Glycomics and Lipidopics

Extended Abstract

Rescuing Canavan Disease by Redirecting Metabolic Processing: Support for the Astrocyte Hypothesis of Canavan Disease Generation and A Possible Human Cure

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Abstract:

Canavan disease (CD) is a globally occurring but rare human spongiform leukodystrophy that is associated with inborn errors affecting the activity of aspartoacylase (ASPA), an enzyme highly expressed in oligodendrocytes that hydrolyzes N-acetylaspartate (NAA). Lack of ASPA activity is associated with the inability of oligodendrocytes to build or maintain axon-enveloping myelin sheaths. The primary source of NAA in brain is neurons, cells that synthesize but cannot catabolize it. Neurons also synthesize N-acetylaspartylglutamate (NAAG) from NAA and glutamate but cannot catabolize this substance as well. For their metabolism, these substances are released to extracellular fluid and are metabolized by oligodendrocyte ASPA and astrocyte NAAG peptidase respectively. A hypothesis developed suggested that the cause of the leukodystrophy component in CD was due to release of NAAG by neurons at white matter nodes of Ranvier, its catabolism by astrocytes forming NAA and increased osmotic-hydrostatic pressure as a result of its buildup at these nodes due to the lack of ASPA activity. In this communication, we provide evidence supporting this hypothesis and comment on the cause and possible cure for human CD. We bring attention to a recent study in which a human adeno-associated virus genetic aspartoacylase (ASPA) construct was serendipitously inserted into the "wrong" cell and by redirecting its metabolic processing rescued a murine model of Canavan disease (CD). After achieving an overall cure including enhanced motor performance, these authors were prompted to "hypothesize" that ASPA expression in a non-oligodendrocyte glial cell, astrocytes, might be involved. This was subsequently ascertained using appropriate cell markers. In this communication, we provide metabolic, physiological and cellular contexts as well as a plausible mechanism for evaluating this remarkable finding. CD is an autosomal recessive disease due to inborn errors resulting from more than 100 different mutations in which oligodendrocyte expressed ASPA is inactive. CD is a rare disease in that there are only several hundred human cases worldwide at any given time. Spatially, it is distributed among all races of the world, but is especially prevalent in the Ashkenazi Jewish population of northern Russia. ASPA and N-acetyl-L-aspartate (NAA), its natural substrate synthesized by neurons and maintained at high mM levels in both gray matter (GM) and white matter (WM), are clearly important for normal brain function as evidenced by these inborn metabolic errors. NAA is present in every human brain thus far examined except one, and in almost every other vertebrate brain examined. CD is manifested clinically as a spongiform leukodystrophy and is characterized by early onset, megalocephaly and a progressive loss of functions, generally leading to early mortality. Children with CD may appear relatively normal at birth but fail to reach typical milestones in development. An early subjective sign is the loss of ability to maintain their head in an upright position when being held. Other important clinical characteristics of CD are the buildup of high mM concentrations of NAA in brain, signs of WM pathology and presence of high concentrations of NAA in urine. Many therapeutic approaches to treatment of CD have been tried but with little success. Among these are the use of lithium in both animals and humans. Lithium treatment rapidly brings brain and urine NAA down to normal levels, but fails to achieve rescue of the spongiform demyelination in CD. There are still rarer mild cases of CD, with some individuals surviving for decades and having normal or near normal functionality although presenting with elevated NAA in brain and urine. The values for residual enzyme activity were derived from transfecting HEK293 cells with mutated genes and measuring residual expressed ASPA activity against the normal gene. These cases, although imperfectly associated with the amount of residual activity of the ASPA enzyme protein, are highly significant in that they show (1) that an elevated level of NAA in whole brain by itself does not cause CD, and (2) that a small amount of residual ASPA activity (<1 to 12.4 %) may rescue CD. The imperfect association between residual ASPA protein activity and mild cases of human CD may be explained by the presence of a second non-specific acylase that has been observed to be expressed by cultured rat astrocytes and that has some activity against NAA. The Tri-Cellular Metabolism of NAA ,ASPA and NAA are part of a unique tri-cellular metabolic system in brain wherein NAA is made in neurons from acetyl Co-enzyme A

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and L-aspartate by NAA synthase and is then used to synthesize an L-glutamate (Glu) adduct, the neurotransmitter N-acetylaspartylglutamate (NAAG), via NAAG synthase [6]. Neurons cannot further metabolize either of these substances and for their hydrolysis they must be released to extracellular fluid (ECF). NAAG is targeted to the metabotropic glutamate receptor 3 (mGluR3) on the surface of astrocytes and upon docking is cleaved into Glu and NAA by NAAG peptidase. The Glu can be converted into glutamine for recycling to neurons, but the NAA product cannot be further hydrolyzed by astrocytes since they do not express ASPA and it must be released to oligodendrocytes which do express this enzyme. This system requires the coordinated functioning of four enzymes, two anabolic and two catabolic, expressed in three different cell types and a specific receptor for its completion. Inborn Errors in NAA and NAAG Metabolism and their Related Human Brain Pathologies Both NAA and NAAG are dynamic and turn over every 14-16 h. While the function of this entire system is as yet unclear, one hypothesis suggests that the role of the astrocyte surface mGluR3-NAAG peptidase complex is to liberate Glu from NAAG to signal via astrocytes a neuron's ongoing requirements for energy and oxygen in order to increase focal blood flow. NAAG is a neurotransmitter specifically targeted to astrocytes, the key cellular components in neurovascular coupling in both GM and WM. If this is the case, then any reduction in this process could be detrimental, and any enhancement beneficial to neuron function. The complete absence of ASPA activity results in the severe form of human CD and the complete absence of NAA synthase activity in a single known human case results in hypoacetylaspartia, an inborn error where neither NAA nor NAAG are produced. In both of these human conditions, there are profound clinically observed negative consequences. However, in rodents, the effects of similar enzyme deficits, natural or engineered, are much less severe.

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