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A Unique Neuronal Molecule
in the Central Nervous System
ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY

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N-ACETYLASPARTATE
A Unique Neuronal Molecule in the Central Nervous System

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NAA, NAAG AND CANAVAN’S DISEASE: THE FIRST INTERNATIONAL SYMPOSIUM ON N-ACETYLASPARTATE

Natcher Conference Center, NIH, Bethesda Maryland. September 13th and 14th, 2004. This volume chronicles the first comprehensive meeting held on N-acetylaspartate and Canavan's disease. The Symposium featured presentations by leading investigators and a poster session. The meeting was organized by the National Institute of Mental Health and was co-sponsored by the National Institute of Neurological Disorders and stroke, the National Institute of Child Health and Human Development, and the Office of Rare Diseases at the National Institute of Health, Bethesda, Maryland USA.

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PREFACE: A BRIEF REVIEW OF N-ACETYLASPARTATE

John R. Moffett and Aryan M.A. Namboodiri

1. INTRODUCTION

The First International Symposium on N-acetylaspartate (NAA) was held on September 13th and 14th, 2004 in the Natcher Conference Center at the NIH, in Bethesda Maryland. The event showed clearly that NAA was no longer an obscure object of study by a few scattered researchers, and that instead it had become a subject of great interest to hundreds of scientists and clinicians from around the world. This newfound attention for one of the brain’s most concentrated amino acid derivatives was a long time in coming, since the first report of NAA in the brains of cats and rats by Tallan and coworkers in 1956.1 The Symposium also covered N-acetylaspartylglutamate (NAAG), a related dipeptide comprised of NAA and glutamate, which was first identified definitively as a brain-specific peptide by Miyamoto and colleagues in 1966.2 Despite decades of research on the roles both these molecules play in the nervous system, their functions remain enigmatic, and controversial.

NAA in particular is a study in controversy, with virtually no consensus on its principle metabolic or neurochemical functions after nearly five decades of research. There is relative unanimity on one point; NAA is not thought to be a neurotransmitter or neuromodulator that is released synaptically upon neuronal depolarization. Beyond that, there is little consensus. At least four basic hypotheses have been offered for the principle role of NAA in the nervous system: 1) an organic osmolyte that counters the “anion deficit” in neurons, or a co-transport substrate for a proposed “molecular water pump” that removes metabolic water from neurons, 2) a source of acetate for myelin lipid synthesis in oligodendrocytes, 3) an energy source in neurons, and 4) a precursor for the biosynthesis of NAAG. These functions are not mutually exclusive, and indeed, NAA certainly serves multiple functions in the nervous system.

The two findings that catapulted NAA into mainstream scientific consciousness were the connection to the fatal hereditary genetic disorder known as Canavan disease, and the

* Uniformed Services University of the Health Sciences, 4301 Jones Bridge Rd, Bethesda MD, 20814 USA; email, jmoffett@usuhs.mil.
prominence of the NAA-signal in magnetic resonance spectroscopy (MRS). In the case of Canavan disease, it was found that a mutation in the gene for the enzyme aspartoacylase (ASPA) resulted in the inability to remove acetate from NAA, leading to a failure of CNS myelination, and preventing CNS maturation. In the case of the prominent NAA signal in MRS, it has been found that the levels of NAA in various parts of the brain correlated with neuronal health or dysfunction. Low levels of NAA as detected by MRS have been interpreted to indicate neuronal/axonal loss, or compromised neuronal metabolism. High levels of brain NAA were found in many Canavan patients, suggesting that excess NAA may have toxic effects in the CNS. The First International Symposium on NAA covered these, and many other issues involving the roles played by NAA in normal and pathological brain function. The following sections briefly outline several of the various hypotheses proposed by different research groups on the possible functions of NAA in the nervous system.

1.1. NAA and the Osmolyte Hypothesis

Perhaps the most controversial hypothesis regarding the possible functions of NAA in the nervous system is the neuronal osmolyte hypothesis. It is controversial because this hypothesis has relatively little definitive experimental support, but paradoxically is one of the most cited hypotheses in recent NAA literature. In the 1960s, several research groups proposed, based on the high concentration of NAA in the brain, that it might function to counter the so-called “anion deficit” in neurons. In the 1990’s, Taylor and colleagues proposed that NAA acts as a neuronal osmolyte involved in active neuronal volume regulation, or possibly in acid-base homeostasis. Using microdialysis, they demonstrated that NAA concentrations in the extracellular fluid increased in response to hypoosmotic stress. However, the changes in extracellular NAA concentrations were modest as compared with the substantially larger increases in extracellular taurine concentrations under the same conditions. Indeed, if NAA is involved significantly in neuronal osmoregulation, it is but one of many organic osmolytes that are responsible for maintaining water homeostasis in the brain. More recently, Davies et al. showed that under hypotonic conditions extracellular taurine levels increased almost 20 fold, whereas under the same conditions, extracellular NAA increased by only a few percent. NAA efflux of that magnitude could occur by simple leakage along the concentration gradient when neurons are subjected to osmotic stress. As such, NAA may be a minor contributor to neuronal volume regulation, whereas taurine and other osmolytes provide the predominant regulation of water homeostasis in the brain.

More recently, Baslow and colleagues have proposed a modified version of the neuronal osmolyte hypothesis in which NAA acts as a cotransport substrate for an as yet undescribed molecular water pump which removes excess metabolic water from neurons. The aspects of NAA that suggest that it could be involved in such a function include; 1) high concentration, 2) high intraneuronal to extracellular gradient, and 3) the fact that osmotic stress increases the extracellular NAA concentrations to a small degree (see chapter by Verbalis, this volume). Nonetheless, to date no proteins have been described in neurons that act to cotransport NAA and water out of neurons. However, it has been shown that the sodium-dependent NaDC3 transporter moves NAA into glial cells (also see chapter by Ganapathy and Fujita, this volume).
1.2. NAA as Acetate Carrier for Myelin Lipid Synthesis

One hypothesis on the role of NAA in the nervous system that has mounting experimental support is the acetate hypothesis of myelin lipid synthesis in oligodendrocytes. In the 1960’s, D’Adamo and coworkers showed that the acetate moiety of NAA was incorporated into brain lipids during CNS development, indicating that it was likely to be involved in the myelination of axons. In the early 1990’s Burri and colleagues showed that the acetate group from NAA was preferentially incorporated into brain lipids during brain development, and in the mid 1990’s Mehta and Namboodiri corroborated that radiolabeled NAA and acetate were incorporated into acetyl CoA and brain lipids. In 2001, Ledeen and colleagues showed that radiolabeled NAA was transported down the optic nerve, and the acetate group was incorporated into the ensheathing myelin of the optic axons. Recently, Namboodiri and coworkers demonstrated in the mouse model of Canavan disease (aspartoacylase knockout) that the rate of lipid synthesis was significantly reduced, and that free acetate levels in the brain were almost 5-fold lower than in wild-type mice (see Namboodiri et al., this volume). Taken together, these data provide strong evidence for NAA as being a major source of free acetate and acetyl CoA for lipid synthesis in the brain during development, and that NAA-derived acetate is critical for proper CNS myelination.

It is important to state that the acetate-lipid synthesis hypothesis does not address the presence of the extraordinarily high levels of NAA in adult neurons. A theory that pertains directly to the high concentrations of NAA in the neurons of adult animals posits that NAA is intimately involved in neuronal energy metabolism.

1.3. NAA in Neuronal Energy Metabolism

The first report that NAA might be involved in brain metabolism was by Buniatian and coworkers in 1965. The idea that NAA is involved in energy metabolism in the nervous system is based on a number of facts, including that 1) NAA is synthesized by aspartate N-acetyltransferase in neuronal mitochondria, 2) traumatic brain injury causes rapid and partially reversible decreases in NAA concentrations, 3) inhibition of mitochondrial respiration results in the simultaneous decrease of NAA production, ATP production and oxygen consumption in isolated brain mitochondria, and 4) NAA levels in the striatum of rats and primates were significantly decreased after the animals were treated with a mitochondrial toxin (3-nitropropionate). Recently, Madhavarao and coworkers proposed a model whereby NAA is associated with neuronal energy production (see Namboodiri et al. and Madhavarao and Namboodiri, this volume). In this model, aspartate aminotransferase, the enzyme that synthesizes NAA, facilitates removal of excess aspartate from neuronal mitochondria via acetylation, thus favoring α-ketoglutarate formation from glutamate, and energy production via the citric acid cycle.

1.4. NAAG Biosynthesis

NAAG biosynthesis has been studied for well over a decade, and yet to date, no NAAG biosynthetic enzyme has been isolated and characterized. The presence of a “NAAG synthetase” enzyme has been demonstrated indirectly in tissue culture and tissue explants, but no data on the incorporation of radiolabeled precursors into NAAG have been reported in tissue homogenates. Protein synthesis inhibitors have no effect on
incorporation of radiolabeled precursors into NAAG, indicating that NAAG is not synthesized ribosomally as a portion of a protein, and then later cleaved to generate the active molecule. Because the ability of neurons to synthesize NAAG is lost when tissue explants are homogenized, it seems clear that “NAAG synthase” is either sequestered in a membrane compartment where optimal conditions are maintained for enzyme activity, or that the mixing of extracellular and intracellular constituents brings the enzyme in contact with salts, proteases or inhibitors that render it inactive.

1.5. NAA Toxicity in Canavan Disease

An additional controversy concerning NAA is that it is toxic to neurons when the concentration is substantially elevated in the brain, as in the case of many Canavan disease patients. Canavan disease (CD) is a fatal, hereditary leukodystrophy that compromises myelination in the CNS. CD is caused by mutations in the gene for ASPA, an enzyme that currently is thought to function exclusively to hydrolyze NAA into L-aspartate and free acetate. However, ASPA is strongly expressed in other tissues such as kidney, even though the only known substrate, NAA, is present predominantly in the nervous system. Despite the established connection between mutations in the gene for ASPA in CD, and the lost capacity to deacetylate NAA, the specific connection between ASPA deficiency and the failure of proper CNS development is unclear. It has been proposed that lack of deacetylase activity against NAA leads to toxic increases in the concentration of NAA in the brain. Indeed, the level of extracellular NAA may be a critical factor in determining if it has toxic effects. For example, Pliss and colleagues reported that injection of 0.25 micromoles/ventricle of NAA into the lateral cerebral ventricles of rats did not induce any detectable neuronal death in the hippocampus, whereas Akimitsu et al. reported that intraventricular injection of 8 micromoles of NAA into the lateral ventricle of rats induced strong seizures. Seizures are one of the symptoms of CD, but it has not been conclusively demonstrated that elevated NAA levels are responsible for the seizure activity. The question of the toxicity of NAA has not been fully answered, but it is possible that exceptionally high levels of NAA in the brains of some CD patients may be involved in some of the pathogenesis, possibly by inducing seizure activity.

1.6. NAA and Magnetic Resonance Spectroscopy

Finally, it is important to note that there are two distinct NAA research communities; one group involved in basic research into the neurochemistry of NAA, and another group which employs MRS techniques for the non-invasive analysis of NAA levels in the brain with respect to clinical applications. NAA levels measured by MRS have been shown to be changed in a number of neurological disorders. These studies have mostly detected decreases in NAA, with the exception of Canavan disease which involves accumulation of NAA in the brain. The diseases and disorders in which NAA levels are decreased include stroke, Alzheimer’s disease, epilepsy, brain cancer, multiple sclerosis and AIDS dementia complex. Earlier, the decreases in NAA were interpreted to represent irreversible loss of neurons. However, more recent evidence indicates that these decreases also could represent reversible mitochondrial dysfunction. In support of this view, some evidence has been presented showing that NAA levels are restored when patients recover clinically. Two recent reports have shown that NAA levels in the brain
are increased in rats after prolonged administration of haloperidol,45 and in human children with sickle cell disease.46

The First International Symposium on NAA brought basic researchers and clinicians together, and offered these two research groups an opportunity to interact and discuss ideas, and while none of the controversies surrounding NAA neurochemistry were resolved, both groups benefited greatly from the exchange. The following proceedings from the first international NAA Symposium detail ongoing research in numerous laboratories, and present the interactions and exchanges between some of the top researchers in this field.

2. REFERENCES


## CONTENTS

1. **A BRIEF OVERVIEW OF N-ACETYLASPARTATE AND N-ACETYLASPARTYLGLUTAMATE** ................................................................. 1
   Joseph T. Coyle

2. **EXPRESSION OF N-ACETYLASPARTATE AND N-ACETYLASPARTYLGLUTAMATE IN THE NERVOUS SYSTEM** ............... 7
   John R. Moffett and Aryan M. A. Namboodiri

3. **N-ACETYLASPARTATE METABOLISM IN NEURAL CELLS** ........... 27
   Kishore K. Bhakoo, Timothy Craig and Daniel Pearce

4. **NAA SYNTHESIS AND FUNCTIONAL ROLES** ................................. 49
   Chikkathur N. Madhavarao and Aryan M. A. Namboodiri

5. **IDENTITY OF THE HIGH-AFFINITY SODIUM/CARBOXYLATE COTRANSPORTER NaC3 AS THE NAA TRANSPORTER** .............. 67
   Vadivel Ganapathy and Takuya Fujita

6. **CANDIAN DISEASE: STUDIES ON THE KNOCKOUT MOUSE** ........... 77
   Reuben Matalon, Kimberlee Michals-Matalon, Sankar Surendran and Stephen K. Tyring

7. **FUNCTIONS OF N-ACETYLASPARTATE AND N-ACETYLASPARTYLGLUTAMATE IN BRAIN: Evidence of a Role in Maintenance of Higher Brain Integrative Activities of Information Processing and Cognition** ......................................................... 95
   Morris H. Baslow and David N. Guilfoyle

8. **CONTROL OF BRAIN VOLUME DURING HYPOOSMOLALITY AND HYPEROSMOLALITY** ............................................................... 113
   Joseph G. Verbalis
9. PHYSIOLOGICAL ROLE OF $N$-ACETYLASPARTATE: Contribution to Myelinogenesis

Robert W. Ledeen, Jianfeng Wang, Gusheng Wu, Zi-Hua Lu, Goutam Chakraborty, Markus Meyenhofer, Stephen K. Tyring, and Reuben Matalon

10. DEFECTIVE MYELIN LIPID SYNTHESIS AS A PATHOGENIC MECHANISM OF CANAVAN DISEASE

Aryan M. A. Namboodiri, John R. Moffett, Arun Peethambaran, Raji Mathew, Sreela Namboodiri, Asha Potti, Jeremy Hershfield, Batool Kirmani, David M. Jacobowitz and Chikkathur N. Madhavarao

11. MUTATION ANALYSIS OF THE ASPARTOACYLASE GENE IN NON-JEWISH PATIENTS WITH CANAVAN DISEASE

Bai-Jin Zeng, Gregory M. Pastores, Paola Leone, Srinivasa Raghavan, Zhao-Hui Wang, Lucilene A. Ribeiro, Paola Torres, Elton Ong, and Edwin H. Kolodny

12. DOES ASPA GENE MUTATION IN CANAVAN DISEASE ALTER OLIGODENDROCYTE DEVELOPMENT? A Tissue Culture Study of ASPA KO Mice Brain

Shalini Kumar, Rasika Sowmyalakshmi, Sarah L. Daniels, Ruth Chang, Sankar Surendran, Reuben Matalon and Jean de Vellis

13. QUANTITATION OF NAA IN THE BRAIN BY MAGNETIC RESONANCE SPECTROSCOPY

Peter B. Barker, David Bonekamp, Gerard Riedy and Mari Smith

14. $N$-ACETYL-L-ASPARTATE IN MULTIPLE SCLEROSIS

Gerson A. Criste and Bruce D. Trapp

15. NAA AND HIGHER COGNITIVE FUNCTION IN HUMANS

Ronald A. Yeo, William M. Brooks and Rex E. Jung

16. IN VIVO NMR MEASURES OF NAA AND THE NEUROBIOLOGY OF SCHIZOPHRENIA

Stefano Marenco, Alessandro Bertolino and Daniel R. Weinberger

17. $N$-ACETYLASPARTATE AS A MARKER OF NEURONAL INJURY IN NEURODEGENERATIVE DISEASE

Norbert Schuff, Dieter J. Meyerhoff, Susanne Mueller, Linda Chao, Diana Truran Sacrey, Kenneth Laxer and Michael W. Weiner

18. REGULATION OF NAA-SYNTHESIS IN THE HUMAN BRAIN

IN VIVO: Canavan’s Disease, Alzheimer’s Disease and Schizophrenia

Kent Harris, Alexander Lin, Pratip Bhattacharya, Thao Tran, Willis Wong and Brian Ross
19. MAGNETIC RESONANCE SPECTROSCOPY FOR MONITORING NEURONAL INTEGRITY IN AMYOTROPHIC LATERAL SCLEROSIS ......................................................... 275
Sanjay Kalra and Douglas L. Arnold

20. HYPOACETYLASPARTIA: CLINICAL AND BIOCHEMICAL FOLLOW-UP OF A PATIENT ........................................................................ 283
Alessandro P. Burlina, B. Schmitt, U. Engelke, Ron A. Wevers,
Alberto B. Burlina and Eugen Boltshauser

21. CELLULAR LOCALIZATION OF NAAG ........................................................................ 289
Suzannah Bliss Tieman

22. SYNTHESIS OF N-ACETYLASPARTYLGLUTAMATE (NAAG) AND N-ACETYLASPARTATE (NAA) IN AXONS AND GLIA OF THE CRAYFISH MEDIAL GIANT NERVE FIBER ..................................................................... 303
Edward M. Lieberman, Mohit Achreja and Albert K. Urazaev

23. NAAG AS A NEUROTRANSMITTER ........................................................................ 317
Barbara Wroblewska

24. GLUTAMATE CARBOXYPEPTIDASE II (NAALADase) INHIBITION AS A NOVEL THERAPEUTIC STRATEGY ......................................................... 327
Ajit G. Thomas, Krystyna M. Wozniak, Takashi Tsukamoto, David Calvin,
Ying Wu, Camilo Rojas, James Vornov and Barbara S. Slusher

25. N-ACETYLASPARTYLGLUTAMATE (NAAG) IN SPINAL CORD INJURY AND DISEASE ........................................................................ 339
James L. Meyerhoff, Debra L. Yourick, Barbara S. Slusher and
Joseph B. Long

26. N-ACETYLASPARTYLGLUTAMATE (NAAG) IN PELIZAEUS-MERZBACHER DISEASE ........................................................................ 353
Alessandro P. Burlina, Vanni Ferrari, Alberto B. Burlina, Mario Ermani,
Odile Boespflug-Tanguy, Enrico Bertini, and the Clinical European
Network on Brain Dysmyelinating Disease (ENBDD)

27. CONCLUDING REMARKS ........................................................................ 361

INDEX ........................................................................................................ 365