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# Metabolism of N-Acetyl-L-Aspartate in Rat Brain

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The abundance and developmental regulation of N-acetylaspartate (NAA) in brain suggest that it plays an important role in brain metabolism. Previous studies demonstrated that NAA transports acetate from the mitochondrion to the cytoplasm where it is utilized for lipid synthesis, however, the metabolic fate of NAA-derived aspartate is not established. To investigate NAA metabolism, rats were injected intracranially with N-([$^2H_3$]acetyl)-L-[$^{15}$N]aspartate ([$^2H_3$,$^{15}$N]NAA) and whole brain metabolites were analyzed using gas chromatography and mass spectrometry techniques (GC/MS). The rapid decline of [$^2H_3$,$^{15}$N]NAA was associated with a rapid appearance of [$^{15}$N]glutamate, indicating rapid transamination of the [$^{15}$N]aspartate that was derived from the enzymatic hydrolysis of [$^2H_3$,$^{15}$N]NAA. Inability to detect [$^{15}$N]NAA in brain extracts in several experiments indicates that the $^{15}$N moiety is not reutilized for NAA synthesis and suggests one metabolic role of NAA may be the transport of amino nitrogen from the mitochondrion to the cytoplasm.

**KEY WORDS:** N-acetylaspartate; brain metabolism; aspartate; oxalacetate; amino nitrogen metabolism.

## INTRODUCTION

N-Acetylaspartate (NAA) is synthesized in the mitochondrion and transported to the cytoplasm, where it is hydrolysed to aspartate and acetate (1,2). The high brain levels of NAA (*5.6* μmol/g of brain), the third most abundant free amino acid in the CNS (3), as well as the observation that these brain levels and metabolism of NAA are under developmental control (4), led to the suggestion that NAA serves an important function in brain (5). One metabolic function of NAA is the transfer of acetate from the mitochondrion to the cytoplasm where it is utilized for lipid synthesis and, in this regard, is the primary acetate shuttle in brain in lieu of the citrate shuttle (6–9). The metabolism of NAA-derived aspartate in brain is not established (5). We have used stable isotopes to study the in vivo metabolism of brain NAA. Utilizing [$^{15}$N]NAA, we have found that one metabolic role of NAA may be the transfer of amino nitrogen from the mitochondrion to the cytoplasm.

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## EXPERIMENTAL PROCEDURE

[$d_6$]Acetic anhydride (98 atom % enrichment) and L-[$^{15}$N]aspartic acid (99 atom % enrichment) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Ion exchange resins were purchased from Bio Rad Laboratories, Inc. (Melville, NY, USA). Fischer 344 female rats were purchased from Charles River Laboratories (Wilmington, MA, USA). Other chemicals were reagent grade.

N-[$^2H_3$]acetyl-L-[$^{15}$N]aspartic acid ([$^2H_3$,$^{15}$N]NAA) was prepared by the acetylation of [$^{15}$N]aspartic acid with [$^2H_6$]acetic anhydride and [$^2H_3$,$^{15}$N]NAA was separated from reactants utilizing a cation exchange column (AG 50W X-8, $H^+$ form) to remove unreacted [$^{15}$N]aspartic acid followed by lyophilization to remove acetic acid and acetic anhydride. Confirmation of the expected product, [$^2H_3$,$^{15}$N]NAA, was accomplished utilizing gas chromatography/mass spectrometry (GC/MS). The purity routinely exceeded 95%.

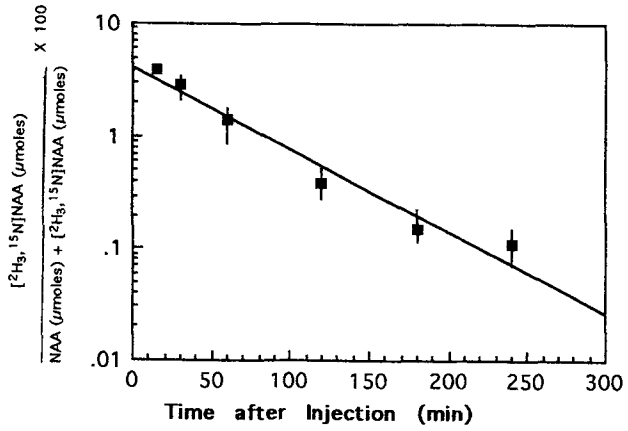


**Fig. 1.** Rats were injected with [$^2H_3$,$^{15}N$]NAA, sacrificed at various times after injection (3 rats/time point), and the brain was removed and homogenized individually. NAA was isolated from the soluble fraction, and the mole fraction of [$^2H_3$,$^{15}N$]NAA determined by GC/MS. Fig. 1 is a composite of 3 separate experiments with the vertical axis representing the mole fraction of [$^2H_3$,$^{15}N$]NAA with respect to total NAA in brain and is expressed as a percent. Regression analysis of the data was performed using an exponential function (R = 0.980). Vertical bars represent the range of values at each time point.

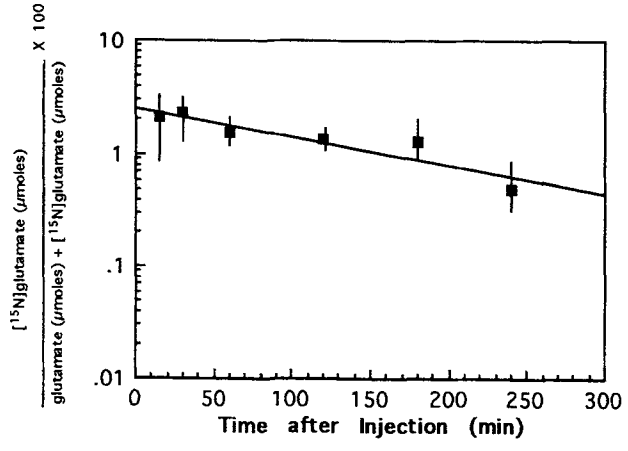


**Fig. 2.** Experimental data was obtained from the experiments described in Fig. 1. Glutamate was isolated from the soluble brain fraction, and the mole fraction of [$^{15}N$]glutamate determined by GC/MS. Fig. 2 is a composite of 3 separate experiments with the vertical axis representing the mole fraction of [$^{15}N$]glutamate with respect to total glutamate in brain and is expressed as a percent. Regression analysis of the data was performed using an exponential function (R = 0.923). Vertical bars represent the range of values at each time point.

Fischer 344 female rats, 29 days old, were injected intracranially with 15 μl of [$^2H_3$,$^{15}N$]NAA (0.42 μmol/μL). The intracranial injection was accomplished using a 50 μl syringe which was modified with a guard over the needle that permitted a needle tip penetration through the skull of <2mm (10). This allowed the solution to be injected into the cerebral spinal fluid rather than into brain tissue. Rats were sacrificed (3 animals per time point) at 15, 30, 60, 120, 180, and 240 minutes after injection. The brain was rapidly removed, weighed, frozen in liquid $N_2$, and stored frozen until analysis. The brains then were homogenized in cold (4°C) water and centrifuged at 50,000 *g* for 50 minutes in a RC-28S Sorvall centrifuge. An aliquot of the soluble fraction was deproteinated with 10% perchloric acid and then neutralized with 10% KOH containing 50 mM $K_2CO_3$, lyophilized, reconstituted with 20 mM Tris buffer, pH 8.6, and passed through a column containing anion exchange resin (AG 1-X8, $Cl^-$ form). After washing the column with water, a fraction containing NAA was eluted with 1N HCl and lyophilized. The level of isotopically-labeled NAA is determined as the mole fraction (%) of total NAA. A composite of replicate experiments was constructed by setting the mole fraction at 15 minutes equal to 4%; normalizing the mole fraction at other time points in the experiment to this value; and determining the average mole fraction (%) for each time point of replicate experiments.

Glutamate was isolated as a component of the partially purified NAA fraction (described above) and an aliquot of this fraction was utilized for the GC/MS determination of labeling in this amino acid. In order to obtain a composite of the glutamate data in replicate experiments while maintaining the relationship between glutamate and NAA abundance, the glutamate data for an individual experiment were normalized with the same factor used to normalize the NAA data of that experiment.

Glutamine was isolated from a deproteinated aliquot of the soluble fraction of brain homogenate (described above). This solution was passed through a column of ion exchange resin (AG 50W-X8, $H^+$ form); the column was washed with water; and the glutamine was eluted with cold 3 M $NH_4OH$.

## RESULTS AND DISCUSSION

In a preliminary experiment, rats were injected intracranially with 15 μl Fast Green dye solution and were sacrificed either 5 or 15 minutes after injection. Five minutes after intracranial injection above the cerebral cortex, all areas of the brain showed some staining, including the white matter areas contralateral to the injection site and tissue lining the ventricles. After 15 minutes, staining was more pronounced throughout the brain indicating that the intracranial bolus injected over the cerebral cortex was rapidly distributed throughout the brain tissue.

To investigate the metabolism of NAA, rats received an intracranial injection of [$^2H_3$,$^{15}N$]NAA and were sacrificed at various times after injection. Fifteen minutes after injection, the mole fraction of [$^2H_3$,$^{15}N$]NAA, relative to whole brain NAA, was 2 to 4%. As shown in Fig. 1, a composite of 3 experiments, the mole fraction of [$^2H_3$,$^{15}N$]NAA in whole brain decreased rapidly from 4% at 15 min to approximately 0.5% at 240 min. The designation of "M+1" glutamate refers to [$^{15}N$] glutamate, i.e., glutamate with a molecular weight of 1 atomic mass unit greater than the naturally occurring amino acid. As shown in Fig. 2, formation of this species from [$^{15}N$]NAA was relatively rapid, reach-

ing a maximum by 30 min and declining steadily thereafter. Other isotopic forms of glutamate were not detected. Also undetectable in brain extracts were isotopically-labeled glutamine and M+1 NAA. Essentially no [$^2H_3$]NAA could be detected.

Several aspects of these observations are of interest. Although NAA does not readily cross the cell membrane (5), it is possible to transfer a small, but significant amount into the cell by injecting a bolus intracranially (this is the reverse of what occurs in Canavan's disease where elevated cellular levels lead to the loss of a portion of the cellular NAA to the extracellular fluid). The [$^2H_3$,$^{15}$N]NAA, which decreased rapidly following injection, was not a reflection of extracellular [$^2H_3$,$^{15}$N]NAA included as a contaminant during the isolation of brain NAA. This statement is based on the following details. (1) As estimated below, the formation of [$^{15}$N]glutamate from the transamination of NAA-derived [$^{15}$N] aspartate can account for most if not all of the observed decrease in [$^2H_3$,$^{15}$N]NAA. (2) This transamination must have occurred subsequent to the deacylation of [$^2H_3$,$^{15}$N]NAA. And (3), the deacylation of [$^2H_3$,$^{15}$N]NAA is specifically catalyzed by aspartoacylase (EC 3.5.1.15), an intracellular enzyme (11).

The essential absence of [$^2$H]NAA species which would indicate a lack of recycling of [$^2$H]acetate is commensurate with previous reports which indicated that the acetate formed from NAA deacylation is efficiently utilized for the synthesis of lipids (6,8,9). The appearance of M + 1 glutamate, which would result from transamination of [$^{15}$N]aspartic acid and α-ketoglutarate, presents the possibility that glutamate and oxalacetate, via the oxalacetate-malate shuttle, could be translocatated to the mitochondrion and via transamination could provide aspartate for the synthesis of NAA. Although the experimental protocol cannot provide an indication as to whether the oxalate derived from aspartate is reutilized for the synthesis of aspartate portion of NAA, the inability to detect [$^{15}$N]NAA in any of our experiments suggests that the NAA-derived aspartate nitrogen is not reutilized in the synthesis of NAA. Furthermore, this suggests that an additional role of NAA in brain metabolism may involve a transfer of amino nitrogen from the mitochondrion to the cytoplasm.

The proportion of NAA-derived aspartate nitrogen transferred to α-ketoglutarate can be estimated (neglecting steady-state dynamics) from the relative abundance of NAA and glutamate in brain and the maximum enrichment (mole fraction) of [$^2H_3$,$^{15}$N]NAA and [$^{15}$N]-glutamate observed in whole brain. The brains (~1.3g) contained ~7.3 μmole of NAA and ~15 μmole of glutamate (3). With a maximum enrichment of [$^2H_3$,$^{15}$N]NAA equal to 4% (see Fig. 1), the brain contains ~0.3 μmole of [$^2H_3$,$^{15}$N]NAA. If, after the deacylation of [$^2H_3$,$^{15}$N]NAA, all of the NAA-derived [$^{15}$N]aspartate was transaminated to form 0.3 μmole of [$^{15}$N]glutamate the maximum enrichment with respect to whole brain glutamate would be ~2%. Based on this estimation and the experimental data in Fig. 2, it appears that most, if not all, of the NAA-derived [$^{15}$N]aspartate undergoes transamination resulting in the formation of [$^{15}$N]glutamate.

In summary, rats were injected intracranially with [$^2H_3$,$^{15}$N]NAA and whole brain metabolism was studied using GC/MS. The rapid decline of [$^2H_3$,$^{15}$N]NAA was associated with a rapid appearance of [$^{15}$N]glutamate, reflecting transamination of the [$^{15}$N]aspartate that was derived from the enzymatic hydrolysis of [$^2H_3$,$^{15}$N]NAA. Inability to detect [$^{15}$N]NAA in brain extracts in several experiments indicates that the $^{15}$N moiety is not reutilized for NAA synthesis and suggests one metabolic role of NAA may be the transport of amino nitrogen from the mitochondrion to the cytoplasm.

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