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Glutamine in the central nervous system: function and dysfunction

Jan Albrecht [1], Ursula Sonnewald [2], Helle S. Waagepetersen [3], and Arne Schousboe [3]

[1] Department of Neurotoxicology, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland, [2] Department of Neurosciences, Norwegian University of Science and Technology, Trondheim, Norway, [3] The Neurobiology Unit, The Danish School of Pharmaceutical Sciences, Copenhagen, Denmark

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1. ABSTRACT

Glutamine (Gln) abounds in the central nervous system (CNS), and its interstitial and cerebrospinal fluid (CSF) concentrations are at least one order of magnitude higher than of any other amino acid. Gln transport from blood to the brain is insufficient to meet the demand of the brain tissues for this amino acid. This demand is met by intracerebral Gln synthesis from glutamate (Glu), a reaction carried out by glutamine synthetase (GS), an enzyme residing in astrocytes. A major proportion of astroglia-derived Gln is shuttled to neurons where it is degraded by phosphate-activated glutaminase (PAG) giving rise to the excitatory neurotransmitter amino acid Glu, which is also a precursor of the inhibitory neurotransmitter γ-aminobutyric acid (GABA). Glu released from neurons is taken up by astrocytes, and reconverted to Gln, closing the so called "glutamate – glutamine" cycle. A portion of Gln serves as an energy metabolite, and part of it leaves the brain to blood. Gln efflux from astrocytes, its neuronal uptake and egress to the blood via the cerebral capillary endothelial cells is mediated by different amino acid carriers showing i) considerable preference for Gln, ii) distribution between astrocytes and neurons that favors astrocyte-to-neuron fluxes of the amino acid. The Gln -specific carriers also largely contribute to Gln efflux from the brain to the vascular bed. Excessive accumulation of Gln in brain cells may be deleterious to brain function. In hyperammonemia associated with acute liver failure, excess Gln leads to cerebral edema, which largely results from its interference with mitochondrial function and partly from its osmotic action. Future analyses of the roles of Gln in both normal and abnormal cerebral metabolism and function will have to account for its newly recognized direct involvement in the regulation of gene transcription and/or translation.

2. INTRODUCTION

The complexity of the brain has long been acknowledged. However, the more we learn, the more complex the metabolic issues become. Some dogmas, which have served to guide our experiments in the past, have proven to be wrong and it is clear that we have to re-evaluate our understanding of most of the issues. The truth of the above statements becomes striking if one looks at the evolution of views on the diverse roles of Gln in brain metabolism in norm and pathology. It must be emphasized that many metabolic pathways involving Gln is common to the CNS and other mammalian tissues. The present review aims at summarizing events that are specific to the functioning of the CNS. Therefore, the roles of Gln as a precursor or product of an array of molecules whose significance is not specific to the CNS will not be discussed here. The only apparently ubiquitous events associated with Gln to be discussed in this review are ammonia metabolism and energy balance. As will be referred to in sections 4 and 5, Gln is the molecule that couples ammonia metabolism to the synthesis of neurotransmitter amino acids Glu and GABA. In the process, Gln fine-tunes the communication between neurons that propagate the signals and astrocytes which are their active associates. Gln metabolism produces compounds that are direct precursors of the tricarboxylic acid cycle intermediate α-ketoglutarate and as such contribute to meeting the high energy demand of the brain (section 5). Clearly, the diverse roles of Gln in the CNS function, but also the modes in which its excess results in CNS dysfunction (section 6) cannot be appreciated without information on the distribution of Gln in the different compartments of the CNS, which is the subject of the forthcoming section.

3. GLUTAMINE (GLN) CONTENT AND REGIONAL DISTRIBUTION

Gln is relatively evenly distributed between the different brain regions, and its tissue content is in the same order of magnitude as that of the other major amino acids. Among 15 different rat brain regions analyzed, Gln content ranges from ~5 nmoles/mg in the hippocampus to ~7 nmoles/mg in the cerebellum, which is slightly less than the Glu content and marginally higher than the aspartate (Asp) or taurine (Tau) content (1). Among 17 rabbit brain regions, Gln content ranges from ~4 nmoles/mg in the frontal cortex to ~ 8 nmoles/mg in the hippocampus; for comparison, Glu and Tau contents is in the different regions are in the range of ~4- ~20, and ~8 – ~40 nmoles/mg, respectively (2,3). Gln content in the extracellular fluid (microdialysates) [GLN_{ECF}] does not show high variation between the different species and regions of the brain either, ranging somewhere between 0.2 – 0.5 mM (4,5); it reaches 1.5 mM in the spinal cord (6). However, [GLN_{ECF}] exceeds by at least one order of magnitude the extracellular content of other amino acid (see the above references). The cerebrospinal fluid (CSF) concentration of Gln is ~0.5 mM which exceeds the levels of other amino acids by ~10-100 times (6-8). In fact, Gln is the only amino acid whose arterial blood concentration (~0.5 mM; ref. 9 and references therein) is very close to [GLN_{ECF}]. As will be discussed later in the context of regulation of Gln transport, these flat Gln gradients are of significance in ensuring well-controlled movement of the amino acid between the different CNS compartments.

4. DISTRIBUTION OF GLN SYNTHESIZING- AND DEGRADING ENZYMES AND GLN CARRIERS

4.1. Glutamine synthetase

Inward transport of Gln across the blood-brain barrier is relatively modest, the Km for Gln influx from plasma into cerebral cortex being two orders of magnitude higher than Km for any major large neutral amino acid (10). Accordingly, a majority of CNS Gln is synthesized endogenously, in a reaction of Glu amidation with ammonia catalyzed by glutamine synthetase (GS). Immunocytochemical studies performed by Norenberg and colleagues three decades ago have provided unambiguous evidence that GS is specifically located in astrocytes of different brain regions (11, 12). GS immunoreactivity has been identified in a selected group of oligodendrocytes in rat and bovine brain areas, but the staining intensity was relatively weak and restricted to isolated areas (13). To the authors' knowledge, GS has never been located in neurons, either *in situ* or in culture. Studies with neuron-astrocytic co-cultures have provided evidence that GS expression in astrocytes is positively modulated by neurons, and the effect appears to be mediated by neuron-derived Glu or other trophic factors, but may also involve direct contact of astrocytes with the nerve cell matrix (14-16). Interestingly, in the hippocampus, GS immunostaining is strongest in astrocytic processes that engulf glutamatergic nerve terminals (17), and shows a specific layered distribution corresponding with the Glu binding sites (18). In a recent study, Fonseca et al. (19) incubated astrocytes in culture with changing concentrations of the Gln- hydrolyzing enzyme, glutaminase, which *in situ* abounds in neurons (see Section 4.2), in this way constructing a "virtual glutamatergic neuron" model. GS expression and activity in the astrocytes was correlated with the extracellular Glu concentration, which underscored the key role of GS-mediated Gln synthesis in astrocytes as a controlling step of Glu metabolism in the CNS. Vice versa, inhibition of Gln synthesis with a specific GS inhibitor, L-methionine-D/L-sulfoximine (MSO) in hippocampal slice cultures increased Gln immunoreactivity in glia and at the same time decreased the Glu immunoreactivity in adjacent nerve terminals, which in turn was associated with a several-fold increase of the latter in glia (20). This study bespeaks the key role of uninterrupted Gln synthesis in the maintenance of the synaptic (neurotransmitter?) pool of Glu.

4.2. Phosphate-activated glutaminase

Phosphate-activated glutaminase (PAG) catalyzes the hydrolysis of Gln to Glu and ammonia, which is the major route of Gln degradation in the CNS. Subcellular fractionation and immunocytochemical tests revealed that PAG is primarily a mitochondrial enzyme (21-23). Both biochemical (22) and immunocytochemical evidence (23) suggests that the active form of PAG is located externally on the inner mitochondrial membrane,

albeit the presence of a dormant form of PAG in the mitochondrial matrix has not been excluded. While subcellular fractionation (22, 24) and immunocytochemical studies (21) have also suggested the presence of glutaminase in the cytoplasm, significant contribution of the extramitochondrial glutaminase to Gln metabolism has not been ascertained.

Immunocytochemical evidence *in situ* suggests that in the cerebellum PAG is primarily located in neurons, but not in astrocytes (23). Neuronal immunostaining of PAG also predominates in the forebrain albeit diffusely distributed immunoreactivity was detected in glial processes (21). The high PAG activity in neurons is consistent with the compartmentation of Gln metabolism, whose synthesis occurs in astrocytes. However, these *in situ* findings contrast with the high PAG activity that has been measured in cultured cerebellar astrocytes (25, 26). Whether this discrepancy reflects the fact that culture conditions favour PAG expression, or a more difficult access of astrocytic PAG to the immunogold procedure remains a matter of conjecture.

Glutaminase in human and rodent cells occurs in two isoforms coded by different genes: 1) liver-type (LGA) coded by a gene located on chromosome 12 which requires low phosphate for maximum activity and is not inactivated by Glu and ammonia, and 2) kidney-type (KGA), coded by a gene located on chromosome 2, and dependent on high phosphate (27-31). Both izoenzymes have been located in the brain (31). KGA shows a typical mitochondrial localization and its phosphate-dependence, and glutamate- and ammonia-sensitivity are a perfect fit to the characteristics of Glu degradation earlier reported for intact brain mitochondria (25), and cultured astrocytes (26). In addition , the PAG activity is inhibited by endogenous amino acids: N-acetyl-aspartic acid (NAA), taurine (Tau) (32), histidine (His) and lysine (Lys) (33), and by tricarboxylic acid intermediates (34). Whether and in what degree this inhibition reflects the control of Gln transport *in situ* remains to be established.

In contrast to peripheral tissues, LGA is absent from CNS mitochondria but is present in CNS cell nuclei, where it may control Gln availability for regulation of transcription or be a transcription factor itself (31).

4.3. Carriers transporting Gln across cell membranes and blood/brain/CSF barriers

Transport of Gln across the membranes of the CNS cells is mediated by multiple transport systems, whereby the sodium-dependent systems ASC, A and N play dominating roles (35-37). Immunocytochemical analysis and Gln uptake/efflux measurements in vivo and in vitro clearly point to the compartmentation of the major Gln-transporting proteins between astrocytes and neurons. The unidirectional system A transporter SNAT1 (ATA1, GlnT) which catalyzes Gln uptake, is predominantly located in neurons (38-41). Functional studies have demonstrated the role of this transporter in maintaining adequate supply of Gln to glutamatergic terminals and Glu-mediated electrophysiological events. Inhibition of the system A-mediated Gln transport by a specific competitive inhibitor, methyl-amino-isobutyric acid (MeAiB) suppressed discharges in primary hippocampal cultures and hippocampal slices (42), and abolished respiratory activity in isolated brain stem (43). In the latter case an identical suppressing effect was achieved with the GS inhibitor, MSO (43). In vivo, MeAiB caused a substantial accumulation of Gln in the extracellular space (microdialysates) in three different brain regions (5). MeAiB also inhibited the formation of neurotransmitter pools of Glu and GABA from ^{13}C labeled tricarboxylic acid cycle constituents in guinea pig brain slices (44).

Astrocytes are enriched in two bi-directional transporters: system N transporters: SN1 (SNAT3) (37, 45, 46) and in some degree SN2 (SNAT5) (47). Contribution of system ASC transporter –ASCT2 has likewise been suggested on the basis of tests performed in cultured astrocytes: however, its significance in vivo remains to be confirmed (35, 48). None of the above mentioned systems has been detected in neurons. Several properties render SN1(SNAT3) the best candidate to specifically mediated Gln efflux from astrocytes. These include i - activity optimum at physiological Gln concentration (Km ~0.4 mM); ii – independence on a substrate on the trans-side, predisposing it to mediation of net efflux (48); iii – its upregulation by increased intracellular Glu (49). Of note in this context, neuron-derived neurotransmitter Glu provides an overwhelming majority (80-90%) of the substrate pool for Gln synthesis (50). The transport of a portion of astroglia- derived Gln across the blood-brain barrier to the periphery is likewise mediated by system N transporters (51, 52) with some contribution of the sodium-independent L-system that exchanges Gln for large neutral amino acids (for discussion and references see Section 6). Carrier-mediated movements of Gln between astrocytes, neurons and the blood-brain barrier-forming cerebral capillary endothelial cells are schematically outlined in Figure 1.

4.4. Gln transport in mitochondria

Experiments with isolated mitochondria revealed that mitochondrial Gln uptake consists of an active (saturable) and a diffusive (non-saturable) component, whereby the former appears to dominate (22, 53-55). The saturable component is concentrative, osmosensitive and largely stereospecific (56). The uptake is metabolically regulated, i.e. it is partly activated by an electrochemical proton gradient (34, 56), and inhibited by Glu, tricarboxylic acid intermediates (34) and His and leucine (Leu) (33). Inhibition by Glu plus the fact that the uptake is more active in synaptic than in nonsynaptic mitochondria (54, 56) is in agreement with Gln being primarily metabolized in the neurons. Whether Gln uptake to mitochondria is directly coupled with, and a prerequisite of, its intramitochondrial degradation by PAG is a matter of dispute. In favor of this concept, inhibition of mitochondrial Gln uptake by some natural amino acids and their synthetic analogs was found to correlate well with the inhibitory effect of these compounds on Gln degradation (33). On the other hand, factors such as calcium plus phosphate which stimulate PAG inhibit Gln uptake (34), while Tau or NAA inhibit PAG (32) but do not affect Gln

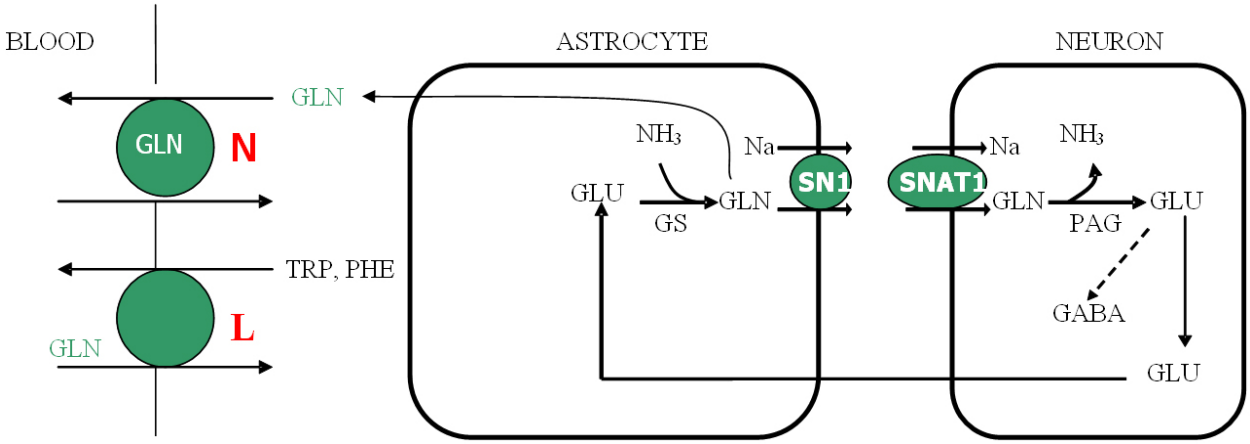

Figure 1. A scheme illustrating the contribution of amino acid carriers to Gln fluxes in the CNS. Newly synthesized Gln exits astrocytes using an N system carrier, SN1 (SNAT3), and is taken up by neurons by an A system carrier SNAT1. A portion of Gln is transported from brain to blood by system N carriers whose identity with the astrocytic carriers remains to be analyzed in detail. Gln also leaves the brain in exchange of large neutral amino acids which is mediated by a sodium-independent system L. Abbreviations: GABA, gamma-amino butyric acid; GLU, glutamate; GLN, glutamine; GS, glutamine synthetase; PAG, phosphate-activated glutaminase; PHE, phenylalanine; TRP, tryptophan.

uptake (33). A recent study has shown that, only a distinct pool of Gln whose mitochondrial uptake is mediated by a His-sensitive carrier undergoes deamidation in the cerebral mitochondria (57).

5. GLN METABOLISM IN THE CNS AS STUDIED USING ^{13}C NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY AND MASS SPECTROMETRY (MS)

^{13}C NMR spectroscopy and mass spectrometry (MS) are excellent tools for studying Gln metabolism and its role in neuronal-glial interaction. In order to define precisely metabolic pathways and particular those involving TCA-cycle reactions it is necessary to be able to determine the intramolecular location of carbon atoms. This is most elegantly achieved using ^{13}C NMR spectroscopy. MS provides a much higher sensitivity but only information about the total number of labeled carbon atoms and not the intramolecular position. A combination of these two methods therefore constitutes a powerful means to elucidate the above mentioned metabolic pathways. Incubating co-cultures of neurons and astrocytes in the presence of [2-^{13}C]acetate and MSO, it could be shown that Gln from astrocytes is an excellent precursor for GABA biosynthesis in neurons (58). Using cultured GABAergic or glutamatergic neurons exposed to different depolarization conditions it is possible to distinguish release from the vesicular and the cytoplasmic neurotransmitter pools, respectively. Thus, exposure of GABAergic neurons to 55 mM K^+ in combination with the GABA transport inhibitor tiagabine leads to release selectively from the vesicular pool while depolarization induced by NMDA in combination with AMPA selectively activates release from the cytoplasmic pool (59). This paradigm was used to investigate the synthetic route of GABA from [U-^{13}C]Gln employing ^{13}C NMR spectroscopy and MS (60). Evidence was provided that 60% of GABA synthesis from Gln occurs via Glu derived from the TCA cycle intermediate α-ketoglutarate. During vesicular release newly synthesized GABA was found to also originate via this biosynthetic mechanism, i.e. around 60% was produced from Glu originating from the TCA cycle constituent α-ketoglutarate. On the other hand, in the cytoplasmic pool, the fraction of GABA synthesized directly from Gln via glutamate, without involvement of the TCA cycle, was found to be more pronounced. In a different set of experiments, a similar depolarization paradigm inducing vesicular release has been employed to study Gln metabolism in glutamatergic neurons incubated in the presence of [U-^{13}C]Gln. Based on labeling patterns of Glu, biosynthesis of the intracellular pool of Glu from Gln was found to involve the TCA cycle to a considerable extent (approximately 50%). The involvement of the TCA cycle was significantly lower for synthesis of Glu in the releasable vesicular pool, perhaps indicating that the vesicular pool may not be in equilibrium with the remaining cellular pool (61).

^{13}C NMR spectroscopy and MS analyses of extracts prepared from cultured astrocytes incubated in medium containing different labeled substrates, such as [U-^{13}C]Glu, [U-^{13}C]glucose and [U-^{13}C]lactate have provided evidence for complex Gln metabolism particularly that related to the TCA cycle and the anaplerotic synthesis of oxaloacetate from pyruvate occurring exclusively in astrocytes (62-64). Thus, labeling patterns of the TCA cycle intermediate citrate and that of Gln originating from another intermediate, α-ketoglutarate, were found to be distinctly different both in the cellular and extracellular pools (65). One of the main tasks of astrocytes is to maintain a low extracellular concentration of Glu (66, 67). Subsequent to uptake via Glu transporters, Glu can be either converted directly to gln and other products, such as GSH, or be metabolized *via* the TCA cycle after conversion to α-ketoglutarate for

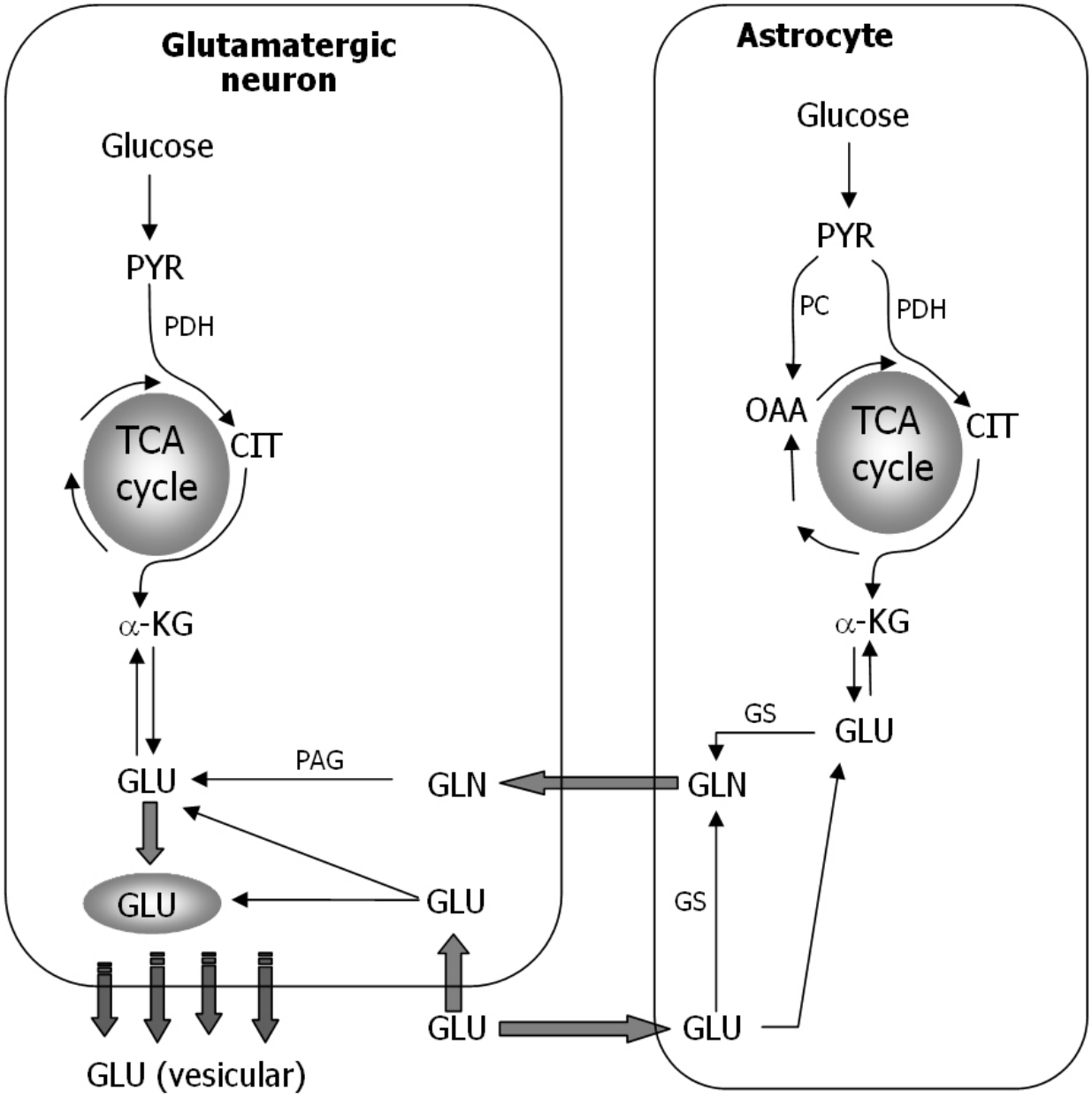

Figure 2. Schematic representation of key metabolic processes involved in glutamate homeostasis related to neurotransmitter release in a glutamatergic synapse interacting with a surrounding astrocyte. The vesicular pool of Glu is highlighted by an ellipse. Glycolysis and TCA cycle metabolism are indicated in both cellular compartments and in the astrocytic compartment pyruvate carboxylation to oxaloacetate *via* pyruvate carboxylase (PC) is included. The glutamate-glutamine cycle including the reactions catalyzed by glutamine synthetase (GS) and phosphate activated glutaminase are indicated in the astrocytic and neuronal compartment, respectively. Abbreviations: CIT, citrate; GLN, glutamine; GLU, glutamate; GS, glutamine synthetase; α-KG, α-ketoglutarate; PAG, phosphate activated glutaminase; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase; PYR pyruvate; TCA, tricarboxylic acid.

energy production and synthesis of various metabolites (Figure 2, for review see ref. 68). The extent to which [U-^{13}C]Glu is converted to gln has been shown to be dependent on its extracellular concentration (69). Incubation of astrocytes with [U-^{13}C]Gln led to formation of labeled Glu, Asp, lactate and alanine thus, demonstrating that [U-^{13}C]Gln was partly metabolized through the tricarboxylic acid cycle, although to a much smaller extent than previously shown for [U-^{13}C]Glu (70, 71).

6. GLN IN NEUROLOGICAL DISORDERS ASSOCIATED WITH HYPERAMMONEMIA

As mentioned before, the central nervous system

(CNS) lacks a complete urea cycle, rendering Gln synthesis as the only route by which CNS cells are able to efficiently neutralize blood-born ammonia or to recycle ammonia generated in the intracellular metabolic pathways (11, 72). Ammonia crossing in excess the blood-brain barrier and penetrating the brain tissues is a causative factor in a group of neurological disorders collectively coined as hyperammonemic encephalopathies. Of these, the most commonly occurring is hepatic encephalopathy (HE) (73). HE associated with viral or toxic liver damage (fulminant hepatic failure; FHF) shows a rapid progress, leading to coma (for reviews see ref. 72-75). There is a consensus that brain edema resulting from astrocytic swelling (76) is a major cause of death in acute HE patients (77, 78), and animals with experimentally-induced HE (79). Liver cirrhosis, the major cause of HE is mostly associated with only minor psychomotoric deficits without significant increase of blood ammonia, the condition being defined as subclinical hepatic encephalopathy (SHE) (80, 81). However, liver cirrhotic patients are highly susceptible to hyperammonemic incidents which result in cerebral edema, coma and death (82).

In the process of ammonia neutralization, Gln in the brain or cerebrospinal fluid increases several fold in hyperammonemic patients (83-85) and in different animal models of fulminant hepatic failure or chronic HE (9, 86-90). Since Gln is a neutral amino acid and is not a ligand of any known neurotransmitter receptor it has long been viewed as a non-toxic ammonia carrier between the different compartments of the CNS, and to the periphery (72, 91). Accordingly, increased astrocytic Gln synthesis during hyperammonemia has been interpreted to reflect efficient ammonia detoxification, effectively protecting neurons against blood-derived ammonia.

This simple view was for the first time challenged by Warren and Schenker (92), who observed that administration of a GS inhibitor, methionine sulfoximine (MSO) into rats decreases their death rate following acute ammonia intoxication. The idea that Gln may be a pathogenic factor in hyperammonemia re-emerged in the early- to mid 90s. Attention has been paid to the good correlation between the elevation of whole brain Gln and brain water content in acutely hyperammonemic animals (93), the correlation also holding for the individual brain regions (88). MSO turned out to be a formidable tool in these studies. Reduction of cerebral Gln accumulation by MSO has been repeatedly reported to coincide with the reversal of different pathophysiologic and metabolic manifestations of experimentally induced FHF and HE. Thus, MSO attenuated cerebral edema (94), and at the cellular level its primary cause, astrocytic swelling, as demonstrated in both *in vivo* and *in vitro* models of hyperammonemia (95-98). Treatment of HE rats with MSO restored the cerebral metabolic rate for glucose in concert with a decrease of cerebral Gln content to the control level (89). Other pathophysiological effects of hyperammonemia that were subject to amelioration by MSO included a rise of intracranial pressure, increased cerebral blood flow (99), reduced CBF responsiveness to hypercapnia (100), or increased extracellular potassium activity (101): each of the above phenomena is related to astrocytic swelling and cerebral edema. Of note, MSO treatment in healthy rats, which *per se* raised plasma ammonia level did not alter cerebral metabolic rate for glucose (102). Most recently, a ^{1}H-NMR examination in cirrhotic patients revealed a good correlation between the cerebral Gln level (Glu/Gln signal) and the severity of neuropsychological effects induced by hyperammonemia in these patients (103).

The contribution of Gln to acute hyperammonemic edema has originally been ascribed to its osmotic effects (104). Accordingly, the absence of edema in chronic hyperammonemic patients or experimental animals has been interpreted to reflect a compensation of the increase of intracellular osmotic due to Gln by a loss from the brain of other osmolytes, e.g. myoinositol and Tau (105). However, a number of experimental data appear to challenge the simple view that Gln acts as a factor producing osmotic imbalance in the ammonia-affected brain. A ^{1}H/^{32}P-NMR-based analysis of the content of organic osmolytes in control rat cerebral cortical slices revealed that Gln accounts for no more than 2% of their total pool (106). Even under extreme ammonia challenge, the cerebral Gln content in HE patients or experimental animals does not rise more than 3-4 fold (9, 83, 84, 86-90), which is not enough to produce by itself significant brain edema. In some models of hyperammonemia an increase of brain water content correlated better with a rise of other low molecular weight osmolytes, such as alanine or lactate (107, 108). Most interestingly, mild hypothermia, a treatment modality based on the principle of sparing energy consumption, normalized ICP and brain cell volume in hyperammonemic rats, without reducing Gln content (108). Based on this observation, hypothermia was employed with success to ameliorate HE symptoms in cirrhotic patients awaiting liver transplantation (reviewed by Vaquero and Blei, ref. 109). In ammonia-treated cerebral cortical slices exposure to NMDA receptor antagonists and inhibitors of downstream effects of NMDA receptor activation, reduced cell volume without elevation of Gln content (110). The results of the latter two studies indicated that the contribution of excess Gln to hyperammonemic edema will be less when the cerebral energy metabolism is slowed down. This interpretation supports direct interference of Gln with the functioning of cerebral mitochondria operating under conditions of normal energy demand (for a discussion see ref. 111).

Paralell to these phenomenological observations, evidence has begun to accumulate that Gln is a mediator of ammonia-induced mitochondrial damage and that astrocytic mitochondria are the primary target. Exposure of isolated rat cerebral mitochondria to Gln concentrations measured in hyperammonemic rats, caused mitochondrial swelling and activation of mitochondrial permeability transition (mPt) (112). This effect was markedly attenuated in the presence of His (112), an amino acid specifically inhibiting the uptake of the Gln pool which is a substrate of PAG (57).

In cultured astrocytes, ammonia-induced formation of free radicals – a marker and carrier of

mitochondrial impairment, and subsequent astrocytic swelling, both were attenuated by MSO treatment (113). Recently, Gln added directly to the astrocytes was found to induce mPt (114) and promote formation of free radicals (115). The latter effect was absent in cultured neurons, confirming astrocytic mitochondria as a primary target of Gln toxicity.

By analogy to what has been suspected to occur at the whole-cell level, the damaging effects of Gln in mitochondria have been initially interpreted to be due to its osmotic action. However, most recent evidence implicates Gln-derived ammonia in Gln-induced damage: Formation of free radicals in Gln-treated astrocytes was counteracted by the PAG inhibitor, 6-diazo-5-oxo-L-norleucine (DON) (115). Ammonia generated from Gln in mitochondria is likely to generate a vicious circle of events. An earlier study revealed that ammonia stimulates the carrier-mediated uptake of Gln in non-synaptic mitochondria (55).

Pathogenesis of hyperammonemic encephalopathies in humans and experimental animals is invariably associated with accumulation in the brain of increased concentrations of large neutral amino acids, including the aromatic amino acids tryptophan (Trp), phenylalanine (Phe) and tyrosine (Tyr) (83, 86, 90, 116). Increased Trp increases the synthesis of compounds implicated in neural inhibition: the inhibitory neurotransmitter serotonin (116, 117) and a sedative, oxindole (118). Pretreatment with MSO prevented the increase of brain content of Trp in portacaval shunted (chronic HE) rats, indicating that the uptake of Trp is promoted by increased Gln content in the brain (89). The underlying mechanism appears to be related to enhanced Trp/Gln exchange, which was observed *in vitro* in cerebral microvessels derived from rats with fulminant hepatic failure (FHF) (119), chronic HE (120), or microvessels from healthy rats which were subsequently treated with ammonium salts (119).

7. PERSPECTIVE

In light of the above presented data the key role of Gln in the metabolism of CNS appears beyond doubt. Until now, Gln has survived the most scrupulous tests verifying its key role in ammonia metabolism, energy production and the synthesis of neurotransmitters Glu and GABA. In this capacity, Gln orchestrates the metabolic communication between astrocytes and neurons. In this interplay, astrocytes are primarily endowed with the task of Gln production and its tunneling to the points of metabolic destination, thus providing neurons with the comfort of a well-controlled and adequately supplied end-user. Distribution of enzymes of Gln metabolism and of Gln-transporting proteins between astrocytes and neurons is subordinated to this goal. The clearly beneficial role of Gln when its level in the CNS remains within the physiological range may turn – and possibly does turn - into primarily detrimental when its production under hyperammonemic conditions exceeds its normal consumption.

Future studies will have to account for the potential role of Gln in regulating gene expression and in controlling the activity of cellular proteins. This relatively recently discovered aspect of Gln function (121, 122) has been analyzed in peripheral tissues but not in the CNS. The idea that Gln may regulate CNS-specific aspects of transcription has been derived from the exclusive presence in brain cell nuclei of the liver-type glutaminase (LGA) (31). Questions amenable for analysis in this context include: i – the existence of mechanisms that redistribute Gln between cell nuclei and other cell compartments, viz. Gln transport across the nuclear membrane ; ii – the role of LGA in the intranuclear Gln metabolism, in particular how LGA activity is coordinated with the Gln requirements for transcriptional events. The potential role of Gln and/or LGA in gene transcription control appears to be underscored by the recent observation of the absence of LGA expression malignant human brain tumors of glial origin (123).

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Abbreviations: CNS: central nervous system; CSF: cerebrospinal fluid; DON: 6-diazo-5-oxo-L-norleucine; ECF: extracellular fluid; FHF: fulminant hepatic failure; GABA: gamma-amino butyric acid; Glu: glutamic acid; Gln: glutamine; GS: glutamine synthetase; HE: hepatic encephalopathy; His: histidine; KGA: kidney-type glutaminase; LGA: liver-type glutaminase; Leu: leucine; Lys: lysine; MeAiB: methyl(amino)isobutyric acid; MS: mass spectroscopy; MSO: methionine sulfoximine; NAA: N-acetyl aspartic acid; NMR: nuclear magnetic resonance; PAG: phosphate-activated glutaminase; SHE: subclinical hepatic encephalopathy; Tau: taurine; Trp: tryptophan

Key Words: Glutamine, Glutamine synthetase, Phosphate-activated glutaminase, Glutamine Carriers, Hyperammonemia, MSO, Magnetic resonance spectroscopy, Review

Send correspondence to: Jan Albrecht, Ph. D., Department of Neurotoxicology, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Pawinskiego St. 5, 02-106 Warsaw, Poland, Tel: 48-22-6685323, Fax: 48-22-6685532, E-mail: jalb@cmdik.pan.pl

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