Glycomics and Lipidopics

Extended Abstract

Evidence that N-acetylaspartylglutamate is the Astrocyte-Targeted Neurovascular Coupling Agent that Regulates Slow Tonic Control of Brain Blood Flow

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Abstract:

The presence of high levels of N-acetylaspartate (NAA) and N-acetylaspartylglutamate (NAAG) in the mammalian brain, and of most of the enzymes that synthesize and hydrolyze them were discovered more than six decades ago. At that time it was also noted that these substances and their anabolic and catabolic enzymes were highly compartmentalized with most NAA and NAAG and their anabolic enzymes present in neurons, and their catabolic enzymes present in macroglia. These early findings have been reviewed. Subsequently, in a review of the properties of metabotropic glutamate receptors (mGluR's) found in brain, it was reported that of the eight members of the three groups of mGluR's, that NAAG was a selective agonist for the group II mGluR3 receptor. At the same time, two papers were published, one that demonstrated for the first time that the enzyme that hydrolyzed NAA was only expressed in oligodendrocytes , and the other that showed that the enzyme that hydrolyzed NAAG was expressed "exclusively in astrocytic glial cells". The "nagging" question of the function of NAAG was considered in 1997 and revisited in 2015. The hypothesis that NAAG was intimately involved in neuron-astrocyte communication was first suggested in 1999 [5] and it was speculated that NAAG, via the action of its substrate-specific astrocytic peptidase, "may be an important mediary of neuronal-glial communication". The expanded NAAG functional hypothesis was a logical step in the evolution of the concept in that it considered the entire NAA-NAAG metabolic sequence as a unique linked tri-cellular system and stated that "NAAG in the CNS may have a …primary role" in "neuronal-glial cell-specific signaling and communication". In 2005 it was observed for the first time that by inhibiting NAAG peptidase *in vivo*, the astrocytic enzyme that hydrolyzes NAAG, that there was a prolonged global reduction in the proton magnetic resonance blood oxygen level-dependent (BOLD) signal indicating a reduction in global cerebral blood flow (CBF), but with little or no effect on physical activity. In 2006 the hypothesis was expanded to suggest that NAAG functioned "to control focal or regional hyperemia" by stimulating astrocytes to synthesize and release second messengers to the vascular system via cyclooxygenase-1 (COX-1) synthesized prostaglandins. A number of recent studies support aspects of the original hypothesis. In 2013, it was demonstrated that the mGluR3 receptor was the predominant mGluR receptor present in mature murine and human astrocytes and that other mGluRs were very low or absent. Thus, not only is NAAG uniquely targeted to the mGluR3 receptor, but it may be the only mGluR receptor on the surface of mature astrocytes, the cells that are integral to regulating near-field CBF. Additional support for the hypothesis was developed in 2015, when the presence of a neuronal efflux transporter that transports both NAA and NAAG into ECF by a non-synaptic mechanism was reported , thus providing a possible mechanism for their continuous release to oligodendrocytes and astrocytes respectively. The brain exhibits a remarkable feature in that there is a high degree of functional specialization in a relatively small organ. As a result there are many different small neuronal "neighborhoods", independent of neuron type or connectivity, that require different temporal allocations of CBF in order to supply sufficient quantities of glucose (Glc), O_2 and nutrients, and also to serve as a sink for waste products CO_2, H_2O and generated heat. This is accomplished by an intricate system of chemical feedback signals between neurons, astrocytes and the vascular system. This cellular association has been termed the "neurovascular unit" and the crosstalk between the cells called neurovascular coupling (NVC). In this process, glutamate (Glu) plays an important role in activating astrocytes to initiate signaling to the vascular system. In 2015, it was reported that there were two different types of NVC, one rapid and phasic in response to abrupt changes in neuron synaptic activity, inducing astrocyte Ca^{2+} oscillations and eliciting immediate vascular responses. The other, slow and tonic and independent of regional changes in neuronal synaptic activity, using resting intracellular Ca^{2+} and continuous release of COX-1 generated second prostaglandin messengers. Many neurovascular coupling mechanisms are known that can regulate phasic changes in CBF, but how the brain accomplished tonic control of CBF was reported to be unknown. In this analytical review, we bring together evidence of a signaling mechanism that

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matches the criteria for tonic regulation CBF and suggests that the function of neuronal release of NAAG in brain is to regulate tonic control of CBF. The Tri-Cellular Metabolism of NAA and NAAG Metabolism NAA and NAAG are among the highest concentration amino acids and dipeptides present in brain, and are almost exclusively found in neurons. Within neurons, the ratio of NAAG to NAA is lowest in gray matter (GM) and highest in white matter (WM). NAA is synthesized from L-aspartate (Asp) and acetyl Co-enzyme A (AcCoA) by NAA synthase with Glc the source of acetate (Ac) in AcCoA. NAA is the only known precursor of NAAG, a non-excitatory form of Glu synthesized from NAA and Glu in neurons by NAAG synthase. Importantly, because of their genesis, the rates of synthesis of both NAA and NAAG always reflect the rate of neuronal Glc oxidation, with about 1 molecule of NAAG synthesized for every 320 molecules of Glc oxidized. This is shown in equation 1. Eq 1. (Neurons, NAA and NAAG synthases) 11520 ADP + 11520 P + 320 Glc + 1920 O_2 + 11 Asp + 1Glu 1920 CO_2 + 1920 H_2O+11520 ATP + 10 NAA + 1 NAAG Most neurons in brain synthesize NAA and NAAG and store large quantities of both substances. However, neurons cannot catabolize either of these substances. For their catabolism, they are exported to extracellular fluid (ECF). NAA is targeted to oligodendrocytes where it is hydrolyzed by aspartoacylase (ASPA) liberating Ac and Asp (Eq 2), and NAAG is targeted to the mGluR3 receptor on the astrocyte surface where the Glu is cleaved by NAAG peptidase, (Eq 3.). Eq 2. (Oligodendrocytes, ASPA) NAA Ac + Asp Eq 3. (Astrocytes, NAAG peptidase) NAAG NAA + Glu NAA is also a byproduct of astrocyte NAAG hydrolysis but astrocytes cannot further metabolize it. For its catabolism it must be liberated to ECF and hydrolyzed by oligodendrocyte ASPA. The unique tri-cellular metabolism of NAA and NAAG with two synthetic and two hydrolytic enzymes distributed between three cell types, and the NAAG-mGluR3-NAAG peptidase Glu release mechanism on the astrocyte surface has been called the "operating system" of the brain. This is because failure of several parts of the system in humans has been observed to lead to abnormal brain function. The group II mGluR3 is also unique among mGluR's in that there is an astrocyte-targeted neuron-dedicated neurotransmitter (NAAG) and an associated specific enzyme (NAAG peptidase) for its hydrolysis The mGluR3 is a G-protein Gi/Go bound receptor negatively coupled to adenylate cyclase that does not trigger Ca^{2+} increases in astrocytes, thus excluding its involvement in rapid synaptic events.

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