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Roles of Glutamine Synthetase Inhibition in Epilepsy

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Abstract

Glutamine synthetase (GS, E.C. 6.3.1.2) is a ubiquitous and highly compartmentalized enzyme that is critically involved in several metabolic pathways in the brain, including the glutamine-glutamate-GABA cycle and detoxification of ammonia. GS is normally localized to the cytoplasm of most astrocytes, with elevated concentrations of the enzyme being present in perivascular endfeet and in processes close to excitatory synapses. Interestingly, an increasing number of studies have indicated that the expression, distribution, or activity of brain GS is altered in several brain disorders, including Alzheimer's disease, schizophrenia, depression, suicidality, and mesial temporal lobe epilepsy (MTLE). Although the metabolic and functional sequelae of brain GS perturbations are not fully understood, it is likely that a deficiency in brain GS will have a significant biological impact due to the critical metabolic role of the enzyme. Furthermore, it is possible that restoration of GS in astrocytes lacking the enzyme could constitute a novel and highly specific therapy for these disorders. The goals of this review are to summarize key features of mammalian GS under normal conditions, and discuss the consequences of GS deficiency in brain disorders, specifically MTLE.

Keywords

Astrocytes; Glutamate; Hippocampus; Seizures; Temporal lobe epilepsy

Introduction

Glutamine synthetase (GS; a.k.a. glutamate ammonia ligase, GLUL, EC 6.3.1.2) is a key enzyme involved in nitrogen metabolism, acid–base homeostasis, and cell signaling across multiple species of prokaryotes and eukaryotes [1–3]. One of the main roles of GS in

vertebrates is to metabolize glutamate and ammonia to glutamine according to the following reaction:

This reaction is biologically significant because high concentrations of glutamate and ammonia are toxic to the central nervous system (CNS) [4-7]. Moreover, a continuous supply of glutamine is required for several physiological processes, including synthesis of glutamate and GABA, synthesis of proteins, and osmoregulation [8]. Because GS is the only known enzyme in humans capable of synthesizing glutamine, alterations in the expression and activity of the enzyme are likely to have significant biological effects.

Indeed, recent studies have shown that systemic mutations of the GS gene in humans result in brain malformations, seizures, multiorgan failure, and early death [9, 10]. There is also emerging evidence to suggest that brain GS may be implicated in the pathogenesis of several neurological disorders and psychiatric conditions including Alzheimer's disease [11, 12], hepatic encephalopathy [13], suicide/depression [14, 15], schizophrenia [16], and epilepsy [17-19].

The goal of this review is two-fold; first, to summarize key features of GS under normal conditions, and second, to discuss the potential involvement of GS in brain disorders, particularly epilepsy.

GS is a Ubiquitous, Yet Highly Compartmentalized Enzyme

The importance of GS for life is underscored by its ubiquitous presence in a wide range of organisms, including microbes [20, 21], plants [22], animals (Elliott [51]), and humans [23]. Among the two main forms of GS, GSI and GSII, the latter is present in eukaryotes and prokaryotes. In contrast, GSI has only been detected in prokaryotes [24]. The human GS gene (GLUL) is localized to chromosome 1q31. A GS pseudogene (GLULP) is present on chromosome 9p13, and three GS-like genes are present on 5q33 (GLULL1), on 11p15 (GLULL2), and on 11q24 (GLULL3) [25]. The biological significance of the pseudogene and GS-like genes is not fully understood. Although there are several GLUL gene variants among eukaryotes, the ammonia binding site and the amino acid residues that interact with glutamate, magnesium/manganese ions, and ATP, are highly conserved [26]. Thus, herbicides, drugs, and other toxins that interfere with these sites are likely to do so across multiple species [26].

The GS enzyme in humans is structurally comprised of two ring-like pentamers forming an active decamer [26]. In humans and many vertebrates, the enzyme is abundantly present in a limited set of hepatocytes surrounding the terminal hepatic veins [27], in skeletal muscle cells, and in many (but not all) GFAP-positive astrocytes in the brain [28-30]. An even finer compartmentation of GS protein is evident in immunostained sections of the brain. GS is strongly expressed in astrocytic processes surrounding glutamatergic nerve terminals in the hippocampal formation [31]. GS is also particularly abundant in a laminar pattern in the molecular layer of the dentate gyrus, corresponding to glutamate binding sites [32].

GS is Essential for the Glutamine-Glutamate-GABA Cycle Metabolism

Glutamate and GABA are the primary excitatory and inhibitory neurotransmitters in the brain. To ensure the necessary fidelity of excitatory and inhibitory synaptic transmission, a carefully regulated synthesis, cellular release, and extracellular uptake of glutamate and GABA is required. Thus, an intimate metabolic relationship exists between glutamate and

GABA. This relationship, which is known as the glutamine-glutamate-GABA cycle, includes GS and glutamine as well as a number of other metabolic pathways and transporter proteins. The glutamine-glutamate-GABA cycle involves the following steps (Fig. 1): (1) Glutamate in astrocytes is metabolized with ammonia and ATP to form glutamine via the GS reaction described earlier [33]. (2) Glutamine is transported from astrocytes into the extracellular space via specialized transporter proteins, particularly the system N transporters (SN1/SNAT3 and SN2/SNAT5) [34]. (3) Extracellular glutamine is imported to glutamatergic neurons via the system A transporter subtype 2 (SAT2/SNAT2) [35], and to GABA-ergic neurons via system A transporter subtype 1 (SAT1/SNAT1) [36]. (4a) Glutamine in glutamatergic neurons is converted to glutamate via the mitochondrial enzyme phosphate activated glutaminase (PAG) [37, 38], and glutamate is imported into synaptic vesicles via vesicular glutamate transporters (VGLUT) [39-42]. (4b) Glutamate is released from synaptic vesicles followed by binding to extracellular glutamate receptors present on neurons and glial cells. The extracellular glutamate is then taken up primarily by astrocytes via astrocytic glutamate transporters (EAAT1/GLAST and EAAT2/GLT1) [43]. (4c) Finally, glutamate is converted to glutamine by GS in the cytoplasm of astrocytes, thus completing the *glutamine-glutamate (or glutamate-glutamine) cycle*. (5a) Glutamine taken up by GABAergic neurons is converted to glutamate via PAG and then to GABA via glutamic acid decarboxylase isoforms 65 or 67 (GAD65/67) [44]. (5b) GABA is imported into synaptic vesicles via vesicular GABA transporters (VGAT) [45]. (5c) GABA is released from either the synaptic vesicles or from the cytoplasm through reversal of GABA transporters, then binds to extracellular GABA receptors, and is subsequently taken up by neurons and glial cells via GABA transporters [46, 47]. The GABA transporter subtype GAT3 (slc6a11) is preferentially present on astrocytes, whereas GAT1 and GAT2 are mainly expressed on neurons. (5d) GABA may then enter the TCA cycle as succinate, which eventually gives rise to alpha-ketoglutarate. Glutamate dehydrogenase (GDH) may further convert alpha-ketoglutarate to glutamate, which in astrocytes is metabolized to glutamine via GS [48]. This completes the *glutamine-glutamate-GABA cycle*.

Importantly, the glutamine-glutamate-GABA cycle represents a simplistic view of a complex process involving numerous additional metabolic pathways closely linked to the cycle. Moreover, a highly efficient spatial arrangement of enzymes and transporter molecules in functional microdomains are likely to exist and adds to the complexity of the cycle concept. However, despite these limitations, the glutamine-glutamate-GABA cycle provides an attractive and testable reference framework for studies of brain metabolism and neurotransmission, as demonstrated by numerous seminal discoveries on the topic [49-51].

GS is Critical for Ammonia Detoxification in the CNS

Considerable amounts of ammonia are formed in the human body every day, mainly from the action of bacterial enzymes on colonic content and from the hydrolysis of glutamine in the small and large intestines [52]. Smaller amounts of ammonia are produced from breakdown of body protein and from metabolism of glutamine to glutamate via PAG [37]. Most of the ammonia derived from the intestines is taken up and degraded by the liver via the urea cycle and hepatic GS. However, even with normal liver function, as much as 35 $\mu\text{mol/L}$ of ammonia can remain in the plasma. The concentration of plasma ammonia is even higher in cases of liver failure, and in patients with congenital defects in GS or the urea cycle. Because ammonia is neurotoxic and readily crosses the blood brain barrier, a system for efficient removal of brain ammonia is required [5, 53, 54]. The brain lacks a urea cycle, and therefore depends on the GS reaction in astrocytes for most of its ammonia removal [55]. The importance of astrocytes in ammonia detoxification is further emphasized by their anatomical relationship with the blood-brain barrier. The perivascular astrocyte endfeet, which surround the abluminal domain of microvessel-associated endothelial cells, constitute

a metabolic “buffering” compartment between the blood and the rest of the brain. Thus, blood-derived ammonia enters the astrocytic compartment for metabolism by GS, limiting further progression into the neuronal compartment.

A number of CNS Disorders are Associated with Alterations in GS

Given the ubiquitous presence and discrete distribution of GS it is not surprising that alterations in the enzyme result in significant biological effects. However, it is only recently that the potential involvement of GS in human pathological conditions has begun to be appreciated. Haberle and colleagues reported two cases of congenital GS deficiency due to homozygous mutations in the GS gene (R324C and R341C) [9, 10]. The newborns had extensive brain malformations with almost no cerebral activity on EEG other than short bursts of theta waves and generalized seizures. They exhibited severe enteropathy and necrolytic erythema of the skin and died from cardiac failure and multi-organ failure 2 days and 4 weeks after birth [9, 10]. Glutamine was largely absent from their serum, urine, and cerebrospinal fluid. So far, only one patient with genetic GS deficiency has been known to live beyond early infancy despite having encephalopathy and seizures [56]. The profound morbidity associated with genetic deficiencies in GS is further demonstrated by studies of transgenic mice. Animals with prenatal excisions of the GS gene in all cell types die during early embryonic development [57], while mice with gene deletions selectively in GFAP-positive astrocytes live until postnatal day 3 [58].

Several cases of non-genetic (secondary) deficiencies in GS have been reported. Studies of brains from patients with Alzheimer’s disease demonstrate reduced activity of GS [11, 12], increased concentrations of oxidatively modified GS protein [11, 59, 60], and alterations in the cellular and subcellular distribution of GS protein [61, 62]. Brain GS mRNA is also reduced in patients with depression [63], in depressed suicide subjects [14, 15], and in non-depressed individuals who committed suicide [14]. Alterations in brain GS mRNA are also present in patients with schizophrenia [16]. Finally, we and others discovered that GS protein and activity are severely reduced in the hippocampal formation [18, 19] and amygdala [64] in patients with mesial temporal lobe epilepsy (MTLE). The significance of the latter findings will be discussed below.

GS is Deficient in Astrocytes in Patients with MTLE

MTLE is one of the most common forms of drug-resistant, localization-related epilepsies in humans. The seizures in MTLE appear to involve a network of temporal lobe and limbic structures including the hippocampal formation, amygdala, entorhinal cortex, lateral temporal neocortex, medial thalamus, and inferior frontal lobes [65]. Furthermore, one of the hallmarks of MTLE is a well-characterized pathology known as hippocampal sclerosis, which is recognized by a pattern of selective neuronal loss and glial alterations in the hippocampal formation [66-68].

Studies using in vivo brain microdialysis in patients with MTLE have shown that the sclerotic and epileptogenic hippocampal formation contains five-fold higher concentrations of extracellular glutamate interictally, when compared with the non-sclerotic and non-epileptogenic hippocampal formation [69, 70]. Moreover, during a seizure, the extracellular glutamate concentration increases six-fold above the interictal level in the hippocampal formation, and remains markedly elevated for several hours after the cessation of seizure activity [71]. Finally, the interictal extracellular glutamate concentration is considerably higher in patients with hippocampal sclerosis (MTLE) than in patients without this pathology (non-MTLE), despite the 60–80 % neuronal loss and doubling of glial density in the sclerotic hippocampal formation [70, 72-74]. The glutamate excess in MTLE is likely to be of critical importance for the pathophysiology of the disease due to the excitatory and

excitotoxic properties of the neurotransmitter [7, 75-77]. Therefore, a key question is: What causes the glutamate excess in MTLE?

GS is severely deficient in the sclerotic hippocampal formation in patients with MTLE (Fig. 2) [18, 19]. Analysis of surgically resected brain tissue from patients with MTLE revealed a nearly 40 % loss of GS protein and enzyme activity in astrocytes in the sclerotic hippocampal formation. A similar loss of GS was not present in surgically resected hippocampal formations from patients with other types of temporal lobe epilepsy (non-MTLE) or in hippocampal formations from diseased subjects with no history of epilepsy [18]. These findings led us to hypothesize that a deficiency in glutamine synthetase in astrocytes slows the glutamate-glutamine cycle metabolism and causes chronic accumulation of glutamate in astrocytes and the extracellular space. Studies of patients with MTLE using magnetic resonance spectroscopy have supported this hypothesis by demonstrating slowed glutamine-glutamate cycling in the sclerotic versus the non-sclerotic hippocampal formation [78]. Furthermore, sustained inhibition of hippocampal glutamine synthetase using continuous infusion of methionine sulfoximine (MSO) in the hippocampal formation of rats results in recurrent seizures, increased concentrations of glutamate in hippocampal astrocytes, and neuropathological changes that resemble hippocampal sclerosis in some animals (Fig. 3) [17, 79, 80].

What are the Metabolic and Functional Consequences of Brain GS Deficiency?

Because GS is an integral part of a complex and highly compartmentalized metabolic system, it is likely that alterations in the expression and activity of GS will result in multifaceted metabolic and functional effects. Furthermore, due to the tight homeostatic control of most metabolic pathways, it is also plausible that alterations in GS are accompanied by one or more counter-regulatory (“compensatory”) mechanisms. Thus, to theoretically predict the downstream effect of alterations in GS is difficult, and only carefully designed studies are expected to accurately address this issue. We will therefore limit our discussion of possible consequences of GS deficiency to a few plausible scenarios that either have been or can be tested experimentally.

Effects on Glutamine, Glutamate, and GABA

Numerous studies of patients and animals with genetic GS deficiencies, and experiments involving chemical inhibition of GS through enzyme blockers such as methionine sulfoximine (MSO), have demonstrated that a loss of GS protein or enzyme activity leads to reduced synthesis of glutamine [10, 56, 57, 81, 82]. Because glutamine is critically involved in the glutamine-glutamate-GABA cycle, a highly relevant question is whether a lack of glutamine will affect the concentrations of glutamate and GABA, the main excitatory and inhibitory neurotransmitters in the brain.

It can be argued that loss of GS in the brain leads to decreased metabolism of glutamate to glutamine with accumulation of glutamate in the cytoplasm of *astrocytes*. Indeed, glutamate increases and glutamine decreases in astrocytes in organotypic slice cultures of the hippocampus after treatment with MSO [82]. Glutamate also increases in hippocampal astrocytes after chronic infusion of MSO into the hippocampus in rats [79]. Possible consequences of the increased astrocytic glutamate are: (1) impaired uptake of extracellular glutamate due to a reduced concentration gradient of the amino acid across the plasma membrane [18]; (2) release of glutamate into the extracellular space by astrocytes [83-85]; and (3) metabolism by oxidation of excess astrocytic glutamate via other pathways such as the TCA cycle [50, 86]. The contribution of each of these mechanisms to the increased

astrocytic and extracellular fluid glutamate observed in vivo in MTLE remains to be established.

Glutamine is used for the synthesis of glutamate in neurons via the PAG reaction [37, 87]; hence, inhibition of GS in normal animals leads to acute *decreases* in neuronal and extracellular brain glutamate [81, 88, 89], similar to findings seen with the PAG inhibitor 6-diazo-5-oxo-L-norleucine [90]. These studies may appear to contradict our hypothesis that a deficiency in GS leads to increased extracellular glutamate in MTLE [18]. However, it should be noted that the cited studies were performed in normal animals using acute and widespread inhibition of GS by MSO. This situation is quite different from patients with drug-resistant MTLE which is characterized by several years (sometimes decades) of seizure activity, profound neuropathological changes in the hippocampal formation, and spatially restricted deficiencies in GS in the hippocampal formation (mainly in CA1, CA3, and the dentate hilus) [18, 19, 66, 67]. In human MTLE, it is possible that compensatory events specific to the epileptogenic hippocampal formation provide sufficient glutamine for neuronal synthesis of glutamate, perhaps by increased uptake of glutamine from the blood or via other mechanisms. Furthermore, the loss of GS in human MTLE is spatially restricted to specific areas such as CA1, CA3, and the dentate hilus, whereas other regions such as CA2 and subiculum are less affected and therefore may produce glutamine [18]. Recent studies have also suggested that GS is redistributed at the subcellular level in MTLE. In chronically epileptic pilocarpine-treated rats, GS is expressed more in proximal astrocytic branches than in distal branches, and GS expressing astrocytic cell bodies are located in closer proximity to vascular walls [91]. This finding suggests a highly compartmentalized action (and lack of action) of GS in the epileptogenic hippocampal formation with potentially different effects on the glutamine-glutamate metabolism in areas near microvessels compared to areas near synapses.

GABAergic neurotransmission is also influenced by inhibition of GS, partly because glutamine is a precursor for the synthesis of GABA. Blockade of GS by MSO results in decreased synthesis of neuronal GABA [92] and impaired release of synaptic GABA under physiological conditions [93]. Furthermore, inhibition of GS by MSO directly influences GABAergic neurotransmission by altering GABA(A) subunit composition; however, the mechanism and functional significance of this effect is not known [94].

Effects on Brain Excitability

Several studies indicate that inhibition of, or deficiency in GS can increase brain excitability and lead to seizures. More than 60 years ago it was observed that domestic animals developed seizures after being fed bleached (agenized) flour [95]. The bleaching process oxidized methionine in the flour to MSO [96], and later investigations demonstrated that systemic administration of MSO to rats and mice inhibits GS and results in severe convulsive seizures [97-100]. Moreover, chronic infusion of MSO locally into the hippocampus of rats results in inhibition of GS, and subsequent recurrent seizures [17, 80]. Finally, patients with mutations of the GS gene typically develop severe, intractable seizures [9, 10].

Although the studies described above strongly implicate a deficiency in GS in the pathophysiology of epilepsy, the definite role of GS in MTLE remains to be fully established. Importantly, most studies investigating the role of GS in epilepsy involve widespread (systemic) deficiencies in GS, whereas the deficiency observed in MTLE is highly localized to specific anatomical and subcellular compartments in the brain [18, 19, 64, 91]. The observation that intrahippocampal infusion of MSO in rats leads to a clinical and pathological state similar to MTLE suggests that a focal deficiency in brain GS is important in the disease process (Fig. 3) [17, 80]. However, the effects of MSO are not

limited to GS inhibition, and it is possible that the convulsant properties of the chemical are caused by other mechanisms. For example, treatment of animals with MSO increases the content of glycogen in astrocytes [97] and depletes the tissue stores of glutathione [101]. Alterations in brain glutathione [102] and glycogen (discussed later) [98, 103] have been associated with seizures; however, the exact role of these compounds in epilepsy are not completely understood. MSO can also induce glutamate release independent of GS inhibition in cortical slices [104, 105]. Further studies, using more specific inhibitors or targeted gene knockouts of GS are therefore needed to fully address the role of GS inhibition in epilepsy.

Effects on Ammonia and Cell Swelling

Because GS is one of the key enzymes responsible for metabolism of brain ammonia, it is expected that ammonia is increased in areas of the brain where GS is deficient, such as the amygdala, and the hippocampal areas CA1, CA3, and dentate hilus in MTLE [18, 19, 64]. However, the metabolic and functional consequences of such highly localized ammonia elevations are not known, because most studies of brain ammonia have involved conditions of severe hyperammonemia, which is typically observed in conditions of liver failure (hepatic encephalopathy) and urea cycle defects. These studies have suggested that GS serves as an important and beneficial detoxifying enzyme for physiological amounts of brain ammonia. However, when plasma ammonia is present at high concentrations, metabolism via the GS pathway may be detrimental to the brain. This notion is based on the idea that the excessive amounts of glutamine produced by GS during hyperammonemia serves as a “Trojan horse” for the following reasons [106]. First, accumulation of glutamine in astrocytes leads to swelling of the cells with cytotoxic brain edema and possible release of glutamate as clinically important consequences [8, 107]. Second, the increased brain glutamine may be taken up by neurons and converted back to glutamate and ammonia via PAG, which is preferentially localized to neuronal mitochondria [37, 87, 108]. Accumulation of ammonia in mitochondria has been proposed to induce oxidative/nitrosative stress and result in opening of the mitochondrial transition pore. These events can lead to cell dysfunction and cell death [106]. Notably, administration of MSO to animal models of hyperammonemia ameliorates many of the toxic effects of this condition, suggesting that GS inhibition may in fact be neuroprotective during conditions of hyperammonemia [13]. The effects of GS inhibition are clearly multifaceted and further studies are required to assess the functional consequences of localized increases of brain ammonia thought to be present in MTLE.

Effects on Energy Metabolism

Interestingly, seizures induced acutely by systemic convulsant doses of MSO in ventilated rodents differ from those produced by GABA receptor antagonists (e.g., bicuculline, pentylenetetrazol, or flurothyl) in their effects on brain glucose and energy metabolism. Whereas GABA antagonists induce rapid loss of phosphocreatine and glycogen, and large increases in lactate (~6–8-fold)—all signs of intense glycolytic activation [109–114], MSO-induced seizures do not produce such changes. Instead, levels of phosphocreatine and ATP are maintained at or near normal during MSO-induced seizures, while levels of glucose and glycogen are elevated, with only a modest rise in lactate (<twofold) [98]. Because GS is a major pathway for free ammonia disposal, inhibition of GS by systemic injection of MSO leads to hyperammonemia, which in itself can induce convulsions [115]; MSO however, counteracts many of the effects of hyperammonemia such as astrocyte swelling and a rise in extracellular potassium ions and glutamine [13].

While the difference in metabolic response to MSO has led to questioning whether GS inhibition is related to seizure generation, we note that unlike GABA-antagonist induced

seizures, which play out with an intact astroglial glutamate uptake and clearance system, i.e., an operational glutamine-glutamate-GABA cycle [116], MSO blocks the major pathway of glial glutamate clearance and neuronal glutamate/GABA replenishment. Thus, the increases in glucose and glycogen, and diminished lactate observed during MSO-induced seizures could be explained if the metabolic pathways are linked directly to the energetics of astroglial glutamate clearance [117-120].

An intriguing link between brain glycogen metabolism and glutamine synthesis is highlighted by the dramatic effect of MSO on glycogen levels. Glycogen is the major storage form of glucose, comprising a heterogeneously sized population of high molecular weight molecules, but is limited in supply to approximately 3–10 $\mu\text{mol/g}$. Glycogen is normally synthesized only in astrocytes [121, 122], but formation is seen in some neurons under pathophysiological conditions since the necessary enzymes are present. Glycogen levels reflect the concerted actions of glycogen synthase (E.C.2.4.1.11) and glycogen phosphorylase (E.C.2.4.1.1), and both enzymes are highly regulated via cyclic-AMP dependent protein phosphorylation involving protein kinases, phosphatases, and allosteric modulators, including IMP, AMP, and glucose. In MSO-treated mice the rise in glycogen at seizure-onset has been attributed to reduction in the activated form of phosphorylase, suggesting that decreased degradation is involved [103]. Glycogen accumulation is greatest in the perivascular endfeet [123], which is also a site of high expression of glutamate transporters and GS. Increased gluconeogenesis from glutamate has been proposed to explain MSO-induced glycogen accumulation [124], although a study using primary cultures of astrocytes found that direct synthesis of glycogen from glucose in the medium could account for this [120]. These investigators first noted the possible link between GS inhibition (and the interruption of the flow of glutamate-to-glutamine) and glycogen accumulation. Of note, MSO-induced glycogen accumulation was not seen in white matter [98], which has been shown to have much lower rates of glutamine-glutamate-GABA cycling compared to grey-matter regions that are enriched in nerve terminals [125]. MSO-induced glycogen accumulation is also much greater in vivo, where glutamine synthesis and cycling rates are high [126], in contrast to brain slices where only small increases were seen (14–28 %) [98, 127], an observation consistent with the slow rate of glutamine synthesis from neuron-derived glutamate reported in the unstimulated slice [128]. Noteworthy is the recent report of high glycogen levels in epileptic hippocampus of quickly frozen tissue removed during neurosurgery for intractable TLE [129], although its relation to neuropathology is unclear because only ‘normal’ appearing cortical grey and white matter (and not hippocampal tissue) was available for comparison. Thus, it remains to be shown whether glycogen levels are elevated in the human hippocampus in TLE.

Brain glycogen concentration shows an inverse relationship with activity. Higher levels are seen under deep anesthesia [130] and during sleep, with lower levels observed under waking states and sleep deprivation [131]. Glycogen is rapidly degraded during physiologic brain activation and its turnover is increased with activity [132-135]. In studies of the rat optic nerve in vitro, glucose deprivation leads to glycogen breakdown in astrocytes and formation of lactate, which can be utilized by axons [136]. The shunting of glucose through glycogen in astrocytes has been proposed as a possible explanation of the value of the oxygen-to-glucose index (OGI) and its change during increased activity [118], effectively linking the flow of glucose through astroglial glycogen as a source of rapid glycolytic ATP formation needed for the extrusion of sodium ions accumulated during glutamate uptake for rapid ECF glutamate clearance [117, 118, 135]. Astrocytes may also provide glycogen-derived glucose or lactate to neurons as oxidative fuels [137-139], as well as support de novo synthesis of glutamate and glutamine in astrocytes to replenish neuronal neurotransmitter pools [140-142].

Glycogen accumulation has been shown to delay the rate of propagation of spreading depression induced by insults such as high potassium or oxygen-glucose deprivation [127], which could play an important role in delaying the onset and spread of seizures [143, 144]. Thus, understanding the MSO-induced increase in glycogen levels could have potential therapeutic benefits. Because the chronic MSO-infused hippocampal formation in rats replicate some of the finding in human MTLE, future studies are needed to assess the levels of glycogen in this epilepsy model to more firmly link glial deficits in glutamine-glutamate cycling to glycogen and epilepsy.

Conclusions

GS is a ubiquitous and highly compartmentalized enzyme that is critically involved in several metabolic pathways, including the glutamine-glutamate-GABA cycle and metabolism of ammonia in the brain. Not surprisingly, an increasing number of studies suggest that alterations in the expression or activity of GS may underlie a variety of brain disorders, including Alzheimer's disease, depression, schizophrenia, and epilepsy. However, because GS is an integral part of a complex metabolic system, the effects of GS perturbations are multifaceted and difficult to predict. Furthermore, the type of perturbation varies among each specific disorder, and includes alterations in GS mRNA, posttranslational modifications of the protein, decreased enzyme activity, redistribution of the protein within astrocytes, aberrant expression in neurons, and changes limited to specific areas of the brain. Controlled studies using targeted mutations of the GS gene, or highly specific, focally delivered enzyme inhibitors, are expected to better address the role of GS during physiological and pathological conditions. If a deficiency in GS is indeed critically involved in the development of brain disorders such as MTLE, then restoration of GS in astrocytes deficient in the enzyme, could represent a novel and potentially efficacious therapeutic approach.

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Fig. 1.

Diagram of GS and its role in the glutamine-glutamate-GABA cycle and ammonia metabolism. Key enzymes, transporter molecules, and metabolic intermediates are shown. Note the metabolic relationship among astrocytes, GABAergic neurons, and glutamatergic neurons as indicated by the glutamine-glutamate-GABA cycle (purple arrows to the left) and the glutamine-glutamate cycle (red arrows to the right). Abbreviations: *ADP* adenosine diphosphate, *ASAT* aspartate aminotransferase, *ATP* adenosine triphosphate, *GABA* γ -aminobutyric acid, *coA* coenzyme A, *GAT3* GABA transporter subtype 3, *GAD65/67* glutamate decarboxylase isoforms 65/67, *GDH* glutamate dehydrogenase, *MCTs* monocarboxylate transporters, *PAG* phosphate activated glutaminase, *SAT1/2* system A transporter subtypes 1/2, *SN1* system N transporter subtype 1, *VGAT* vesicular GABA transporter, *VGLUT* vesicular glutamate transporter

Fig. 2.

GS is deficient in astrocytes in the hippocampal formation in patients with MTLE. **a–c** Immunohistochemistry of GS in the hippocampal formation from a non-epilepsy (autopsy control) subject reveals intense labeling for GS in the cell body and processes of astrocytes throughout the hippocampal formation (*arrows* in **b** and **c**). Note that the labeling extends into the finest and most distal astrocyte processes. **d–f**: In contrast, GS is virtually absent from astrocytes in several areas of the hippocampal formation in patients with MTLE, particularly in CA1 (**f**). In areas where GS is present (such as in the subiculum), the protein frequently does not extend into the most distal astrocyte processes (*arrow* in **e**). The loss of GS was confirmed by Western blots and enzyme activity studies. *Scale bar* in **A** = 0.5 mm (same magnification as **d**), **B** = 100 μ m (same magnification as **c**, **e** and **f**). Abbreviations: *alv* alveus, *CA1* hippocampal subfield Cornu Ammonis 1, *dg* dentate gyrus, *sub* subiculum. (Adapted from [18] and reproduced with permission from The Lancet/Elsevier B.V.)

Fig. 3. Chronic infusion of MSO into the hippocampal formation of rats causes inhibition of GS, and in some cases neuropathological changes that resemble hippocampal sclerosis as reported earlier [17, 80]. **a–d:** a silver staining technique for labeling degenerating neurons and fiber tracts reveals numerous stained (injured) neurons and fibers preferentially in area CA1 in a rat infused with MSO into the hippocampal formation (**b, d**) whereas a rat infused with 0.9 % NaCl does not reveal stained (injured) tissue elements (**a, c**). Magnification: A = B; C = D. Abbreviations: *CA1–3* hippocampal subfields Cornu Ammonis 1–3, *MSO* methionine sulfoximine. (Adapted from [17] and reproduced with permission from Brain/Oxford University Press)