Amino Acids (2010) 39:1139–1145
DOI 10.1007/s00726-010-0656-6

MINIREVIEW ARTICLE

Evidence that the tri-cellular metabolism of *N*-acetylaspartate functions as the brain's "operating system": how NAA metabolism supports meaningful intercellular frequency-encoded communications

Morris H. Baslow

Received: 24 January 2010 / Accepted: 7 June 2010 / Published online: 19 June 2010

Abstract *N*-acetylaspartate (NAA), an acetylated derivative of L-aspartate (Asp), and *N*-acetylaspartylglutamate (NAAG), a derivative of NAA and L-glutamate (Glu), are synthesized by neurons in brain. However, neurons cannot catabolize either of these substances, and so their metabolism requires the participation of two other cell types. Neurons release both NAA and NAAG to extra-cellular fluid (ECF) upon stimulation, where astrocytes, the target cells for NAAG, hydrolyze it releasing NAA back into ECF, and oligodendrocytes, the target cells for NAA, hydrolyze it releasing Asp to ECF for recycling to neurons. This sequence is unique as it is the only known amino acid metabolic cycle in brain that requires three cell types for its completion. The results of this cycling are two-fold. First, neuronal metabolic water is transported to ECF for its removal from brain. Second, the rate of neuronal activity is coupled with focal hyperemia, providing stimulated neurons with the energy required for transmission of meaningful frequency-encoded messages. In this paper, it is proposed that the tri-cellular metabolism of NAA functions as the "operating system" of the brain, and is essential for normal cognitive and motor activities. Evidence in support of this hypothesis is provided by the outcomes of two human inborn errors in NAA metabolism.

Keywords Aquaporins · Brain · Molecular water pumps · Neurons · Free water · Bound water

M. H. Baslow (✉)
Center for Neurochemistry, Nathan S. Kline Institute for Psychiatric Research, 140 Old Orangeburg Road, Orangeburg, NY 10962, USA
e-mail: Baslow@nki.rfmh.org

Abbreviations

Ac	Acetate
AcCoA	Acetyl coenzyme A
Asp	Aspartate
APQ4	Aquaporin 4
ASPA	Aspartoacylase
BBB	Blood brain barrier
ECF	Extracellular fluid
fMRI	Functional magnetic resonance imaging
fMRS	Functional magnetic resonance spectroscopy
Glc	Glucose
Gln	Glutamine
Glu	Glutamate
GRM3	Metabotropic Glu receptor 3
MWP	Molecular water pump
NAA	*N*-acetylaspartate
NAAG	*N*-acetylaspartylglutamate

Introduction

N-acetylaspartate (NAA), an acetylated derivative of L-aspartate (Asp), and *N*-acetylaspartylglutamate (NAAG), a dipeptide derivative of NAA and L-glutamate (Glu), are metabolically linked substances that are synthesized by neurons in brain (Baslow 2007). However, neurons cannot catabolize either of these substances, and so their metabolism requires the participation of three cell types for its completion. These include neurons, where NAA and NAAG are synthesized and then released to extra-cellular fluid (ECF) upon neurostimulation, astrocytes, the target cells for NAAG, which can hydrolyze NAAG but not NAA, and oligodendrocytes, the target cells for NAA, which can

hydrolyze NAA but not NAAG. In this cycling, the NAA by-product of astrocyte NAAG hydrolysis, along with NAA exported directly to ECF by neurons, are hydrolyzed by oligodendrocytes liberating Asp, that is recycled to neurons, and acetate (Ac), that is further metabolized by oligodendrocytes. This sequence is unique as it is the only tri-cellular amino acid metabolic cycle known to occur in brain.

The results of this cycling are two-fold. First, stimulation-released NAA and NAAG both play a role in maintaining neuronal osmotic and structural integrity by transporting metabolic and other water to ECF against a water gradient for its removal from the brain (Baslow 2007). Second, the released NAAG initiates astrocyte-mediated signaling to capillary endothelial cells inducing a focal hyperemic response, thus coupling the rate of neuronal activity with blood flow and providing stimulated neurons with the energy required to transmit meaningful frequency-encoded messages (Baslow and Guilfoyle 2007). In this paper, a hypothesis is presented that the tri-cellular metabolism of NAA serves as the "operating system" in brain, and is essential for maintenance of normal cognitive and motor functions. Evidence is provided based on the outcomes of two human inborn errors in NAA metabolism that strongly supports this hypothesis.

Discussion

NAA and NAAG metabolism

NAA was first discovered as a bound form of Asp in cat brain in 1954 (Tallan et al. 1954) and its structure reported in 1956 (Tallan et al. 1956). NAAG was first discovered in horse brain in 1965 (Curatolo et al. 1965). In 2000, the unique tri-cellular metabolism of these two substances in brain was described for the first time (Baslow 2000).

NAA metabolism and function

NAA is synthesized in neurons from acetyl-coenzyme A (AcCoA) and Asp. The AcCoA is derived from the oxidation of glucose (Glc) and is joined to Asp by an NAA synthase, aspartate *N*-acetyltransferase 8L (Wiame et al. 2010). NAA, a dedicated form of Asp that blocks the use of this pool of Asp for any other cellular metabolic pathways, builds to high concentrations in neurons where it is maintained at a level of about 20 mM, and with an intracellular/extracellular NAA gradient of >200. Upon stimulation, NAA is released to ECF down its gradient and is targeted to oligodendrocytes, the only cell type that can catabolize NAA, where it is hydrolyzed by aspartoacylase (ASPA) (Bitto et al. (2007) liberating Asp and Ac. The Ac is taken up by oligodendrocytes for further metabolism, and the Asp is actively taken up by neurons for recycling into NAA. In this process, for each molecule of NAA released, at least 32 molecules of NAA-obligated water are also transported to ECF against a water gradient. Upon NAA hydrolysis, the NAA-obligated water is released as free water for removal from brain via aquaporin-4 (AQP4) free water channels present on the surfaces of astrocytes and vascular capillary endothelial cells at the glia limitans of the blood brain barrier (BBB) (Baslow and Guilfoyle 2007). This mechanism is needed since neurons are especially insulated from the movement of free water across their plasma membranes. In that, they do not express AQP4 free water channels (Agre et al. 2002), nor any of the other 12 known AQPs in any abundance (Buffoli 2010), and experimentally appear to be largely water impermeable (Andrew et al. 2007). Neurons also do not form gap junctions with their glial chaperone cells for movement of either free or bound water (Rash et al. 1997). Thus, neurons appear to rely on the co-transport of solute-bound water, both for influx and efflux of water, in order to regulate their osmolarity. These kinds of pumps wherein a solute travels down its gradient across a plasma membrane and co-transports water against a water gradient have been called "molecular water pumps" (MWP's) (Baslow 1999; Meinild et al. 1998). As part of this co-transport process, neuronal NAA is released at about 6%/h so that all NAA turns over every 16 h or about 1.5 times per day (Baslow 2007).

NAAG metabolism and function

There is only one metabolic pathway available for NAA in neurons, and that is to form an adduct of NAA and Glu. NAAG is synthesized from NAA and Glu by an NAAG synthase, forming a dedicated pool of Glu that also cannot be further metabolized, and NAAG is maintained at a concentration of about 1 mM. Like NAA, NAAG is liberated to ECF upon stimulation at about 6%/h. NAAG is a non-excitotoxic form of the neurotransmitter Glu that is targeted to astrocytes (Baslow 2009a) where it first docks with the astrocyte surface Glu metabotropic receptor 3 (GRM3). NAAG is then hydrolyzed by NAAG peptidase releasing Glu which activates the GRM3 receptor. In this process, NAA-water is liberated back to ECF, and the Glu moiety taken up and converted into relatively non-excitatory glutamine (Gln) by astrocytes prior to recycling it back to neurons. In this process 21 of the 53 molecules of NAAG-obligated water are also released as free water to ECF. The activation of the GRM3 receptor initiates Ca^{++} oscillations in astrocytes that result in release of second messengers to the endothelial cells that form the capillaries of the vascular system. This activation initiates a focal hyperemic response, increasing the availability of Glc and O_2 to stimulated neurons, as well as providing increased water sink capacity for free water (Baslow 2007).

The normal operation of the NAA–NAAG tri-cellular metabolic system

The normal operation of the tri-cellular metabolism of NAA and NAAG in brain which couples the signaling activity of neurons with the inter-compartmental movement of water and supply of energy is illustrated in Fig. 1.

In this cartoon, the normal NAA and NAAG intercellular metabolic cycles are shown as dashed arrows, and their interactions with the vascular system as solid arrows. Since NAA is synthesized and released at about ten times the rate of that of NAAG (Baslow 2007), NAA is the major transporter of neuronal metabolic water to ECF, where its hydrolysis releases its bound water as free water. However, the role of NAAG is equally important to brain function in that its stimulatory effect on astrocytes affects both the rate at which free water can be transported out of the brain and the rate at which energy can be supplied to stimulated neurons. Thus, the tri-cellular metabolism of NAA provides neurons with a homeostatic mechanism operating on a focal level where the rate of neurostimulation affects the rate of blood flow, the blood volume, and the availability of energy (Smith et al. 2002). These dynamic neurostimulation-induced focal changes in blood flow and blood volume can be observed as areas of "brain activation" using blood oxygenation-level dependent (BOLD) "functional" magnetic resonance imaging (fMRI) as well as several other techniques (Baslow and Guilfoyle 2007). Other fMRI and functional MR spectroscopy (fMRS) studies have been used to demonstrate the dynamic changes in levels of NAA, Glc, and the ratio of bound to free water that are induced by neurostimulation. The modular nature of this system, which requires four cell types for its metabolic and physiological functions, also suggests that the NAA–NAAG system may define the basic unit of brain structure.

Interaction of NAA and NAAG metabolism with cognitive and motor activities

The tri-cellular metabolism of NAA and NAAG operates as a linked system

While there have been many attempts to try to understand the functions of NAA and NAAG in brain, most have tried

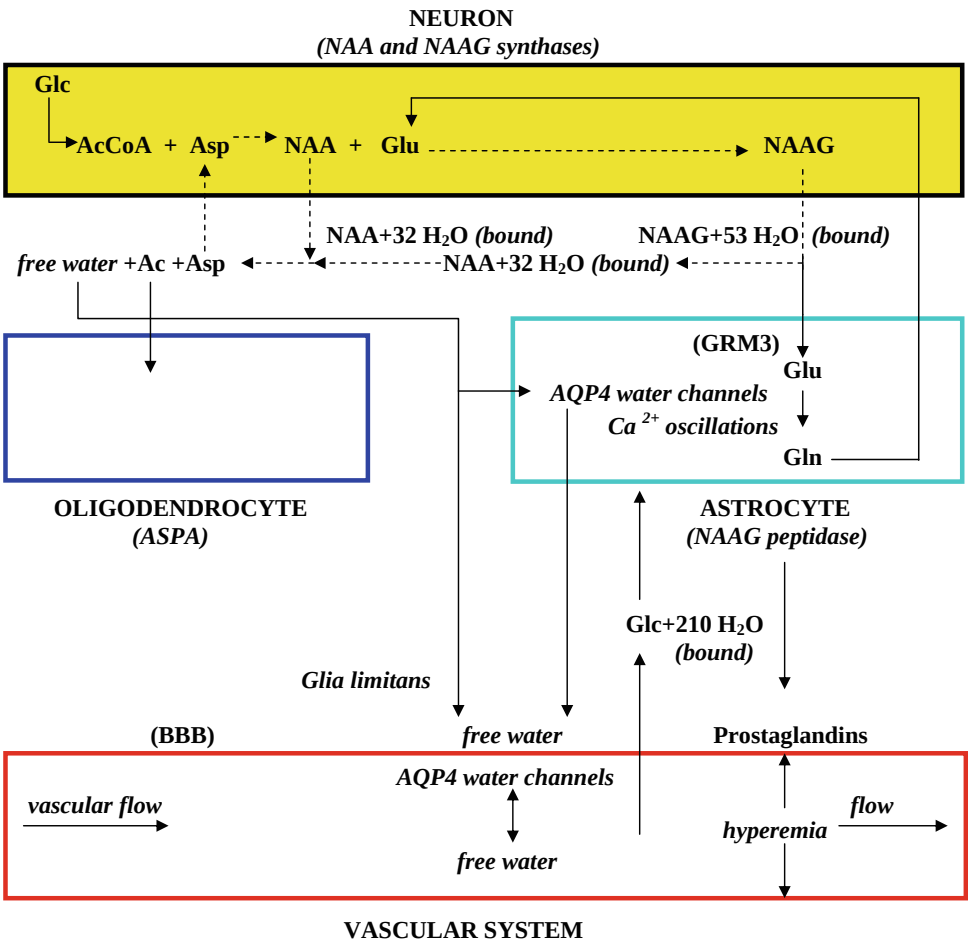

Fig. 1 Overview of normal brain metabolism of NAA, NAAG, Glc, and Glu showing their coupling with hyperemia, energy availability, and the movement of free and bound water

to do this by analyzing the physiological roles of these substances and their metabolism separately rather than as linked components in the overall functioning of the brain. Based on what is known about the complex tri-cellular metabolism of the NAA–NAAG system, it is reasoned that their roles in brain can only be fully understood if one considers their individual functions as part of an overall scheme that allows neurons to communicate successfully with one another (Baslow 2009b).

Structural and energy requirements of neurons

The basic function of neurons is to communicate, and they do this using energy to generate coherent intracellular electrophysiological signals, in the form of action potentials or "spikes", at various encoded frequencies. These signals are then translated into a variety of frequency-encoded neurochemical signals transmitted to other neurons at synapses, and subsequently interpreted at some level in the central nervous system neural network for both cognitive and motor functional activities (Baslow 2009b). In order to carry out their basic communication function, two things are required by neurons. First, their complex morphology within the neural network must be maintained, and second, a timely access to energy must be available. To maintain their structural integrity, neurons have to regulate the movement of water so that they do not swell and compromise any of their complex physical inter-neuronal connections. In order to provide adequate energy for spiking, neurons, which contain limited energy resources, must have a mechanism for rapidly increasing energy availability from the vascular system as a function of the rate of spiking. These operations involving the transport of water and supply of energy clearly depend on a close coordination between neuron activity and vascular system dynamics.

NAA metabolism functions as the "operating system" in brain

Based on the unique tri-cellular metabolism of NAA and on the likely physiological roles of NAA and its anabolic product, NAAG, the reasons for the linked metabolism of NAA and NAAG in neurons and the unusual inter-compartmental metabolism of NAA and NAAG become apparent. Simply stated, NAA and NAAG, both appear to function as MWP's in transporting neuronal metabolic water to ECF and in doing so they participate in the maintenance of the structural integrity of neurons. The additional physiological role of released NAAG as a neurotransmitter to astrocytes provides neurons with focal and timely access to the large amounts of energy required for neuronal signaling activity and with additional sink capacity for its metabolic water. Taken together, these functions insure that neurons are able to continuously transmit meaningful messages both within the brain and to the rest of the body, regardless of the bioenergetic demands made on any specific part of the brain and its neural network at any given time. This analysis leads to the hypothesis presented in this article which proposes that the tri-cellular metabolism of NAA serves as the primary "operating system" for brain function. As a corollary, it also leads to the conclusion that any failure in this operating system should result in impaired cognitive and motor functions.

Inborn errors in the metabolism of NAA and NAAG

There are two human inborn errors in the metabolism of NAA that result in cognitive and motor function impairments. One is Canavan disease (hyperacetylaspartia), an osmotic disease where the absence of aspartoacylase activity in oligodendrocytes results in the build up of NAA and NAAG in brain ECF. The other is the lack of NAA synthase activity in neurons (hypoacetylaspartia) which results in the absence of both NAA and NAAG in neurons. The biochemical, metabolic and physiological consequences of these inborn errors are assessed in terms of the etiology of these profoundly debilitating brain diseases and their association with failure of the brains' NAA "operating system".

Canavan disease (hyperacetylaspartia)

Canavan disease (CD) is a rare globally distributed early-onset spongiform leukodystrophy (Baslow 2007; Matalon et al. 1988). It is genetic in nature, and the syndrome results from a number of different mutations in the gene that encodes for ASPA, all of which result in inactive forms of this enzyme instead of the normal enzyme that is expressed in oligodendrocytes. The CD mutations are autosomally inherited and recessive traits, and usually, but not always, result in early mortality. CD is a progressive disease, and clinical symptoms associated with the typical CD phenotype are observed within the first few months of life. These early symptoms are characterized by initial apathy and difficulty in holding the head erect, as well as in an abnormally large head size. As the disease progresses, there is usually optic atrophy, a pronounced megaloencephaly, ataxia, myoclonus and some form of epilepsy. At later stages, there is considerable psychomotor and mental retardation and a failure to meet all normal developmental milestones such as holding the head up, grasping at objects, speech and walking. Before death there may be spasticity and paralysis. In CD, the most important clinical clue to disease diagnosis, and one that distinguishes it from

all other leukodystrophies, is the finding of a 20–30% increase in levels of NAA in the brain and the presence of NAA and NAAG in blood and urine of these patients. Pathologically, CD is an encephalitis periaxialis diffusa in which the myelinating oligodendrocytes in white matter fail, and in doing so leave the axons intact but unsheathed and therefore without normal function. The spongiform condition is due to the presence of large vacuoles that form mostly in white matter and primarily in the intramyelinic spaces of disrupted myelin sheaths.

While the CD phenotype is complex, the etiology of the disease and its clinical course are predictable based on the hypothesis that the tri-cellular metabolism of NAA functions as the primary operating system in the brain, and that a specific function of exported NAA is that of a MWP. The metabolic sequence that is disrupted in CD is shown in Fig. 2.

In this cartoon, the point of metabolic block in CD is shown as a bar, and the missing parts of the normal NAA–NAAG inter-compartmental cycles are shown in gray. As a result of the absence of ASPA activity in CD, there is a failure of the NAA-water ASPA release mechanism. This failure, coupled with the inability of NAA or NAAG to easily pass through the BBB, results in the build up of both NAA and NAAG in ECF. Since these substances are associated with large amounts of obligated water, an osmotically-induced pathology would be anticipated. Thus, the etiology of the CD syndrome can be traced directly to the failure of the NAA-water ASPA release mechanism and its osmotic consequences.

Based only on the hypothesized NAA brain "operating system" paradigm, failure of the ASPA water release mechanism can be predicted to result in a syndrome that includes elevated levels of NAA and NAAG in brain, megalocephaly due to water retention, and based on a known "osmotic demyelination syndrome", extensive white matter spongiform vacuolization and resulting leukodystrophy (Baslow and Guilfoyle 2009). Furthermore, based on a requirement for maintenance of the structural integrity of neurons in order to carry out their normal functioning, the consequences of edema and demyelination would also be expected to result in disrupted inter-neuronal communications leading to profound loss of normal cognitive and motor functions. Clearly, the theoretical effects of absence of ASPA activity in the "operating system" model predict to a large degree the phenotype associated with CD.

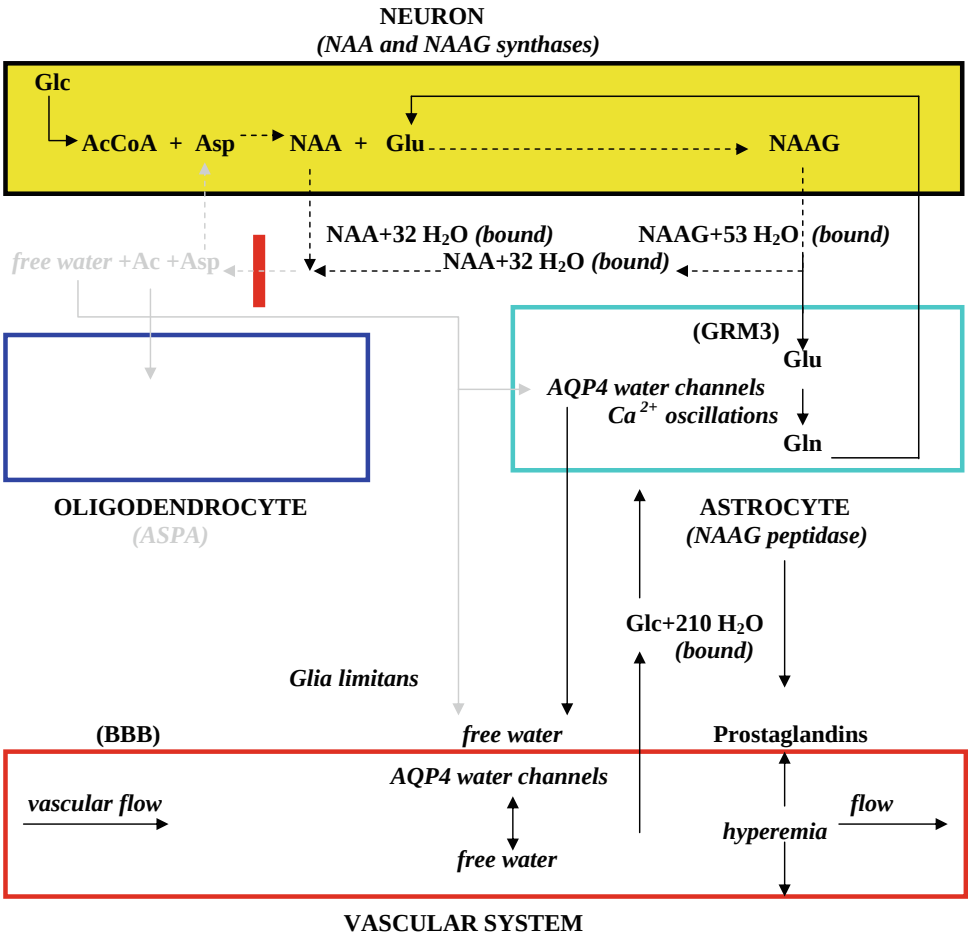

Fig. 2 Overview of NAA metabolism in Canavan disease (hyperacetylaspartia), an osmotic disease where the absence of ASPA activity results in the buildup of NAA–H_2O and NAAG–H_2O in ECF with osmotic consequences

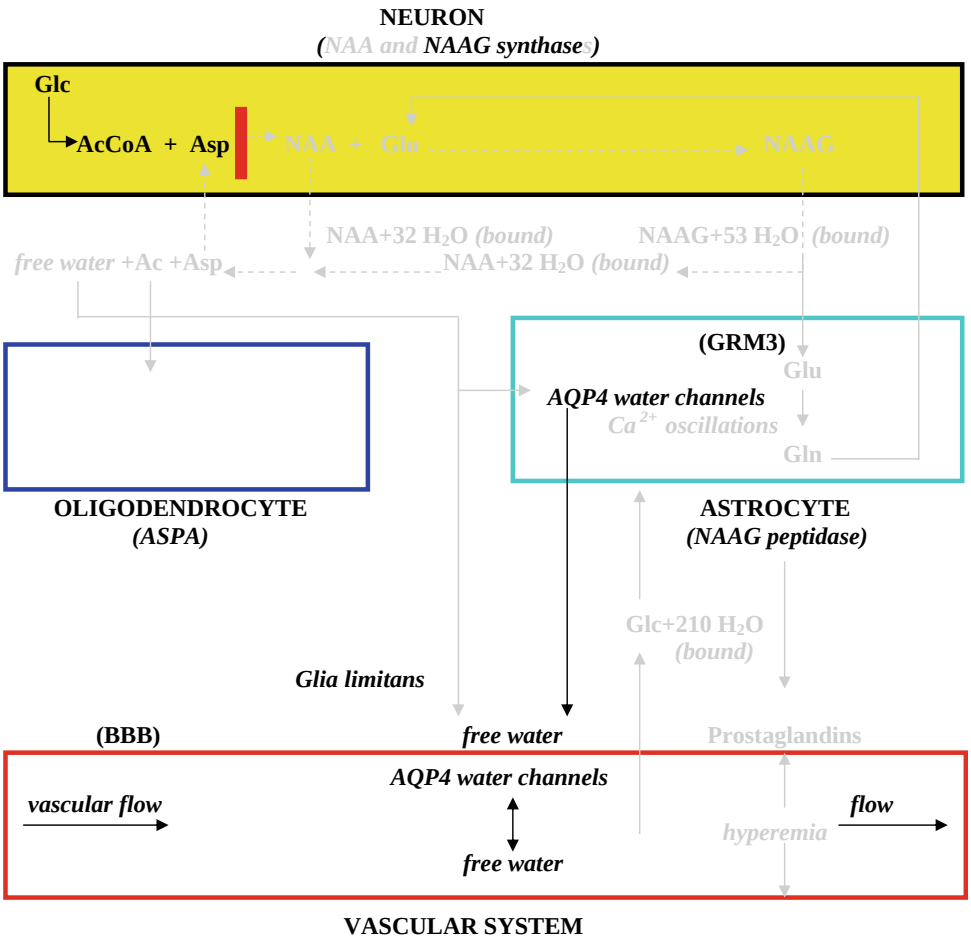

Fig. 3 Overview of NAA and NAAG metabolism in brain in hypoacetylaspartia where the absence of neuronal NAA synthase effectively decouples ongoing neuronal activity from required energy supplies

Hypoacetylaspartia

A second inborn error in NAA metabolism has been found in a singular human case of lack of brain NAA and NAAG (Martin et al. 2001). While the early effects in this case were reported to be comparatively mild, a follow-up of this patient revealed that by age 8, the effects were profound, with microcephaly, marked abnormalities in motor performance, language ability and cognition, and with episodic status epilepticus (Boltshauser et al. 2004). This patient is presently 12 years of age and has provided DNA used in a study to find the nature of the defect responsible for the disease syndrome. Genetic analysis of the DNA in this singular case revealed that the patient was homozygous for a 19 bp deletion in the genetic code for production of *N*-acetyltransferase 8L, the enzyme that synthesizes NAA from AcCoA and Asp (Wiame et al. 2010), and this appears to be the primary deficiency associated with this disease. The metabolic sequence that is now known to be disrupted in hypoacetylaspartia is shown in Fig. 3. In this cartoon, the point of metabolic block is shown as a bar, and the missing pathways in the NAA–NAAG system in gray.

In terms of the hypothesized NAA "operating system" model, this defect would effectively inactivate the entire NAA and NAAG MWP system, and importantly, it would decouple the homeostatic NAAG–astrocyte mechanism used by stimulated neurons to signal their requirements for additional energy for ongoing communication activities. Inactivation of the entire NAA–NAAG MWP system should not affect water movement in neurons since there are many other MWP's that are available to cells. However, based on the model, one would anticipate that the failure to synthesize NAA or NAAG would be manifested primarily by the inability of stimulated neurons to obtain energy in a timely manner either on a focal or a global level, thus affecting the precision of inter-neuronal signaling. This should result in cognitive and motor abnormalities, adversely affecting performance, a predictable outcome that is reflected in the clinical phenotype associated with this disease.

Conclusions

While there is at present little question regarding the unique tri-cellular metabolism of NAA and NAAG (Baslow 2000), there is as yet no general consensus regarding the specific roles of either NAA or NAAG in brain, especially that of NAA (Moffett et al. 2007). In this paper, it is proposed that neuron-generated NAA and NAAG function primarily as a metabolically linked system in order to provide for two basic needs of neurons. These include removal of the large amounts of metabolic water produced as a result of their ongoing signaling activities, and the timely supply of adequate energy resources to insure their ability to transmit meaningful frequency-encoded signals. Clearly, both these functions are required for maintenance of the brain's normal cognitive and motor activities and both depend on the dynamic interaction between stimulated neurons and the vascular system. As a hypothesis, it is suggested that the unique tri-cellular metabolism of NAA functions as the primary "operating system" in brain that continuously carries out an important neuron–vascular system dialogue that allows the brain to carry out its complex and integrated activities. Evidence is presented that in two inborn errors in NAA metabolism where the neuron–vascular system dialogue fails for different reasons, there are profound deficits in brain function. Finally, based on the operating system paradigm presented in this paper, it has also been possible to identify the probable etiology in each of these inborn errors and match expected outcomes of their specific metabolic blocks with their observed clinical syndromes. It is hoped that the NAA-operating system concept developed in this paper will lead to novel insights into how the brain functions, and perhaps be of use in understanding the etiologies of several additional neuropathies as well as in identifying potential targets for specific drug therapies such as are currently being investigated in the case of the astrocyte surface GRM3–NAAG peptidase complex (Baslow 2006, 2008).

Conflict of interest statement None.

References

Agre P, King LS, Yasui M, Guggino WB, Ottersen OP, Fujiyoshi Y, Engel A, Neilsen S (2002) Aquaporin water channels-from atomic structure to clinical medicine. J Physiol 542:3–16

Andrew RD, Labron MW, Boehnke SE, Carnduff L, Kirov SA (2007) Physiological evidence that pyramidal neurons lack functional water channels. Cereb Cortex 17:787–802

Baslow MH (1999) The existence of molecular water pumps in the nervous system. A review of the evidence. Neurochem Int 34:77–90

Baslow MH (2000) Functions of *N*-acetyl-L-aspartate and *N*-acetyl-L-aspartylglutamate in the vertebrate brain. Role in glial cell-specific signaling. J Neurochem 75:453–459

Baslow MH (2006) NAAG peptidase as a therapeutic target: Potential for regulating the link between glucose metabolism and cognition. Drug News Perspect 19(3):145–150

Baslow MH (2007) *N*-Acetylaspartate and *N*-acetylaspartylglutamate, Chap 14. In: Lajtha A (ed) Handbook of neurochemistry and molecular neurobiology, vol 6, 3rd edn, Amino acids and peptides in the nervous system, pp 418, Springer Science, New York, pp 305–346

Baslow MH (2008) The astrocyte surface NAAG receptor and NAAG peptidase signaling complex as a therapeutic target. Drug News Perspect 21(5):251–257

Baslow MH (2009a) A novel key-lock mechanism for inactivating amino acid neurotransmitters during transit across extracellular space. Amino Acids (doi10.1007/s00726-009-0232-0)

Baslow MH (2009b) The languages of neurons; an analysis of coding mechanisms by which neurons communicate, learn and store information. Entropy 11(4):782–797

Baslow MH, Guilfoyle DN (2007) Using proton magnetic resonance imaging and spectroscopy to understand brain "activation". Brain Lang 102(2):153–164

Baslow MH, Guilfoyle DN (2009) Are astrocytes the missing link between lack of brain aspartoacylase activity and the spongiform leukodystrophy in Canavan disease? Neurochem Res 34(9):1523–1534

Bitto E, Bingman CA, Wesenberg GE et al (2007) Structure of aspartoacylase, the brain enzyme impaired in Canavan disease. PNAS 104(2):456–461

Boltshauser E, Schmitt B, Wevers RA, Engelke U, Burlina AB, Burlina AP (2004) Follow-up of a child with hypoacetylaspartia. Neuropediatrics 35(4):255–258

Buffoli B (2010) Aquaporin biology and nervous system. Curr Neuropharmacol 8:97–104

Curatolo A, D'Archangelo P, Lino A, Brancati A (1965) Distribution of *N*-acetylaspartic and *N*-acetylaspartylglutamic acids in nervous tissue. J Neurochem 12:339–342

Martin E, Capone A, Schneider J, Hennig J, Thiel T (2001) Absence of *N*-acetylaspartate in the human brain: impact on neurospectroscopy? Ann Neurol 49:518–521

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

Meinild A-K, Klaerke DA, Loo DDF, Wright EM, Zeuthen T (1998) The human Na^+-glucose cotransporter is a molecular water pump. J Physiol 508(1):l5–21

Moffett JR, Ross B, Arun P, Madhavarao CN, Namboodiri AM (2007) *N*-acetylaspartate in the CNS: from neurodiagnostics to neurobiology. Prog Neurobiol 81:89–131

Rash JE, Duffy HS, Dudek FE, Bilhartz BL, Whalen LR, Yasumura T (1997) Grid-mapped freeze-fracture analysis of gap junctions in gray and white matter of adult rat central nervous system, with evidence for a "panglial syncytium" that is not coupled to neurons. J Comp Neurol 388(2):265–292

Smith AJ, Blumenfeld H, Behar KL, Rothman DL, Shulman RG, Hyder F (2002) Cerebral energetics and spiking frequency: the neurophysiological basis of fMRI. Proc Natl Acad Sci USA 99:10765–10770

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

Tallan HH, Moore S, Stein WH (1956) *N*-acetyl-L-aspartic acid in brain. J Biol Chem 219:257–264

Wiame E, Tyteca D, Pierrot N, Collard F, Amyere M, Noel G, Desmedt J et al (2010) Molecular identification of aspartate N-acetyltransferase and its mutation in hypoacetylaspartia. Biochem J 425:127–136