Epilepsy Currents
2019, Vol. 19(6) 400-401

DOI: 10.1177/1535759719875468
journals.sagepub.com/home/epi
SAGE

An Ancient Enzyme Takes a Hit in Epilepsy

Phosphorylation of Glutamine Synthetase on Threonine 301 Contributes to Its Inactivation During Epilepsy

Huyghe D, Denninger AR, Voss CM, et al. *Front Mol Neurosci.* 2019;12:120. doi:10.3389/fnmol.2019.00120. eCollection 2019. PMID: 31178690.

The astrocyte-specific enzyme glutamine synthetase (GS), which catalyzes the amidation of glutamate to glutamine, plays an essential role in supporting neurotransmission and in limiting NH_4^+ toxicity. Accordingly, deficits in GS activity contribute to epilepsy and neurodegeneration. Despite its central role in brain physiology, the mechanisms that regulate GS activity are poorly defined. Here, we demonstrate that GS is directly phosphorylated on threonine residue 301 (T301) within the enzyme's active site by cAMP-dependent protein kinase (protein kinase A). Phosphorylation of T301 leads to a dramatic decrease in glutamine synthesis. Enhanced T301 phosphorylation was evident in a mouse model of epilepsy, which may contribute to the decreased GS activity seen during this trauma. Thus, our results highlight a novel molecular mechanism that determines GS activity under both normal and pathological conditions.

Commentary

Glutamine synthetase (GS, glutamate-ammonia ligase, E.C. 6.3.1.2), which catalyzes the condensation of ammonia and glutamate to glutamine, is a fascinating enzyme because of its ancient origin, ubiquitous presence in nature, and emerging involvement in several diseases, including epilepsy. GS is encoded by one of the oldest genes known to exist, likely originating more than 3500 million years ago in the "pre-prokaryotic" era, that is, the period between the origin of life and the divergence of prokaryotes and eukaryotes.[1] Moreover, all extant organisms express GS, and absent or reduced enzyme levels are respectively deadly, or sometimes associated with a variety of pathological conditions such as Alzheimer disease,[2] schizophrenia,[3] and epilepsy.[4-7] Even though GS is widely distributed in nature, the expression in mammals is restricted to a surprisingly small number of cell types. In the central nervous system, for instance, GS is almost exclusively found in astrocytes[8] and is thought to play a critical role in ammonia detoxification and in regulation of the excitatory and inhibitory neurotransmitters glutamate and γ-aminobutyric acid (reviewed in the study by Eid et al[9]).

Severe loss-of-function mutations of the GS gene are often associated with considerable mortality and morbidity, and the small number of humans reported with such mutations have suffered from multi-organ failure, encephalopathy, and epilepsy.[10] In line with these observations, transgenic mice with complete loss of GS activity are embryonic lethal,[11] and mice with brain-restricted GS loss die 3 days after birth,[12] suggesting that a critical level of GS activity is necessary for life. In contrast, an increasing number of studies have suggested that incomplete deficiencies in GS are relatively common, compatible with life, and sometimes associated with dysfunction and disease. For example, the activity of GS is partially reduced in the epileptogenic hippocampal formation in humans with refractory mesial temporal lobe epilepsy,[4,7] in the amygdala in some patients with refractory neocortical epilepsies,[6] and in the neoplastic tissue in some patients with glioblastoma-associated epilepsy.[5] Furthermore, chemical inhibition or genetic deletion of GS focally in the hippocampus and neocortex of rodents causes epileptic seizures and neuropathological changes similar to human mesial temporal lobe epilepsy,[13,14] suggesting that a partial reduction in brain GS activity is sufficient to cause epilepsy. Thus, it has been proposed that preventing or reversing the brain GS deficiency could potentially be used to treat epilepsy. However, effective approaches have been lagging partly because the molecular mechanisms of the GS deficiency are incompletely understood.

Using carefully designed in vitro and in vivo approaches, Huyghe and colleagues report here that phosphorylation of GS near the active site of the enzyme, on threonine 301 (T301), contributes to the enzyme inactivation during epilepsy.[15] Mammalian GS is a homomeric decamer protein comprising 2 stacked pentameric rings with 5 identical, active sites.[16] By reviewing the protein sequence of the murine and human

isoforms of the enzyme, the authors discovered 2 consensus sites for protein kinase A (PKA)–dependent phosphorylation on T301 and serine 343 (S343), near the active site of the enzyme.

To assess whether phosphorylation of GS regulates the enzyme activity, the authors first created several mutated versions of mouse GS and showed that substitution of threonine on 301 with alanine completely abolished GS phosphorylation, indicating that T301 is the primary phosphorylation site of PKA-mediated phosphorylation of GS in vitro. Next, using a combination of enzyme kinetic studies, mass spectrometry, and site-directed mutagenesis, the authors showed that PKA-mediated phosphorylation of GS led to an approximately 40% reduction in enzyme activity in vitro and that phosphorylation of T301 accounted for the majority of this reduction.

To determine whether GS phosphorylation also occurs in the normal brain in vivo, the authors first purified GS from mouse brain extracts using immunoprecipitation and then analyzed the purified GS by mass spectrometry, which revealed 2 phosphorylated protein segments from the purified GS protein. Using additional confirmatory assays, the authors demonstrated that mouse brain GS is phosphorylated at T301 and S343, as predicted from the in vitro experiments, but that phosphorylation of T301 has the highest impact on GS activity.

Finally, the authors used the systemic kainic acid mouse approach (a commonly used model of status epilepticus and subsequent mesial temporal lobe epilepsy) to determine whether a 60-minute episode of status epilepticus would cause phosphorylation of GS and reduced activity. Intriguingly, T301 phosphorylation, but not S343 phosphorylation, was significantly increased in the hippocampus in the seizing animals versus saline-injected controls. Moreover, the increased phosphorylation was accompanied by an approximately 25% reduction in hippocampal GS activity.

Taken together, the studies by Huyghe and colleagues are of potentially high translational relevance because they demonstrate a novel molecular mechanism by which brain GS may lose its activity in some of the most common forms of epilepsy. Although additional studies are needed to test for this mechanism in the brain of patients with GS-deficient epilepsies, the results are encouraging and suggest that PKA inhibition may have a role in preventing and treating epilepsy as well as other conditions associated with GS inhibition.

By Tore Eid

ORCID iD

Tore Eid https://orcid.org/0000-0001-9217-6393

References

1. Kumada Y, Benson DR, Hillemann D, et al. Evolution of the glutamine synthetase gene, one of the oldest existing and functioning genes. *Proc Natl Acad Sci U S A*. 1993;90(7):3009-3013.
2. Smith CD, Carney JM, Starke-Reed PE, et al. Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. *Proc Natl Acad Sci U S A*. 1991;88(23):10540-10543.
3. Steffek AE, McCullumsmith RE, Haroutunian V, Meador-Woodruff JH. Cortical expression of glial fibrillary acidic protein and glutamine synthetase is decreased in schizophrenia. *Schizophr Res*. 2008;103(1-3):71-82.
4. Eid T, Thomas MJ, Spencer DD, et al. Loss of glutamine synthetase in the human epileptogenic hippocampus: possible mechanism for raised extracellular glutamate in mesial temporal lobe epilepsy. *Lancet*. 2004;363(9402):28-37.
5. Rosati A, Marconi S, Pollo B, et al. Epilepsy in glioblastoma multiforme: correlation with glutamine synthetase levels. *J Neurooncol*. 2009;93(3):319-324.
6. Steffens M, Huppertz HJ, Zentner J, Chauzit E, Feuerstein TJ. Unchanged glutamine synthetase activity and increased NMDA receptor density in epileptic human neocortex: implications for the pathophysiology of epilepsy. *Neurochem Int*. 2005;47(6):379-384.
7. van der Hel WS, Notenboom RG, Bos IW, van Rijen PC, van Veelen CW, de Graan PN. Reduced glutamine synthetase in hippocampal areas with neuron loss in temporal lobe epilepsy. *Neurology*. 2005;64(2):326-333.
8. Martinez-Hernandez A, Bell KP, Norenberg MD. Glutamine synthetase: glial localization in brain. *Science*. 1977;195(4284):1356-1358.
9. Eid T, Gruenbaum SE, Dhaher R, Lee TW, Zhou Y, Danbolt NC. The glutamate-glutamine cycle in epilepsy. *Adv Neurobiol*. 2016;13:351-400.
10. Haberle J, Gorg B, Rutsch F, et al. Congenital glutamine deficiency with glutamine synthetase mutations. *N Engl J Med*. 2005;353(18):1926-1933.
11. He Y, Hakvoort TB, Vermeulen JL, Lamers WH, Van Roon MA. Glutamine synthetase is essential in early mouse embryogenesis. *Dev Dyn*. 2007;236(7):1865-1875.
12. He Y, Hakvoort TB, Vermeulen JL, et al. Glutamine synthetase deficiency in murine astrocytes results in neonatal death. *Glia*. 2010;58(6):741-754.
13. Eid T, Ghosh A, Wang Y, et al. Recurrent seizures and brain pathology after inhibition of glutamine synthetase in the hippocampus in rats. *Brain*. 2008;131(pt 8):2061-2070.
14. Zhou Y, Hassel B, Eid T, Danbolt NC. Axon-terminals expressing EAAT2 (GLT-1; Slc1a2) are common in the forebrain and not limited to the hippocampus. *Neurochem Int*. 2019;123:101-113.
15. Huyghe D, Denninger AR, Voss CM, et al. Phosphorylation of glutamine synthetase on Threonine 301 contributes to its inactivation during epilepsy. *Front Mol Neurosci*. 2019;12:120.
16. Krajewski WW, Collins R, Holmberg-Schiavone L, Jones TA, Karlberg T, Mowbray SL. Crystal structures of mammalian glutamine synthetases illustrate substrate-induced conformational changes and provide opportunities for drug and herbicide design. *J Mol Biol*. 2008;375(1):217-228.