pressure, intramyelinic edema, and a spongiform degeneration and swelling of astrocytes, with a peculiar elongation of mitochondria within involved astrocytes (Ford, 1960; Adachi and Volk, 1968; Boehme and Marks, 1981). CD has been reported to exist in a number of variants (Matalon et al., 1993). These include a congenital form, in which the disease is apparent at birth or shortly thereafter; a much more common infantile form, in which symptoms appear after the first 6 mo; and a juvenile form, in which the disease appears after approx 5 yr. CD can also exist in severe or mild forms, affecting both rate of progression and longevity (Shaag et al., 1995). *N*-acetylaspartic aciduria was first linked to a case of progressive CNS atrophy, probably CD, in a 6-yr-old child by Kvittengen et al. (1986), who proposed that the excretion of *N*-acetylaspartate (NAA) was caused by a defective ability of the CNS to degrade this compound. Subsequently, CD was found to be uniquely associated with *N*-acetylaspartic aciduria, and with a biochemical lesion of NAA metabolism involving an *N*-acetyl-L-aspartate amidohydrolase (aspartoacylase) deficiency, which results in the accumulation of NAA in extracellular fluid (ECF), including plasma and its excretion in urine (Matalon et al., 1988, 1995; Kaul et al., 1991). The gene controlling the synthesis of aspartoacylase has been identified on chromosome 17, and there are several different mutations, with distinct population distributions, that can produce aberrant aspartoacylase with reduced enzymatic activity (Kaul et al., 1993, 1994a,b; 1996; Shaag et al., 1995), but these investigators have as yet been unable to provide an explanation of just how the CNS and systemic aspartoacylase deficiency results in the observed CD syndrome. Although the enzymatic deficit associated with this disease is genetic and biochemically profound, the onset of the severe disease does not appear *in utero*, but instead appears postpartum, in infancy, usually within months after normal birth, with the majority of patients rapidly deteriorating and dying within the first 3 yr of life (Canavan, 1931; Adachi and Volk, 1968). NAA and its associated metabolic enzymes appear early in the gestation period, when the CNS is being formed (Birken and Oldendorf, 1989), and elevated NAA in amniotic fluid of the CD fetus confirms the early onset of the biochemical lesion (Bennett et al., 1993; Elpeleg et al., 1994). Therefore, it would appear that the delay of onset of the disease itself may be caused by some biochemical protection afforded to the homozygous fetus by the heterozygous maternal carrier during the period of gestation. Although the function of NAA in the brain of vertebrates is presently unknown, as a result of the observed genetic defect in CD, Matalon et al. (1993) suggest that NAA and aspartoacylase play a significant role in brain biology. It is also obvious that the other two components that comprise the NAA biochemical system, its precursor amino acid and its synthetic enzyme, must share in this significant role. It is to be hoped that elucidation of this role could also illuminate a new and important area of CNS function in normal individuals.

Of the scores of inborn errors of metabolism found in the human brain (Scriver and Rosenberg, 1973), many are aminoacidopathies that are associated with a primary enzyme deficiency. These enzyme deficits generally manifest themselves in a secondary amino aciduria or hypo- or hyperamino acidemia, which becomes the clinical marker for the specific disease. Finally, there is a tertiary effect that results in a hypo- or hyperamino acidia at the tissue level that is responsible for the metabolic consequence and observed syndrome that is a result of the initial biochemical lesion. In some of these cases, the overall effect on metabolism may be benign in nature, whereas in other cases, the primary deficiency may lead to profound metabolic consequences. In this paper, the authors explore the possible metabolic interactions that might occur in CD, which could help to explain how the primary enzyme defect might result in the observed severe clinical symptoms associated with this disease. It is hoped that an understanding of the basic metabolic events associated with NAA may provide clues with regard to where and how CNS metabolism is affected, and point to pos-

sible interventions that might stem the course of the disease process. In this communication, the authors review evidence that there may be two overlapping NAA circulation patterns that are used to provide aspartoacylase to complete the NAA biochemical cycle and produce L-aspartate (Asp): a near-field circulation in the brain and the eye, and a far-field circulation involving the liver and kidney. In addition, the authors develop the concept that it is not the biochemical defect in CD of itself, but rather an induced metabolic deficit resulting from that defect, that is responsible for initiation of the clinical syndrome. The authors also explore the possibility of a maternal–fetal interaction between the heterozygous parent and the homozygous fetus, in the form of a far-field biochemical interaction that delays the onset of the severe symptoms of the CD aspartoacylase defect. Finally, the authors have attempted to evaluate several observed pharmacological and physiological interactions with components of the NAA system in order to gain some insight into how the NAA system might be involved in daily CNS activity in normal individuals.

NAA Metabolism

The Biochemical Cycle

Some recent insights gained with regard to the metabolism of *N*-acetylated amino acids in the vertebrate CNS have provided additional background information with which to evaluate the nature of the biochemical lesion in CD (Baslow, 1997). It has been observed that NAA is a major constituent of the vertebrate brain and eye, characterized by high tissue concentrations, high tissue to ECF gradients, and by a continuous efflux into ECF. NAA is part of a specific biochemical system that consists of NAA; L-aspartate; a synthetic enzyme, L-aspartate *N*-acetyl transferase (EC 2.3.1.17); and a hydrolytic enzyme, *N*-acetyl-L-aspartate amidohydrolase (EC 3.5.1.15). Although the function of the NAA system is as yet unknown, the compartmentalization of the synthetic and hydrolytic enzymes associated with NAA metabolism and the severity of the consequences of the absence of aspartoacylase in CD suggest that it plays a vital role in some membrane function. NAA synthesis is from Asp and AcCoA, and is associated with a cellular mitochondrial fraction. Hydrolysis of NAA to Asp and acetate resides in another compartment, which may be a membrane or ECF, and there is evidence that NAA is cycled between cells and ECF with a major role of aspartoacylase being to hydrolyze NAA so that Asp and acetate are produced, and can then be actively transported back into the cells to complete the biochemical NAA cycle. Synthesis of NAA is not restricted to any single cell type. In addition to the apparent synthesis of large amounts of NAA by cells of the aneural lens in the rat and in fish (Baslow and Yamada, 1997a), studies of specific cell types from the 7-d-old rat CNS, grown in tissue culture, have revealed that NAA can be synthesized by a variety of cell lines. Urenjak et al. (1992) observed that cerebellar granule neurons, a bipotential glial precursor cell found throughout the CNS, oligodendrocyte-type-2-astrocyte progenitors, and immature oligodendrocytes could synthesize NAA. However, in that study, NAA could not be found in cultures of purified meningeal cells, purified cortical astrocytes, or mature oligodendrocytes. Glial cells have been reported to strongly accumulate ^{14}C acetate and ^{14}C Asp against a gradient, whereas there was very little uptake of these substances in neurons. In addition, turnover of Asp in glial cells in the presence of ^{14}C glucose and ^{14}C pyruvate, sources of AcCoA used in the synthesis of NAA, was three to four times that of neurons (Pevzner, 1979). Based on these Asp uptake and NAA synthesis studies, the peculiar cellular morphology presented in CD, and the presence of the NAA system in the aneural lens, it would appear that this system may have very little direct interaction with neurons. Instead, the intercompartmental NAA cycle seems to be more closely associated with cells involved in metabolic and structural support roles, including oligodendroglial myelin-forming cells, neuroglia, and endothelial or epithelial lining cells. Although aspartoacylase in the fish eye appears to be a soluble enzyme in ocular fluids (Baslow, 1997), Kaul et al. (1991), using immunostaining techniques on bovine cerebellum tissue, found aspartoacylase activity to be associated with myelin, and suggest that the enzyme may be membrane bound. The NAA system is dynamic and energy driven, and based on enzyme activity levels and NAA efflux, there may be complete turnover of NAA in the CNS on a daily basis.

The potential for such turnover in the brain can be broadly estimated from the level of tissue NAA and its synthetic and hydrolytic enzyme activities. In the rat, the brain contains 5.25 μmol/g of NAA (Tallan, 1957) and exhibits aspartoacylase activity of 2.05 μmol/g/h (Goldstein, 1976), and NAA synthetase activity of 0.48 μmol/g/h (Truckenmiller et al., 1985). Considering aspartoacylase activity in this species, all of the brain NAA could be hydrolyzed in 2.6 h and based on synthetase activity it could be replaced in 10.9 h.

Relationship Between NAA and N-Acetylhistidine

NAA and *N*-acetyl-L-histidine (NAH), another acylamino acid present in the CNS of vertebrates, in many cases simultaneously, appear to be metabolic analogs (Baslow, 1997). NAH, a major component in tissues of cold-blooded forms, has also been reported to be present in mammalian tissues, including rat brain, rabbit and human lenses, guinea pig and rabbit cardiac muscles, and rat skeletal muscle (Baslow and Yamada, 1997a). Although NAA and NAH have different phylogenetic distributions, they share characteristics of tissue distribution and concentrations, timing of appearance during embryological development, an apparent tissue-to-fluid cycling, and their degradative and synthetic biochemistry. However, the enzymes associated with NAA and NAH comprise two distinct and independent systems that exhibit high specificity for their respective substrates. In addition to their presence in the CNS, both NAA and NAH have also been found to be major components of the aneural and avascular lens (Baslow and Yamada, 1997a). The universal presence of NAA and NAH in the lens, retina, and many other structures of the vertebrate eye suggests that these substances play a key role in visual processes, as well as in CNS function. One important difference between His and Asp, which may be reflected in aspects of their cyclical metabolism, is that His, unlike Asp, is an essential amino acid in vertebrates. A third similar cycling system utilizing two substrates and two compartments is found at the synapse of cholinergic neurons (Baslow and Nigrelli, 1961, 1964). Here, acetylcholine (ACh) is produced and maintained with a high intracellular–extracellular gradient in vesicles at the presynaptic membrane. At the appropriate time, small quantities of ACh are released into the synaptic cleft compartment to perform its signalling function. Recycling is rapidly accomplished by the enzyme acetylcholinesterase, which hydrolyzes the acetyl ester of choline, so that both products can be recycled into neurons to complete the biochemical cycle. In lower forms, where the NAA, NAH, and ACh systems coexist, perhaps in the same cells, these three compartmentalized cycling systems very likely interact to some extent, since they all must compete for the recycled acetyl group, which is formed into AcCoA that is the common acetyl source for their intracellular synthetic enzymes. Formation of ACh from NAA as an acetyl donor by rat brain acetone powders has been demonstrated (Buniatian et al., 1965). In this analysis of the nature of the biochemical defect in CD, data available for various tissues and organs in many different species and for both NAA and NAH have been considered.

The Biochemical Lesion

Potential for Hypoaspartia

As a result of the biochemical defect in CD, the NAA cycle is disrupted and NAA accumulates in ECF, including CSF and plasma, from where it is eventually eliminated via the kidneys. The primary aspartoacylase deficiency results in a secondary *N*-acetylaspartic aciduria and, perhaps of more importance to the function of the NAA cycle, to a continuous depletion of cellular Asp in the CNS. This tertiary effect results in an Asp deficit, which must then be made up by sacrificing and mobilizing other nutritional and metabolic resources in the CNS for production of Asp, if the NAA biochemical cycle is to continue to operate. This is obviously the case, since the *N*-acetylaspartic aciduria is a persistent element in CD. Thus, the primary biochemical lesion in CD, aspartoacylase deficiency, may also induce hypoaspartia in the CNS, which could be an important element in the degenerative process that is associated with the CD syndrome. Hypoaspartia has previously been reported to be a consistent element in familial inherited CNS disease (Pedigree S). In affected individuals who have a chromosome 6p-linked dominant trait,

an olivopontocerebellar atrophy of the cerebellar cortex develops. In these cases, the observed hypoaspartia was universal in nature, evident in the affected cerebellar tissue, in extracerebellar brain areas, and as hypoaspartic acidemia in circulating blood plasma (Kish et al., 1991). Although the metabolic role of the Asp-NAA cycling biochemical system in the CNS remains to be elucidated, it is clearly vital to CNS function. The importance of the maintenance of *N*-acetylamino acids and their metabolism is the brain and the eye, even under extreme conditions, is indicated in a study of organisms that have been starved for prolonged periods. In fish, starved for 8 wk, NAH and its precursor L-histidine (His), which are major constituents of muscle, brain, and eye in this species, disappear from muscle, but at great metabolic expense, are spared in the brain and eye throughout this period (Yamada et al., 1994).

Magnitude of the Aspartate Deficit

A sense of the magnitude of the potential Asp deficit can be obtained from observed plasma NAA concentrations and excretion rates in CD patients. Although not detected in normal individuals, plasma NAA levels in CD patients have been reported to be between 0.92 and 1.00 m*M* (Matalon et al., 1988). These values represent about 20% of the NAA level found in brain, and this NAA is always in a state of flux since the rate of excretion of NAA in urine of CD patients is very high. Using the data of Matalon et al. (1988), it has been calculated that in three CD patients, there was an average excretion of 2.4 mmol of NAA per d per 5 kg of body weight. To put this loss in perspective, we can consider that a 5.0 kg infant with a 1.0 kg brain containing 5.5 m*M* NAA would have its total content (5.5 mmol) of brain-synthesized NAA passed into ECF and excreted every 55 h. A similar conclusion had previously been reached by Kvittingen et al. (1986), who reported that in a 6-yr-old who excreted 3–4 mmol of NAA/d, he was excreting about half the total NAA content of an average adult brain each day. Similar results have also been reported by Burns et al. (1992). They have determined the excretion rate of NAA from a 6 yr-old CD patient at 4.2 mmol/d and calculated that, based on a brain weight of 1200 g, this quantity of NAA represents 65% of the production of a healthy brain NAA pool each day. These authors have also calculated that the brain tissue of this individual synthesized 3.5 mmol of NAA/kg brain each day, giving a daily turnover of 100% of synthesized NAA. In this patient, brain NAA synthetase activity was still high at 6 yr of age, whereas aspartoacylase activity was only 7% of normal, resulting in a substantial CNS loss of Asp in the form of excreted NAA each day. At 100% daily turnover of NAA, this would represent a daily loss of 0.56 g of Asp and, for 1 mo, this loss would be 16.8 g of Asp. In addition, based on the normal static brain levels of Asp at 0.89 mmol/kg (Bremmer et al., 1981) and NAA at 5.5 mmol/kg, the Asp deficit, if all the NAA were excreted daily, would be 6.2 times the free Asp content of the brain. As a result of *N*-acetylaspartic aciduria, there would also be a corresponding deficit of acetate, equivalent to that of Asp, each day. However, the metabolic options available for *de novo* synthesis of acetyl groups appear to be greater than those to replace the lost Asp, and, therefore, the impact of acetate loss would be expected to be less. Glucose serves as a direct source of acetyl groups for AcCoA, and at plasma concentrations of about 5 m*M*, glucose is a very abundant acetate precursor for brain tissue. By the inclusion of an additional transamination step, glucose has also been reported to be the source of both Asp and NAA in the brain via oxaloacetate, possibly with alanine or glutamate as amino acid donors (Winick, 1976).

Bioenergetics and the Aspartate Deficit

Although the daily Asp deficit in the brain, which results from aspartoacylase deficiency and consequent *N*-acetylaspartic aciduria in cases of CD is large, this loss in terms of the bioenergetics of the brain may not be significant. Assuming a cost of three molecules of ATP, two for active transport of Asp and acetate, and another for the resynthesis of AcCoA, for every molecule of NAA cycled at a ratio of 3:1, 16.5 mmol of ATP (8 calories/mmol) would be expended per kilogram of brain for each 5.5 mmol of NAA cycled. Thus, the net energy cost for this system would amount to only 132 calories (0.132 kcal) per cycle. As a result of the biochemical defect in CD, and the observed rate of excretion of NAA, the net energy lost from the system each day would be of the order of 100

calories (0.100 kcal) per kilogram of brain. However, what appears to be more important is that, as a result of the continuous drawdown of NAA that occurs in CD, the brain's ability to perform repetitive near-field Asp-NAA cycles is eliminated, and the equivalent of less than a single normal cycle per day can be accomplished in these individuals. In the rat brain, based on the rate of NAA synthesis in the presence of ample Asp, there could be 2–3 NAA cycles per day. In the fish lens epicortex, the NAH content of 13.4 μmol/g and NAH synthesis activity of 6.1 μmol/g/h (Baslow, 1997), indicates a potential for 10.9 cycles/d. Of course, these estimates based on organ and tissue homogenates are by their nature quite crude, and values might be considerably higher under natural conditions in the microenvironment of the cell. Unfortunately, information for humans with regard to the number of daily cycles that NAA may go through in the CNS of normal individuals is not available at this time. However, even if there were 10 NAA cycles per day in the CNS, with a daily budget of 300 kcal/kg for the brain, the energy cost for the NAA system at <1% of this budget would appear to be nominal.

Although the function of the intercompartmental transfer step in the NAA cycle is not yet known, based on the steep cellular/extracellular gradients that are normally maintained, there is a potential for performing work at a membrane that separates compartments. NAA may or may not be involved with transport at the cell membrane, but if it is, some idea of constraints and boundaries of such a transport system can be evaluated. As an exercise, if the potential work involves a symport or antiport transfer of metabolites, an estimate of the amounts of substance and its energy cost can be calculated. At a transport ratio of 1:1 and 10 Asp-NAA conversions per day, there could be 55 mmol of substance transported per kg of brain at an energy cost of about 1 kcal per day. This amount, although small with regard to the daily transport of major cellular metabolites, still could be substantial. In the initial 3 mo that mark the period of onset of symptoms of infantile CD, approx 5 mol of substance might have been transported per kilogram of brain by the NAA system, if not for the cycling restriction imposed by the disease process. A significant power function would be generated at higher substance to NAA ratios.

Nature of the Residual Aspartoacylase Activity

The observation that some residual aspartoacylase activity remains in CD suggests that the level of remaining activity as well as its location may be important. Perhaps small differences in residual enzyme activity between individuals, associated with different cDNA mutations, play a role in determination of time of onset, the severity of neurodegeneration, and the time course of the disease process. It has also been observed that the NAA and NAH synthetases, as well as the NAA and NAH acylases that are present in ocular fluids and brain, are fairly specific for these substrates. However, these acylases can also hydrolyze a variety of other acetylated amino acids and dipeptides at high rates. Using NAH as an example, whereas His or its 1 and 3-methyl derivatives only can serve as substrates for the synthetic enzyme (Yamada et al., 1995), the acylase has been found to hydrolyze at least 16 different *N*-acetylated amino acids and dipeptides, many at even higher rates than for NAH itself (Yamada et al., 1993). In this study, NAA was also hydrolyzed by the NAH acylase, but only at 3% of the activity on NAH. Based on the relative lack of acylase specificity, the biochemical lesion in CD might also produce a variety of metabolic impacts in the CNS, in addition to producing an Asp deficit, which in turn could amplify and aggravate the effect of the aspartoacylase defect. The nature of the residual aspartoacylase activity in vivo in CD is unknown. It may be caused by residual activity of the mutated specific enzyme, or it may be a result of the presence of a number of nonspecific acylases present in kidney and other tissues that can use NAA as a substrate. Very likely, it is a combination of these factors, and their total impact on NAA hydrolysis may also contribute to the varied degrees of severity that have been observed in CD.

Role of Aspartoacylase

Matalon et al. (1993) have suggested that the role of NAA in the CNS might be to serve as a source of Asp, and that the role of the aspartoacylase is to release it for its metabolic function, which may be related to the formation of arginine. The authors agree that the role of aspartoacylase is to produce Asp, but suggest that the Asp pro-

duced is normally recycled to form NAA once again. As a result of the biochemical deficit in CD, this normal cycle is upset, and NAA accumulates in ECF and plasma. NAA and NAH are relatively inert physiologically, and do not appear to be actively transported against a gradient (Baslow, 1997). Since plasma levels of NAA in CD are only 20% of that found in brain, autointoxication is probably not a factor in evaluating the effect of high plasma NAA concentrations in CD. What is apparent, however, is that the normal high tissue/ECF NAA gradient is greatly reduced, which may have some significance with regard to the disease process. The compartmentalized cyclical metabolism of NAA and NAH appears to be remarkable in only one way; in the extremely high tissue/ECF gradients that are normally maintained as a result of aspartoacylase activity. It is an understanding of this extraordinary feature that may hold the answer to the function of the NAA and NAH biochemical cycles in the CNS and eye of vertebrates. The overall effect of the CD defect is to interrupt the NAA cycle and to eliminate this metabolic characteristic by hindering the rapid hydrolysis of NAA, which in effect reduces the tissue/ECF NAA ratio, and at the same time produces a Asp deficit. Perhaps a good analogy to the NAA system is the situation at a hydroelectric dam. Here, a gradient is formed at the dam boundary, and a great deal of potential energy is stored in the form of water in the upstream compartment. For purposes of energy production, small amounts of water can be released in a controlled fashion and converted into kinetic energy at the dam interface, after which the expended water enters the downstream compartment for eventual recycling. Without a large compartmental gradient, this system can provide very little work, and without a mechanism for recycling, in this case an energy-driven phase change, the system would run downhill and eventually fail. The authors suggest that the key to understanding the function of the NAA cycle lies in the identification of the trigger mechanism, which results in the intercompartmental transfer of NAA down its gradient. In CD, as a result of NAA efflux into ECF and resulting hyper *N*-acetylaspartic acidemia, there may be a >50-fold drop in the intercompartmental NAA gradient, from 275:1 (Swahn, 1990) to about 5:1 (Matalon et al., 1988). At the intercompartmental boundary itself, this gradient may be even lower, and approach 1:1.

In addition to the observed CD aspartoacylase defect on the NAA biochemical cycle, it is of interest that a number of drugs that affect the CNS also can interrupt this cycle, and appear to do so by inhibiting the activity of the acylamino acid acylases. Indirect evidence is available for NAA where injection of iproniazid (100 μg/g), isoniazid (100 μg/g), and imipramine (25 μg/g) all produce an increase of about 25% in brain NAA in the mouse in vivo (Birken and Oldendorf, 1989). For NAH, the effects of CNS drugs on inhibition of acylase activity have been measured in vitro using tuna brain enzyme preparations at pH 7.0 and 30°C. Drugs tested included isoniazid, holothurin, mescaline, phenobarbital, chlorpromazine, promazine, methotrimeprazine, imipramine, trifluoperazine, phenergan, quinacrine, methylene blue, eserine, reserpine, LSD-25, 2-brom-LSD, propoxate, bulbocapnine, benzedrine, serotonin, tubocurarine, librium, and GABA. Of these, only the phenothiazine tranquilizers, compounds related to phenothiazines, and a chemically unrelated tranquilizer, librium, showed inhibitory effects on acylase activity (Baslow and Lenney, 1967). In these studies, 50% acylase inhibition was observed for the phenothiazine tranquilizers, chlorpromazine, promazine, methotrimeprazine, and imipramine at concentrations of 250, 500, 1000, and 500 μg/mL, respectively. However, in this study, isoniazid did not affect acylase activity. Based on these observations, it is possible that some of the drugs that are known to affect CNS function may have some part of this activity because of interactions with acylases of the NAA or NAH systems. In addition, caffeine has been reported to significantly reduce brain NAA, and sodium amytal and GABA to significantly increase brain NAA in vivo, in mice within 20–40 min (Buniatian et al., 1965). Clearly, understanding the function of the ubiquitous NAA and NAH systems might very well lead to a better understanding of drug–CNS interactions in general, and also to the targeted development of specific new drugs that can alter this system for therapeutic purposes. Finally, in many instances involving trauma, Asp in ECF has been observed to increase dramatically. For example, Asp has been found to increase 15-fold in brain ECF during insulin-induced hypoglycemia in the rat in vivo (Sandberg et al., 1986), and about 10-fold

in the CSF of neonate humans exposed to relatively short periods of asphyxia (Riikonen et al., 1992). A twofold increase in plasma Asp has also been noted during migraine attacks (Ferrari et al., 1990). However, these authors, in considering the possible origin of the Asp, did not take into account that the largest store of Asp in the brain is in the form of NAA, and did not give any consideration to aspartoacylase activity as a potential source of the measured increases in Asp. Perhaps this was caused by the failure to recognize the dynamic nature of the NAA biochemical cycle and the rapidity with which NAA in the aspartoacylase compartment can be hydrolyzed. A transient ischemia has been found to cause a significant release of NAA into ECF (Sager et al., 1997). In this study, rats exposed to 20 min of ischemia showed an initial fivefold increase in interstitial NAA, which was followed by a 13-fold increase in the next 20-min recovery period. These authors also indicated that the liberated NAA was cleared from the brain, and that the process may include an extracellular degradation step. Thus, the observed increases in free Asp in ischemia and other conditions may very well be caused by an interaction with the NAA system. The change might be related to a change in the rate of release of NAA to ECF, and its subsequent hydrolysis to Asp and acetate by aspartoacylase. Obviously, a complete understanding of the role of aspartoacylase and the nature of the NAA cycle will also add a new dimension to our ability to interpret findings related to the observation of Asp flux under a variety of physiological conditions.

Possible Maternal–Fetal Interaction

Near and Far-Field Enzyme Activity

An intriguing aspect of CD is that most CD neonates are relatively asymptomatic at birth, and shortly thereafter. Since the biochemical lesion involved in CD is profound and appears early in the gestation period, when NAA metabolism is initiated in the CNS, it would seem that some degree of protection is afforded the developing fetus in utero. For NAA and NAH there is evidence of both a near-field and systemic cycling of these acylamino acids. Although there is more complete information available for NAH in a single fish species *(Nile tilapia)* than for NAA in a mammal (rat), it is clear that for the CNS and the eye, perhaps related to difficulty in obtaining Asp through the blood–brain and blood–ocular fluid barriers, these tissues are served by near-field acylases to hydrolyze their acylamino acids. On the other hand, the heart, skeletal muscle, and several other tissues that synthesize acylamino acids cannot hydrolyze them whereas kidney and liver, which may not synthesize the acylamino acids, contain very high acylase levels (D'Adamo et al., 1973; Yamada et al., 1992). In the Nile tilapia and the rat, acylase activity is high in the CNS, but still the kidney contains 3.7 and 13.9 times the specific acylase activity, respectively, than does the brain tissue in these species. This suggests that the site of acylase activity is of somewhat less importance than its presence in the organism, and that systemic circulation of acylamino acids far-field, via the circulatory system may be a normal part of the metabolism of these acylamino acids in some tissues. Berlinguet and Laliberte (1966) demonstrated that labeled NAA, injected intraperitoneally, did not enter the brain, but that a high level of radioactivity appeared in the kidney. After intracerebral injection the result was similar, leading these authors to conclude that there was a systemic pathway for NAA from the brain to the kidney, probably via plasma, from which it was extracted and hydrolyzed. In these same studies, it was found that labeled Asp did not appear in the kidney, a finding that suggests that there is a differential distribution of NAA and Asp in the blood components. Finally, it was reported, in these studies using mice, that the labeled NAA injected intraperitoneally was found in the kidneys, and in addition, labeled Asp was found in both the kidneys and the brain after only 30 min, thus providing support for a role for the kidneys in the systemic metabolism of NAA. Far-field circulation of NAH appears to be the major pathway for its hydrolysis in the skeletal muscle of the *Nile tilapia*. This species is unique in having a large concentration of NAH in skeletal muscle where a content of 2.82 μmol/g has been observed, but with no apparent acylase activity found in this tissue (Yamada et al., 1992). However, blood plasma in this species contained 0.01 μmol/mL of NAH (Yamada et al., 1994), and liver and kidney could hydrolyze 2.0 and 13.5 μmol/g/h, respectively.

Although the level of circulating NAH is very low, a rapid circulation cycle and high acylase efficiency in passage through liver and kidney could produce significant recycling of His over relatively short periods of time. Incorporation of far-field His into NAH in body tissues including brain and eye is also very rapid. In goldfish, injected intraperitoneally with ^{14}C-His, NAH in the brain, heart, and eye was found to be labeled in <15 min, with peak labeling in this pulse-labeled experiment in 1–3 h (Baslow, 1997). Based on the review of Bremer et al., 1981), Asp that might be produced by kidney aspartoacylase (aminoacylase II) from NAA in humans would be conserved in that organ with very little Asp excreted (0.03–0.06 mmol/24 h). In the kidney tubules, Asp is reabsorbed at 97–98% efficiency, where it is then passed into the circulating blood. Although plasma contains very little Asp, normal erythrocytes concentrate Asp and at 0.264 mol/mL contain 29.3 times the level of plasma Asp. Thus, in humans, circulating erythrocytes may have an additional function in the NAA cycle and could serve as the systemic vehicle, and be a near-field CNS source of this amino acid from both nutritional and from far-field kidney aspartoacylase sources. However, the potential of this resource to provide large amounts of Asp to the CNS in a short period is limited by its relatively poor uptake (Oldendorf, 1971). In addition to the CNS and eye, NAA has also been reported to be found in the spleen, lung, ovary, intestines, thymus, skin, heart, and adrenal medulla (Baslow, 1997).

Taking these factors into consideration, it would appear that a plausible systemic pathway for Asp that is used in the synthesis of NAA can be described. Any NAA that passes into ECF from cells where it is synthesized might be transferred to the circulatory system where it can rapidly reach the kidney. In the kidney NAA can be hydrolyzed to Asp, which is then efficiently reabsorbed into plasma, and then accumulated by erythrocytes and other cellular components so that it can be safely recirculated. In the target organs Asp is removed from plasma, as it is released from erythrocytes, and rapidly taken up by target cells where mitochondrial-associated synthesis of NAA occurs. NAA then passes into the cytosol where a high intracellular compartmental concentration accumulates. Finally, although the function of this step is as yet not understood, NAA passes into another compartment, perhaps a membrane or interstitial fluid, from which it is transferred to plasma for a return to the systemic circulation. In this proposed circulation pattern, the partition of Asp to erythrocytes, and NAA to plasma appears to serve as an effective means to allow circulating NAA to enter the glomerular filtrate of the kidney for processing, whereas at the same time allowing Asp to continuously circulate for use by other tissues and organs. In the CNS and the eye, which are insulated from the general circulation by barriers consisting of epithelial and endothelial cells with tight junctions that line the circulatory system conduits (Christensen, 1990), the systemic circulation pattern is normally bypassed. Only in these organs is near-field acylase provided to hydrolyze NAA so that Asp is produced for incorporation into its dynamic metabolic cycle. The sequence outlined for Asp can also be applied to the systemic circulation of His in the formation and hydrolysis of NAH. One effect of the biochemical lesion in CD is to eliminate the near-field aspartoacylase so that NAA accumulates in ECF and then plasma, and thus enters the systemic circulation where it is observed in the form of a hyper *N*-acetylaspartic acidemia. Unfortunately, in the case of CD, the aspartoacylase deficiency is system-wide, and as a result of the failure of the systemic pathway to recover Asp, NAA is excreted in large amounts. At this point, the *N*-acetylaspartic aciduria that is a characteristic of the disease also becomes apparent.

Role of the Near-Field Acylases

If a far-field circulation of NAA is a normal aspect of NAA metabolism, then the question arises regarding what role the near-field acylases might play. Mammalian plasma is not a rich source of free Asp, and in four mammalian species it was found to be about 0.012 m*M* (Reddy, 1967), and in humans, 0.013 m*M* (Reilmann et al., 1994). These low plasma values might be a reflection of the potential neuroexcitotoxicity of Asp, and be the reason that the circulating erythrocyte Asp pool is so much larger than the plasma pool. Bremer et al. (1981) have collected data from a variety of sources for plasma Asp. The range for 20 men and women is trace–0.005 m*M* Asp in one study. In children, plasma aspartate is reported to be 0.019, 0.002, and 0.010 m*M* for those up to age 4 mo, up to 2 yr, and

up to 10 yr, respectively. Of interest, the placenta of the developing fetus has the ability to concentrate almost all free amino acids present in the maternal plasma. For Asp, there is a twofold increase in plasma Asp of the umbilical vein over that of the circulating maternal plasma. Depending on the content and availability of the larger erythrocyte Asp pool to the ECF surrounding the CNS, this supply may or may not be adequate to replace Asp at the rate it is being utilized. It is difficult to obtain a measure of the amount of free Asp in the brain ECFs vs tissue as a result of the convoluted architecture of the brain and its fluid spaces. However, in the eye, such a comparison can more easily be made, since the lens tissue and aqueous humor are separated from one another by the lens capsule. In the rat, it has been observed that free Asp in plasma, aqueous humor, and lens was <0.001, 0.052, and 0.432 m*M*, respectively. The amount of Asp in the lens was approximately equal to the level of NAA in that organ (Baslow and Yamada, 1997a), and represented 432 and 8.3 times the content of Asp in plasma and aqueous humor. It is obvious that if total lens NAA were to be turned over each day, that plasma, erythrocytes (at 29 times the plasma level), or aqueous humor might not be an adequate source of Asp for such a requirement. Normal human brain, including cerebral cortex and cerebellum, obtained by biopsy, has been found to contain Asp at 0.89 m*M*, or about 16% of the level of brain NAA. As a matter of interest, it was the observation that free Asp in cat brain represented only 26% of the total of free, and an easily hydrolyzable bound form of Asp (Tallan et al., 1954), that led to the discovery of NAA. If the situation in the brain is similar to that of the eye, then it would appear to be quite reasonable, and metabolically efficient, to establish a near-field acylase source with which to rapidly recycle Asp from near-field NAA in both of these important organs. In the eye, it is likely that most of the lens Asp, measured at any given time, has already been repeatedly recycled from ocular fluid NAA. In the eye of the fish, the *Nile tilapia*, the lens contains about 2.8 μmol/g of NAA (Baslow and Yamada, 1997a), and aspartoacylase activity is present in ocular fluid (Yamada et al., 1993) where NAA can be rapidly hydrolyzed, and Asp taken up to resynthesize NAA. As a result, NAA occurs in very low concentration in ocular fluid (0.13 μmol/mL), and the lens/ocular fluid NAA ratio is very high (32:1). The corresponding value for NAH is 59:1 (Baslow and Yamada, 1997b). These values are based on acetylamino acid content of whole lens. Since NAH is known to be concentrated near the lens surface, in <10% of its volume, actual gradients at the lens surface of ten times these values would probably be more realistic. In the human brain, based on a brain NAA level of 5.5 m*M*, and on a value of 0.02 m*M* NAA in normal ventricular fluid (Swahn, 1990), the brain/CSF NAA ratio is 275:1. For rat brain, the brain/interstitial fluid gradient for NAA is 100:1 (Sager et al., 1997). The use of a common reservoir of acylase by a number of organs and tissues has also been observed in microcosm in the fish eye, where it has been found that almost all tissues of the eye, including sclera, choroid, ciliary body and iris, lens, and retina, can synthesize both NAA and NAH, but appear to rely on the presence of acylases in ocular fluids to complete the biochemical cycle (Baslow and Yamada, 1997b). Thus, in the case of NAA, the role of the near-field aspartoacylase appears to be twofold, first, to maintain a high tissue/fluid NAA gradient, and second, to assure the brain and the eye of an adequate supply of Asp, within physiological limits, for use in the intracellular synthesis of NAA as part of the ongoing NAA biochemical cycle.

Sources of CNS Aspartate in the Developing Fetus

If the sources of Asp for the CNS in the developing fetus are examined, there are clear distinctions between normal and CD biochemistry. In the case of the development of a normal fetus, Asp is available from a number of sources, which include both near and far-field recycling from NAA, supplementation from the maternal placental circulation, and *de novo* synthesis from other metabolites (Fig. 1A). In contrast, in the case of the developing CD fetus, aspartoacylase generated near or far-field sources of Asp are mostly absent, and the fetus must rely completely on the maternal placental circulation to provide it with Asp and other metabolites (Fig. 1B). The question of what happens to the fetal NAA in ECF in cases of CD, must also be considered. NAA appears early in the gestation period of the developing mammalian fetus, and its ubiquitous presence in ECFs in cases of CD,

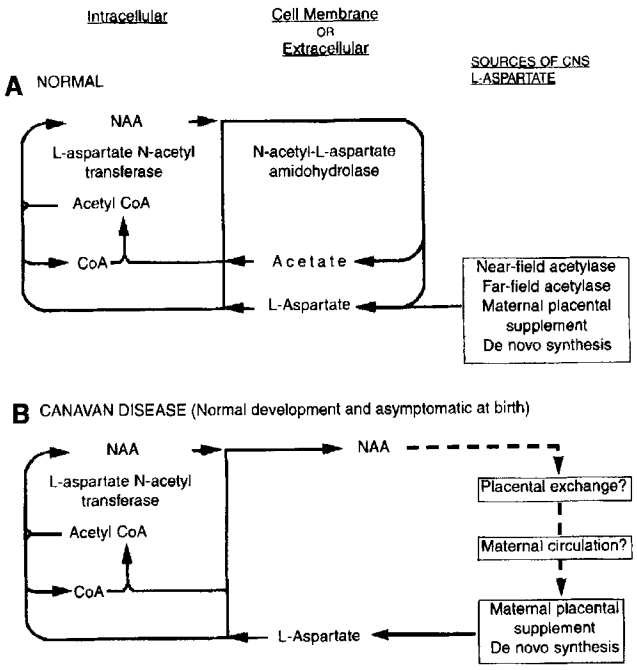

Fig. 1. Schematic representation of the NAA biochemical cycle *in utero*, indicating CNS sources of aspartate in normal **(A)** and in aspartoacylase deficient fetuses **(B)**.

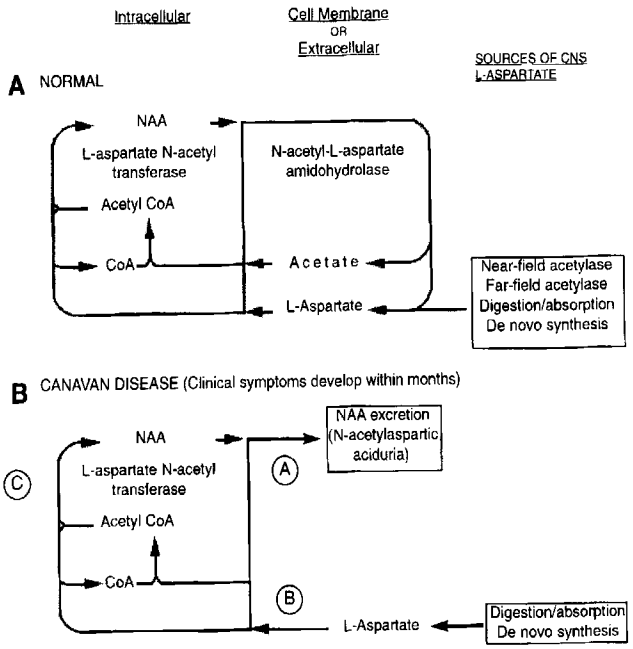

Fig. 2. Schematic representation of the NAA biochemical cycle postpartum, indicating CNS sources of aspartate in normal **(A)** and in aspartoacylase deficient infants (**B**). As a result of the aspartoacylase deficit in the NAA biochemical cycle, there are a number of metabolic consequences: (A) the intercompartmen-tal NAA gradient is reduced at least 20- to 50-fold, (B) recycling of Asp from NAA is eliminated and a near-field Asp deficit is induced, and (C) synthesis of NAA becomes dependent upon far-field nutritional and *de novo* synthetic sources of Asp.

suggests that substantial amounts of NAA should be present in all fetal fluids. Although NAA is elevated in amniotic fluid in CD, at about 6 µmol/L it is present in very low concentration relative to the content of 2–5 m*M* that is present in the CNS (Kelley, 1993), and therefore, cannot account for a large daily production and efflux of this amino acid from that developing tissue. The low level of observed amniotic fluid NAA, along with the relatively normal development *in utero*, suggest that most of the fetal NAA produced in cases of CD is eliminated somehow, and that the mechanism associated with its removal may in some way be connected to viability of the fetus *in utero*. The observation of incipient *N*-acetylaspartic aciduria in neonates with CD is another indication that hyper *N*-acetylaspartic acidemia probably occurs *in utero*, and further supports the hypothesis that some mechanism exists for its removal. Since acetylated amino acids do not appear to be actively transported into cells, it is likely that in CD, fetal plasma NAA reaches the fetal placental circulation and is then excreted, down its gradient by a diffusion process, into the maternal placental circulation. Since the heterozygous mother can synthesize aspartoacylase (Matalon et al., 1989), it is possible that the cells of the maternal portion of the placenta act as a fetal kidney and are the site of NAA hydrolysis. Alternatively, the fetal NAA may enter the maternal circulation along with other excreted products, and be hydrolyzed at other sites prior to Asp becoming available for reuptake by the fetus. If the transfer of NAA via the placental circulation does occur, and if the rate of hydrolysis is adequate, it is possible that the fetal plasma NAA and Asp levels are maintained within the normal range *in utero*, and this may explain the observation that amniotic fluid NAA may not always be found to be elevated in CD (Fig. 2; Matalon et al.,

1993). This possible maternal-fetal interaction is illustrated in Fig. 1B. In any event, even if this were not the case, the mother would be a continuous source of Asp and other metabolites to the fetus for use in NAA synthesis, which could result in reasonably normal development. In this way, the mother would serve as a biochemical surrogate, bypassing the CD impairment, and thus delaying the severe consequences of the CD effect until after birth.

Sources of CNS Aspartate in Neonates

In the normal fetus postpartum (Fig. 2A), most of the sources of Asp are still available, except that the placental source of readily usable metabolites is terminated, and a more complicated source involving an additional digestive step is instituted. On the other hand, at birth, conditions for the CD infant change suddenly and drastically when compared to a normal infant with regard to Asp metabolism. The use of the mother as a biochemical surrogate to counter the aspartoacylase deficit is no longer possible, and the placental supply of readily available Asp and metabolites is terminated abruptly. At birth, the mechanism for excretion of plasma NAA shifts from possible diffusion across the placental membranes, with recycling potential, to the more complex and one-way renal excretion apparatus. In addition, the source of Asp and other metabolites shifts from the maternal circulation, with a transplacental concentrating potential, to a much more variable and complicated intestinal digestion and absorption process as shown in Fig. 2B. Increased postpartum difficulty in obtaining Asp, competition from all cells of the body for this amino acid, and the continuous high demand of the CNS for Asp in CD, as a result of near-field aspartoacylase deficiency and *N*-acetylaspartic aciduria, appear to exacerbate the disease process. It is in this latter condition that severe clinical symptoms of neurodegeneration begin to appear.

A Suggested Therapeutic Intervention

There is no question that in CD, as a result of *N*-acetylaspartic aciduria, that a substantial Asp deficit occurs each day. However, at this time one can only speculate on the potential impact of this deficit on the brain in the near-field. The role that this deficit might have will depend on factors as yet unknown, such as the requirement for Asp by cells that actually synthesize NAA, and the degree to which this requirement can be made up from other sources. However, if a near-field hypoaspartia in the CNS induced by the biochemical lesion associated with CD does occur, then it is likely that this Asp deficiency will interfere with the vital, but still unknown function of the NAA system, and may be responsible for many of the manifestations of CD. In this analysis, the authors have tried to provide a framework to better understand the function of the NAA system in normal individuals, and with which to evaluate the profound effect of the biochemical lesion in CD on that function. Although their conclusions are consistent with the available, sometimes fragmentary, data and they are reasonably comfortable with the inferences made, the authors cannot say with assurance that theirs is the only and correct analysis. Future studies will ascertain whether this is the case. In the meantime, the apparent maternal–fetal protection against the initiation of the CD syndrome and rationale for such protection, based on what appears to be a far-field or systemic aspartoacylase interaction that could eliminate the CNS Asp deficit, may be important and it suggests that a nutritional remedy for CD may be possible. If hypoaspartia is the major cause of the CD syndrome, and if the postulated maternal–fetal mechanism of protection does occur, then a course of treatment becomes evident. To the extent that synthesis of NAA is substrate-limited as a result of hypoaspartia, supplementation of Asp or other suitable precursors should allow an increase in the rate of NAA production to more normal levels. In addition, an increase in NAA production should compensate for the lack of NAA cycling because of the aspartoacylase deficiency, and to some degree increase the NAA gradient at the intercompartmental boundary. Immediate postpartum detection of CD is vital, prior to the formation of irreversible damage that eventually leads to clinical manifestations of the disease. At an early time, CD may respond well to replacement therapy. Such therapy might include appropriate therapeutic doses of Asp, or metabolites that can produce Asp, and acetate,

possibly as a nutritional supplements and perhaps, with Asp in the form of a variety of metal salts (Afanas'ev et al., 1995), to aid in its absorption and to expedite passage through the blood-brain barrier. In addition to the formation of metal salts, Asp can also form ternary metal-2 complexes, under physiological conditions, with itself and perhaps with another amino acid (Baslow, 1997), and thus may provide an alternative form for Asp supplementation. Based on a preliminary analysis, relative to the amount of NAA excreted in urine, a therapeutic dose of Asp or precursor metabolites would be required that delivers the equivalent of at least 0.6 g of Asp to the near-field brain each day. If the normal number of NAA cycles were between 3 and 10/d, the daily brain Asp shortfall could be between 1.8 and 6.0 g/kg. The specific dose needed to achieve this near-field supplement would be a function of other nutritional factors, the form of Asp and other metabolites, and the selected route of administration. Since the brain is probably the major source of urinary NAA in CD, it follows that this metabolic leakage would also be an indicator of brain NAA synthetic activity. Therefore, any nutritional protocol selected should consider use of urinary output of NAA as a noninvasive measure of effectiveness, with the aim being to increase this output, as a reflection of brain NAA synthetic activity, to a level that could be correlated with the rate of NAA synthesis in normal individuals. In light of what is known about the rate of brain NAA synthesis even under the additional metabolic pressures imposed by the aspartoacylase defect in CD, any nutritional protocol that attempts to reduce the output of urinary NAA is likely to be counterproductive. The authors' evaluation of the possible metabolic defect (hypoaspartia) in the CNS in CD could explain the apparent maternal protection afforded to the fetus, and suggests a rationale for both a far-field nutritional Asp supplementation as well as for a far-field recycling of Asp from native NAA. Should nutritional enhancement therapy prove to be of value, then some measure of respite would be possible before more sophisticated treatments, such as transplantation (e.g., normal kidney or liver lobe) or provision of genetically engineered cells that produce aspartoacylase (During, 1996; Levine, 1996), can be instituted in order to alter the clinical course of CD.

Summary and Conclusions

NAA Metabolism

NAA is a metabolic component of the CNS and other tissues of vertebrates, which is part of a specific cycling biochemical system consisting of two amino acid substrates (Asp and NAA), and two enzymes, an NAA synthetase and acylase. In this system, the synthetic and hydrolytic enzymes are compartmentalized. The NAA system also appears to be analogous to another *N*-acetylating, cyclical, and compartmentalized system that uses His instead of Asp. Both of these systems may be present and operate simultaneously in the vertebrate CNS and other tissues.

NAA System Characteristics

The NAA system is characterized by a high CNS tissue concentration, a high tissue to ECF gradient, and by a continuous regulated efflux into ECF. There appear to be two possible intercompartmental circulation patterns for the hydrolysis of NAA and production of Asp, one in the near-fields of the brain and the eye, and the other in the far-field involving a systemic circulation, via plasma, of NAA to the liver and kidney. Turnover of NAA may be several times per day in the CNS and eye.

Role of Aspartoacylase

In the NAA system, aspartoacylase plays a key role in the intercompartmental biochemical cycle in the CNS and other tissues, as it is required for the hydrolysis of NAA and regeneration of Asp. In the CNS, the role of aspartoacylase appears to be twofold, first to maintain a high intercompartmental NAA gradient, and second, to assure the brain and the eye of an adequate Asp supply for use in the NAA cycle.

Possible Site of NAA Function

Tissue distribution and other characteristics of the NAA biochemical system suggest that NAA function is most closely associated with cell populations whose roles are primarily involved with providing metabolic and structural support in a variety of organs.

Potential NAA Interactions

The NAA biochemical system, which appears to be important to normal daily function of the CNS, may also play an important and previously unrecognized role in the CNS with respect to the effects of drugs, interaction with traumatic events, and in the progress of a variety of physiological, as well as genetic disease processes.

Metabolic Consequences of the Aspartoacylase Deficiency in CD

A Continuous Asp Drawdown

There is a daily deficit of Asp in the CNS as a result of *N*-acetylaspartic aciduria, the magnitude of which is about 0.6 g/kg/d, a value that represents 6.2 times the static level of free Asp in the brain. The actual deficit could be much higher depending on the number of daily Asp-NAA cycles that there are in normal individuals.

Reduction in the Intercompartmental NAA Gradient

As a result of NAA efflux into ECF and induced hyper *N*-acetylaspartic acidemia, there may be a 50-fold drop in the intercompartmental NAA gradient, from about 275:1 to 5:1. The gradient at the intercompartmental boundary itself is probably lower approaching 1:1, and thus eliminating a source of potential work at this boundary.

Termination of Intercompartmental NAA Cycling

The ability of the brain to carry out intercompartmental cycling of NAA to Asp is terminated, and instead of multiple cycles per day, there may be the equivalent of less than a single cycle.

Induced Requirement for Nutritional ASP Supplementation

As a result of the termination of recycling of native Asp from NAA, there is a continuous need for additional Asp by cells involved in the synthesis of NAA. This requirement can only be made up in these cells by ECF supplementation of Asp itself, or by a supply of suitable metabolites that can be utilized for the *de novo* synthesis of Asp. The ultimate source of all new Asp used in NAA synthesis in CD is a function of far-field nutritional intake.

Termination of Near- and Far-Field Aspartoacylase Interactions

Both near-fields brain and eye, and far-field systemic circulation patterns involving liver and kidney for the normal metabolism of NAA are terminated.

Potential Onset of the Disease Process

An apparent protection afforded to the developing fetus *in utero*, that may involve a maternal-fetal biochemical interaction in which the mother serves as a biochemical surrogate, bypassing the CD impairment and delaying the severe consequences of CD, is terminated at birth.

Elimination of Alternate Substrate-Aspartoacylase Interactions

Alternate metabolic sequences in which aspartoacylase activity might play a role are eliminated.

Events Leading to the Observed Clinical Symptoms

The genetic aspartoacylase defect in CD results in at least three metabolic lesions in cells that play a role in production and maintenance of the myelin sheath. The progressive deterioration of these cells, as evidenced by astrocyte swelling and glial malfunction and cell loss, is associated with the observed edema, demyelination, and spongiform appearance in the CNS, which is characteristic of the disease. Ultimately, this process leads to failure of neuron function and to presentation of the clinical neurological symptoms that are associated with CD.

Possible Therapeutic Interventions

Postnatal Nutritional Supplements

Based on the apparent maternal–fetal protection, early nutritional intervention to supplement Asp is proposed. This conservative intervention, subject to suitable protocols, should provide either Asp or other suitable precursor metabolites to make up for the ongoing CNS Asp deficit.

Transplantation of Normal Organs

Based on what appears to be a far-field systemic circulation pattern for hydrolysis of native NAA, it is proposed that transplantation of a normal kidney or liver lobe might effectively reduce plasma NAA, and produce sufficient Asp for normal CNS function.

Genetic Engineering

Again, based on the observation of a far-field systemic circulation pattern for NAA, the possibility that genetically engineered, altered somatic cells could make up for the aspartoacylase deficit should be explored.

References

Adachi M. and Volk B. W. (1968) Protracted form of spongy degeneration of the central nervous system (van Bogaert and Bertrand type). *Neurology* **18,** 1084–1092.

Afanas'ev I., Suslova T. B., Cheremisina Z. P., Abramova N. E., and Korkina L. G. (1995) Study of antioxidant properties of metal aspartates. *Analyst* **120,** 859–862.

Baslow M. H. (1997) A review of phylogenetic and metabolic relationships between the acylamino acids, N-acetyl-L-aspartic acid and N-acetyl-L-histidine in the vertebrate nervous system. *J. Neurochem.* **68,** 1335–1344.

Baslow M. H. and Lenney J. F. (1967) a-N-Acetylhistidine amido-hydrolase activity from tuna fish brain: inhibition by phenothiazines. *Seventh Int. Cong. Biochem.* **5,** 1014.

Baslow M. H. and Nigrelli R. F. (1961) Muscle acetylcholinesterase as an index of general activity in fishes. *Copeia* **1,** 8–11.

Baslow M. H. and Nigrelli R. F. (1964) The effect of thermal acclimation on brain cholinesterase activity of the killifish, Fundulus heteroclitus. *Zoologica* **49(1),** 41–51.

Baslow M. H. and Yamada S. (1997a) Identification of N-acetylaspartate in the lens of the vertebrate eye: a new model for the investigation of the function of N-acetylated amino acids in vertebrates. *Exp. Eye Res.* **64(2),** 283–286.

Baslow M. H. and Yamada S. (1997b) Distribution of N-acetyl-L-histidine and N-acetyl-L-aspartate in the vertebrate eye. *J. Neurochem.* **69 (Suppl.),** 5175D.

Bennett M. J., Gibson K. M., Sherwood W. G., Divry P., Rolland M. O., Elpeleg O. N., Rinaldo P., and Jakobs C. (1993) Reliable prenatal diagnosis of Canavan disease (aspartoacylase deficiency): comparison and enzymatic and metabolite analysis. *J. Inher. Met. Dis.* **16,** 831–836.

Berlinguet L. and Laliberte M. (1966) Metabolism of N-acetyl-L-aspartic acid in mice. *Can. J. Biochem.* **44,** 783–789.

Birken D. L. and Oldendorf W. H. (1989) N-Acetyl-L-aspartic acid: a literature review of a compound prominent in H-NMR spectroscopic studies of brain. *Neurosci. Biobehav. Rev.* **13,** 23–31.

Boehme D. H. and Marks N. (1981) Protracted form of Canavan's disease: casehistory and protein kinase activity of membrane fractions. *Acta Neuropathol.* **55,** 221–225.

Bremer H. J., Duran M., Kamerling J. P., Przyrembel H., and Wadman S. K. (1981) Disturbances of amino acid metabolism: clinical chemistry and diagnosis. Urban and Schwarzenberg, Baltimore, MD, p. 536.

Buniatian H. C. H., Hovhannissian V. S., and Aprikian G. V. (1965) The participation of N-acetyl-L-aspartic acid in brain metabolism. *J. Neurochem.* **12,** 695–703.

Burns S. P., Chalmers R. A., West R. J., and Iles R. A. (1992) Measurement of human brain aspartate N-acetyl transferase flux in vivo. *Biochem. Soc. Trans.* **20,** 107S.

Canavan M. M. (1931) Schilders encephalitis periaxialis diffusa. Report of a case in a child aged sixteen and one-half months. *Arch. Neurol. Psychiat.* **25,** 299–308.

Christensen H. N. (1990) Role of amino acid transport and countertransport in nutrition and metabolism. *Physiol. Rev.* **70,** 43–77.

D'Adamo A. F. Jr., Smith J. C., and Woiler C. (1973) The occurrence of N-acetylaspartate amidohydrolase (aminoacylase II) in the developing rat. *J. Neurochem.* **20,** 1275–1278.

During M. (1996) Gene therapy in New Zealand (letter). *Science* **272,** 467.

Elpeleg O. N., Shaag A., Anikster Y., and Jakobs C. (1994) Prenatal detection of Canavan disease (aspartoacylase deficiency) by DNA analysis. *J. Inher. Met. Dis.* **17,** 664–666.

Ferrari M. D., Odink J., Bos K. D., Malessy M. J. A., and Bruyn G. W. (1990) Neuroexcitatory plasma amino acids are elevated in migraine. *Neurology* **40,** 1582–1586.

Ford F. R. (1960) *Diseases of the Nervous System in Infancy, Childhood and Adolescence.* Charles C. Thomas, Pub., Springfield, IL, p. 1548.

Goldstein F. B. (1976) Amidohydrolases of brain; enzymatic hydrolysis of N-acetyl-L-aspartate and other N-acyl-L-amino acids. *J. Neurochem.* **26,** 45–49.

Kaul R., Casanova J., Johnson A. B., Tang P., and Matalon R. (1991) Purification, characterization, and localization of aspartoacylase from bovine brain. *J. Neurochem.* **56,** 129–135.

Kaul R., Gao G. P., Balamurugan K., and Matalon R. (1993) Cloning of the human aspartoacylase cDNA and a common missense mutation in Canavan disease. *Nature Gen.* **5,** 118–123.

Kaul R., Gao G. P., Aloya M., Balamurugan K., Petrosky A., Michals K., and Matalon R. (1994a) Canavan disease: mutations among Jewish and non-Jewish patients. *Am. J. Hum. Gen.* **55,** 34–41.

Kaul R., Balamurugan K., Gao G. P., and Matalon R. (1994b) Canavan disease: genomic organization and localization of human ASPA to 17p13-ter and conservation of the ASPA gene during evolution. *Genomics* **21,** 364–370.

Kaul R., Gao G. P., Matalon R., Aloya M., Su Q., Jin M., Johnson A. B., Schutgens R. B., and Clarke J. T. (1996) Identification and expression of eight novel mutations among non-Jewish patients with Canavan disease. *Am. J. Hum. Gen.* **59,** 95–102.

Kelley R. I. (1993) Prenatal detection of Canavan disease by measurement of N-acetyl-L-aspartate in amniotic fluid. *J. Inher. Metab. Dis.* **16,** 918,919.

Kish S. J., Robitaille Y., el-Awar M., Gilbert J., Deck J., Chang L. J., and Schut L. (1991) Brain amino acid reductions in one family with chromosome 6p-linked dominantly inherited olivopontocerebellar atrophy. *Ann. Neurol.* **30,** 780–784.

Kvittingen E. A., Guldal G., Borsting S., Skalpe I. O., Stokke O., and Jellum E. (1986) N-acetylaspartic aciduria in a child with a progressive cerebral atrophy. *Clin. Chim. Acta* **158,** 217–227.

Levine R. J. (1996) Canavan gene therapy protocol (letter) *Science* **272,** 1085.

Matalon R., Michals K., Sebasta D., Deanching M., Gashkoff P., and Casanova J. (1988) Aspartoacylase deficiency and N-acetylaspartic aciduria in patients with Canavan disease. *Am. J. Med. Genet.* **29,** 463–471.

Matalon R., Kaul R., Casanova J., Michals K., Johnson A., Rapin I., Gashkoff P., and Deanching M. (1989) Aspartoacylase deficiency: the enzyme defect in Canavan disease. *J. Inher. Metab. Dis.* **12,** 329–331.

Matalon R., Kaul R., and Michals K. (1993) Canavan disease: biochemical and molecular studies. *J. Inher. Met. Dis.* **16,** 744–752.

Matalon R., Michals K., and Kaul R. (1995) Canavan disease: from spongy degeneration to molecular analysis. *J. Pediatr.* **127,** 511–517.

Oldendorf W. H. (1971) Brain uptake of radiolabeled amino acids, amines, and hexoses after arterial injection. *Am. J. Physiol.* **221,** 1629–1639.

Pevzner L. Z. (1979) *Functional Biochemistry of the Neuroglia* (Tiplady B., ed.), Plenum, New York, p. 306.

Reddy D. V. N. (1967) Distribution of free amino acids and related compounds in ocular fluids, lens and plasma of various mammalian species. *Invest. Opthalmol.* **6,** 478–483.

Reilmann R., Rolf L. H., and Lange H. W. (1994) Huntington's disease: the neuroexcitatoxin aspartate is increased in platelets and decreased in plasma. *J. Neurol. Sci.* **127,** 48–53.

Riikonen R. S., Kero P. O., and Simell O. G. (1992) Excitatory amino acids in cerebrospinal fluid in neonatal asphyxia. *Pediatr. Neurol.* **8,** 37–40.

Sager T. N., Fink-Jensen A., and Hansen A. J. (1997) Transient elevation of interstitial N-acetylaspartate in reversible global brain ischemia. *J. Neurochem.* **68,** 675–682.

Sandberg M., Butcher S. P., and Hagberg H. (1986) Extracellular overflow of neuroactive amino acids during severe insulin-induced hypoglycemia: in vivo dialysis of the rat hippocampus. *J. Neurochem.* **47,** 178–184.

Scriver C. R. and Rosenberg L. E. (1973) Amino acid metabolism and its disorders, in *Major Problems in Clinical Pediatrics*, vol. X (Schaffer A. L., ed.), Saunders, Philadelphia, PA, p. 491.

Shaag A., Anikster Y., Christensen E., Glustein J. Z., Fois A., Michelakakis H., Nigro F., Pronicka E., Ribes A., Zabot M. T., et al. (1995) The molecular basis of Canavan (aspartoacylase deficiency) disease in European non-Jewish patients. *Am. J. Hum. Gen.* **57,** 572–580.

Swahn C. G. (1990) Determination of N-acetylaspartic acid in human cerebrospinal fluid by gas chromatography-mass spectrometry. *J. Neurochem.* **54,** 1584–1588.

Tallan H. H. (1957) Studies on the distribution of N-acetyl-L-aspartic acid in brain. *J. Biol. Chem.* **224,** 41–45.

Tallan H. H., Moore S., and Stein W. H. (1954) Studies on the free amino acids and related compounds in the tissues of the cat. *J. Biol. Chem.* **211,** 927–939.

Truckenmiller M. E., Namboodiri M. A. A., Browstein M. J., and Neale J. H. (1985) N-Acetylation of L-aspartate in the nervous system: differential distribution of a specific enzyme. *J. Neurochem.* **45,** 1658–1662.

Urenjak J., Williams S. R., Gadian D. G., and Noble M. (1992) Specific expression of N-acetylaspartate in neurons, oligodendrocyte-type-2 astrocyte progenitors, and immature oligodendrocytes in vitro. *J. Neurochem.* **59,** 55–61.

Yamada S., Tanaka Y., Sameshima M., and Furuichi M. (1992) Occurrence of N-acetylhistidine in the muscle and deacetylation by several tissues of

Nile tilapia (Oreochromis niloticus). *Comp. Biochem. Physiol.* [B] **103,** 579–583.

Yamada S., Tanaka Y., Sameshima M., and Furuichi M. (1993) Properties of N-acetylhistidine deacetylase in brain of trout Oncorhynchus mykiss. *Comp. Biochem. Physiol.* [B] **106,** 309–315.

Yamada S., Tanaka Y., Sameshima M., and Furiuchi M. (1994) Effects of starvation and feeding on tissue N-acetylhistidine levels in Nile tilapia Oreochromis niloticus. *Comp. Biochem. Physiol.* [A] **109,** 277–283.

Yamada S., Tanaka Y., and Furuichi M. (1995) Partial purification and characterization of histidine acetyltransferase in brain of Nile tilapia (Oreochromis niloticus). *Biochim. Biophys. Acta* **1245,** 239–247.

Winick M. (1976) *Malnutrition and Brain Development.* Oxford University Press, New York, p. 169.