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A novel key–lock mechanism for inactivating amino acid neurotransmitters during transit across extracellular space

Morris H. Baslow

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Abstract There are two kinds of neurotransmissions that occur in brain. One is neuron to neuron at synapses, and the other is neuron to glia via extracellular fluid (ECF), both of which are important for maintenance of proper neuronal functioning. For neuron to neuron communications, several potent amino acid neurotransmitters are used within the confines of synaptic space. However, their presence at elevated concentrations in extra-synaptic space could be detrimental to well organized neuronal functioning. The significance of the synthesis and release of *N*-acetylaspartylglutamate (NAAG) by neurons has long been a puzzle since glutamate (Glu) itself is the "key" that can interact with all Glu receptors on membranes of all cells. Nonetheless, neurons synthesize this acetylated dipeptide, which cannot be catabolized by neurons, and release it to ECF where its specific physiological target is the Glu metabotropic receptor 3 on the surface of astrocytes. Since Glu is excitotoxic at elevated concentrations, it is proposed that formation and release of NAAG by neurons allows large quantities of Glu to be transported in ECF without the risk of injurious excitotoxic effects. The metabolic mechanism used by neurons is a key–lock system to detoxify Glu during its intercellular transit. This is accomplished by first synthesizing *N*-acetylaspartate (NAA), and then joining this molecule via a peptide bond to Glu. In this paper, a hypothesis is presented that neurons synthesize a variety of relatively nontoxic peptides and peptide derivatives, including NAA, NAAG, homocarnosine (γ-aminobutyrylhistidine) and carnosine (β-alanylhistidine) from potent excitatory and inhibitory amino acids for the purpose of releasing them to ECF to function as cell-specific neuron-to-glia neurotransmitters.

Keywords Brain · *N*-acetylaspartate · *N*-acetylaspartylglutamate · Carnosine · Homocarnosine · Glutamate · Metabotropic glutamate receptor 3

Abbreviations

Ac	Acetate
AcCoA	Acetylcoenzyme A
Asp	Aspartate
ASPA	Aspartoacylase
ATP	Adenosine triphosphate
β-ala	β-Alanine
CD	Canavan disease
Carn	Carnosine
CSF	Cerebrospinal fluid
ECF	Extracellular fluid
ECS	Extracellular space
GABA	γ-Aminobutyric acid
Glu	Glutamate
GRM3	Metabotropic Glu receptor 3
Hcarn	Homocarnosine
NAA	*N*-acetylaspartate
NAAG	*N*-acetylaspartylglutamate

Introduction

The functional significance of the synthesis of *N*-acetylaspartylglutamate (NAAG) from *N*-acetylaspartate (NAA) and glutamate (Glu) by neurons has long been a puzzle (Baslow 2000; Coyle 1997). However, neurons synthesize this acetylated dipeptide neurotransmitter, which cannot be

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

catabolized by neurons, and then release it to extracellular space (ECS) upon stimulation, where its specific physiological target is the Glu metabotropic receptor 3 (GRM3) present on the astrocyte surface (Schoepp et al. 1999). NAAG has a high affinity for this receptor, but itself cannot activate the receptor after docking with it (Woolley et al. 2008). In order to activate the receptor and initiate individual astrocyte Ca^{++} oscillations and subsequent gliotransmissions by release of second messengers such as prostaglandins and ATP to other astrocytes and the vascular system (Bowser and Khakh 2007), the Glu must first be released by the action of astrocyte NAAG peptidase, an enzyme that appears to exist as a linear GRM3-NAAG peptidase surface complex (Baslow 2008). The end result of GRM3 activation is a rapid (<2 s latency) increase in focal cerebral blood flow which serves to satisfy enhanced neuronal requirements for increased energy and removal of waste products (Filosa et al. 2006).

Discussion

The synthesis of NAAG, an archetypal key–lock Glu detoxifying mechanism

Since Glu is an excitotoxic amino acid and also the "key" to activation of all synaptic and glial surface Glu receptors, it is proposed that the formation and release of NAAG by stimulated neurons upon depolarization (Shah et al. 2008) allows Glu to be transported via extracellular fluid (ECF) to nearby glia without the risk of neuronal excitotoxic effects. The metabolic mechanism used by neurons to do this is a key–lock mechanism which is able to detoxify Glu for its intercellular transit. This is accomplished in neurons by first synthesizing NAA from AcCoA and aspartate (Asp), and then joining this *N*-acetylated amino acid via a peptide bond to Glu, forming NAAG for use in an activation sequence at the astrocytic surface GRM3 receptor. The NAAG molecule formed is unique in that it is not only non-excitotoxic when present in ECF, but the NAA component also imparts a specificity for the GRM3 receptor, one of eight known GRM receptors (Schoepp et al. 1999). The structure and synthesis of NAAG are presented in Fig. 1.

After docking, Glu is released from NAAG for its GRM3 activation role by NAAG peptidase, and the Glu is then metabolized by astrocytes to form glutamine prior to recycling it back to neurons (Baslow and Guilfoyle 2007). The released NAA remains in ECF and subsequently diffuses to and is hydrolyzed by oligodendrocyte surface aspartoacylase (ASPA), the acetate (Ac) taken up by oligodendrocytes for energy production and other metabolism, and the released Asp recycled to neurons. NAA is once again synthesized in neurons from glucose-derived AcCoA and recycled Asp, and

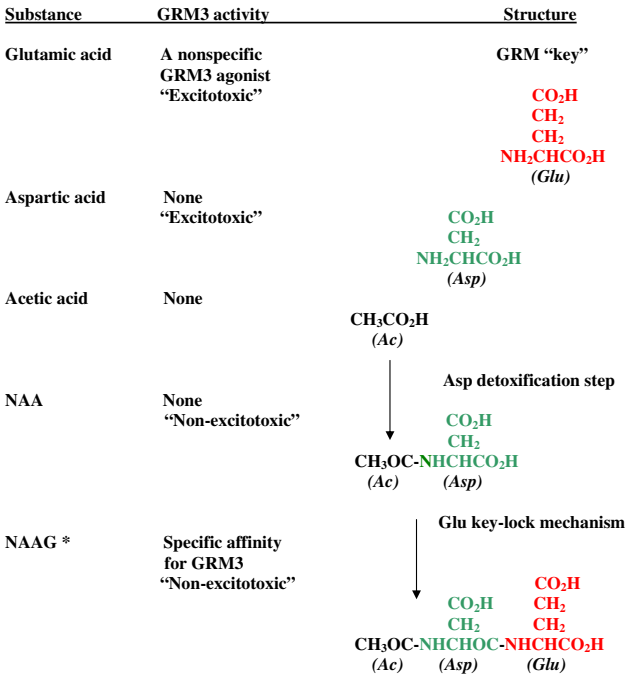

Fig. 1 The chemical synthesis of non-excitotoxic NAAG by neurons

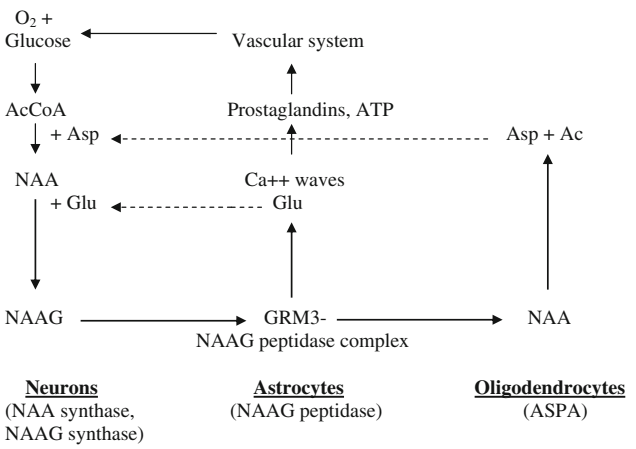

Fig. 2 Metabolic sequences for the NAAG cycle between neurons, astrocytes, and oligodendrocytes in the brain

NAAG is then synthesized from NAA and recycled Glu. This neurotransmitter detoxification system can explain the significance of the synthesis and release of NAAG by neurons, and moreover may represent a common mechanism for using and transporting other neuroactive amino acids to targeted glial receptors via ECF. The intercellular metabolic sequences for the NAAG cycle are shown in Fig. 2.

Synthesis of transport forms of other neuron-to-glia neurotransmitters

Similar neuronal–glial neurotransmitter systems involving intercellular transport of the inhibitory amino acids γ-aminobutyric acid (GABA) and β-alanine (β-ala), using

homocarnosine (Hcarn) (γ-aminobutyrylhistidine) and carnosine (Carn) (β-alanylhistidine) as vehicles, respectively, are known. These structures use histidine ("R") as a deactivating mechanism for β-ala (formula 1) and GABA (formula 2).

Molecular formula 1: (Carn) "R"–NH–CO–CH_2–CH_2–NH_2

Molecular formula 2: (Hcarn) "R"–NH–CO–CH_2–CH_2–CH_2–NH_2

Cellular locations of synthetic and hydrolytic enzymes associated with neuron-to-glia neurotransmitters

Immunohistochemical studies of rat brain (Moffett and Namboodiri 2006), and enzyme studies of cultured rat brain neurons and glial cells have revealed the interconnected relationships between synthetic and hydrolytic compartments for NAAG and other neuron-to-glia neurotransmitters, including Hcarn and Carn as well as NAA (Baslow et al. 1999; Baslow et al. 2001). All of these substances have the potential to transmit real-time neuronal information to specifically targeted glial cells. For NAAG, some of the information transmitted is related to the current level of neuronal activity (Baslow and Guilfoyle 2007). The functions of Hcarn and Carn as neuron-to-glia neurotransmitters are as yet unclear, but evidence from inborn errors in their metabolism suggests that they are very important for normal brain function. A summary of the neuron and glial compartmentalized distribution of these substances and of their synthetic and hydrolytic enzymes in rat brain and in cultured rat brain cells are shown in Table 1.

While these substances are synthesized and stored in neurons, not every neuron type in every area of the brain may synthesize all of them, and in addition, some non-neuronal cells may also be able to synthesize one or more of them as well. However, in general these substances are synthesized by neurons, released to ECF, and astrocytes appear to be a primary target.

Table 1 Cellular locations of neuron-to-glia neurotransmitters and of their synthetic and hydrolytic enzymes in rat brain and cultured brain cells

Cell type[a]	Neurotransmitter	Location	Synthase	Hydrolase
Neurons	NAAG	+	+	−
	NAA	+	+	−
	Hcarn	+	+	−
	Carn	+	+	−
Astrocytes	NAAG	−	−	+
	NAA	−	−	−
	Hcarn	−	−	+
	Carn	−	−	+
Oligodendrocytes	NAAG	−	−	−
	NAA	−	−	+
	Hcarn	−	−	−
	Carn	−	−	+

[a] From their cellular locations and locations of their synthetic and hydrolytic enzymes, the source and targets of these neurotransmitters can be ascertained. Neurons can synthesize all of these substances and maintain them at high cellular to ECF gradients, but generally cannot hydrolyze them. For hydrolysis, they must be released to ECF. Based on the compartmentalization of their catabolic enzymes, upon release they are targeted to specific glial cells. NAA is targeted to oligodendrocytes. NAAG is first targeted to astrocytes, where Glu is hydrolyzed and then the residual NAA diffuses to oligodendrocytes for its hydrolysis. Hcarn is targeted to astrocytes, and Carn is targeted to both astrocytes and oligodendrocytes

Inborn errors of metabolism in targeted neuron-to-glia neurotransmitter systems

In humans, several rare inborn errors in the enzymes that function in synthesis of these neuron-to-glia neurotransmitters, or as hydrolytic release or recycling mechanisms for these substances are associated with profound neurological deficits.

Canavan disease (hyperacetylaspartia): a lack of ASPA activity

Canavan disease (CD) is a recessive genetic neurodegenerative brain disorder associated with many different mutations in the gene encoding ASPA, resulting in the absence of ASPA activity. In humans, the CD syndrome is marked by early onset, hydrocephalus, macroencephaly, head-lag, ataxia, blindness, psychomotor retardation, and widespread spongiform myelin sheath vacuolization with progressive demyelination (Janson et al. 2006). In addition to the hydrocephalus and spongiform vacuolization, the global osmotic nature of the disease is also apparent in the swelling of astrocytes, as well as in increased cerebrospinal fluid (CSF) space. Metabolic hallmarks of the disease include a 20–30% elevation in NAA and NAAG in brain gray matter and white matter (Janson et al. 2006). There are also elevated levels of NAA and NAAG in CSF and increased diffusion of NAA and NAAG from brain ECF down their gradients across the blood brain barrier into the vascular system with daily excretion of large amounts of both substances (Baslow 2007). Since NAA is not only a neuronal substrate for formation of NAAG, but also a product of astrocyte hydrolysis of NAAG, it reasonable to assume that in CD, NAA buildup in ECF decreases the rate of NAAG hydrolysis by end product inhibition and interferes with its GRM3 function. Clinical symptoms generally appear within months following birth, and death usually occurs during early childhood.

Hypoacetyaspartia: a lack of brain NAA and NAAG

There is but a singular known human case of this neurometabolic disease in which there is no measurable NAA or NAAG present in brain or CSF (Burlina et al. 2006). In this individual, there was early onset with delayed developmental milestones within 6 months. At the age of about 9 years in 2004, there was profound neurological dysfunction, truncal ataxia, lack of expressive speech, epilepsy and marked cognitive impairment. Metabolic and other workups showed a moderate delay in myelination and unremarkable blood, CSF and kidney functions. Of interest, small amounts of both NAA and NAAG, in a low normal range were detected in urine.

Homocarnosinosis: a lack of homocarnosinase activity

Hcarn is a dipeptide restricted to the brain whereas Carn is found in brain and other tissues. In homocarnosinosis, homocarnosinase activity is absent in the brain, and there is also a deficiency in serum carnosinase, which hydrolyzes Hcarn at about a rate of 5% of its activity on Carn (Lenney et al. 1983). These patients are afflicted with progressive mental deterioration, spastic paraplegia and retinal pigmentation but without evidence of demyelination. They also have elevated concentrations of Hcarn in brain, CSF and urine (Perry et al. 1979). Clinical symptoms appear early, but patients can live for decades.

Carnosinemia and homocarnosinemia: a lack of carnosinase activity

In humans, carnosinase activity is present in serum. In human cases of carnosinemia and homocarnosinemia, serum carnosinase activity is very low, and brain Hcarn and Carn build up in serum with Carn also excreted in urine (Kramarenko et al. 2001). In one representative case study, clinical characteristics were similar to those of homocarnosinosis, but different in that no pigmented retina or excretion of Hcarn was observed. In this case there was early onset (at about 6 months) with initial evidence of mental and motor retardation, and myopathy with decreased tone of skeletal muscles. At the age of 15 years, there was additional evidence of progressive neurological, electrophysiological and psychological impairments as well as structural changes in the brain.

Conclusions

There are two kinds of neurotransmissions that occur in brain. One is neuron to neuron at synapses, and the other is neuron to glia via ECF, both of which are important for maintenance of proper neuronal functioning. For neuron to neuron communications, several potent amino acid neurotransmitters are used within the confines of the synaptic space. However, their presence at elevated concentrations in the extra-synaptic space could be detrimental to well organized neuronal functioning. In this paper, a hypothesis is presented that neurons synthesize relatively nontoxic peptides and peptide derivatives from these amino acids for the purpose of releasing them to ECF to function as glial cell-specific neurotransmitters. These putative neurotransmitters include NAAG, Hcarn, Carn, and perhaps NAA. Their release by stimulated neurons is dynamic, and they operate via ECF over longer distances and within much longer time-frames (1–2 s) than for synaptic transmissions (1–2 ms). Moreover, their functional importance is attested to by profound neuropathies associated with the rare human inborn errors in their metabolism. The presence of high concentrations of NAA, NAAG, Hcarn and Carn in brain has long presented both metabolic and functional enigmas, and it is hoped that the hypothesis offered in this communication, that these abundant substances are synthesized as relatively nontoxic neuron-to-glia neurotransmitters, will contribute to further elucidating their physiological roles.

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