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Abstract

Brain energy metabolism is often considered as a succession of biochemical steps that metabolize the fuel (glucose and oxygen) for a unique purpose of providing sufficient ATP to maintain the huge information processing power of the brain. However, a significant fraction (10–15%) of glucose is shunted away from the ATP-producing pathway (oxidative phosphorylation) and may be used to support other functions. Recent studies have pointed to the marked compartmentation of energy metabolic pathways between neurons and glial cells. Here, we focus our attention on the biosynthesis of L-serine, a nonessential amino acid that is formed exclusively in glial cells (mostly astrocytes) through re-routing the metabolic fate of the glycolytic intermediate, 3-phosphoglycerate (3PG). This metabolic pathway is called the phosphorylated pathway and transforms 3PG into L-serine via three enzymatic reactions. We first compiled the available data on the mechanisms that regulates the flux through this metabolic pathway. We then reviewed the current evidence that is beginning to unravel the roles of L-serine both in the healthy and diseased brain, leading to the notion that this specific metabolic pathway connects glial metabolism with synaptic activity and plasticity. We finally suggest that restoring astrocyte-mediated L-serine homeostasis may provide new therapeutic strategies for brain disorders.

Introduction

L-serine, one of the most frequent amino acid found in vertebrate's proteins, was first discovered in nature, specifically in silk protein, by German chemist Emil Cramer in 1865 (see ref in [1]). L-serine was initially classified as nutritionally non-essential (dispensable) for humans and animals. From both a functional and a metabolic perspective, L-serine is "essential". It is the precursor of other nonessential amino acids such as glycine and cysteine so that its rate of synthesis is high enough to enable its secondary conversion [2]. L-serine has been known for a long time to sustain cell proliferation [3] as its supplementation can increase cell growth more efficiently than any other amino acid. Increased L-serine biosynthesis is one out of the numerous metabolic changes that have been reported in cancer cells and it is now recognized that a great number of solid tumor cancer cells are highly dependent on L-serine availability [4, 5]. This is mainly due to the fact that L-serine and glycine are critical players of the one-carbon metabolism, a complex metabolic process that generates building blocks for new cellular components, including proteins, lipids and nucleic acids [6, 7]. L-serine metabolism is now considered as a putative target in oncology and inhibitors of the metabolic pathways leading to L-serine biosynthesis could offer new therapeutic opportunities [8, 9].

In the brain, L-serine plays pivotal roles. Genetic conditions leading to a lack of L-serine production induce severe neurological abnormalities (see paragraph on serine deficiency), even if L-serine is produced by most dividing cells in the body. This is mainly because the role of L-serine in the central nervous system is not restricted to the synthesis of building blocks for the construction of new cellular components during cell proliferation. For example, L-serine displays trophic effects on neurons in culture, increasing the length and the complexity of neurites [10, 11]. More importantly, L-serine is critical for neurotransmission. L-serine is the precursor of two very important molecules that regulate the excitatory glutamatergic transmission. These two molecules, glycine and D-serine act as co-agonist of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor. While D-serine gates synaptic NMDARs and facilitates long-term potentiation (LTP, a persistent strengthening of synapses underlying synaptic plasticity), glycine acts mainly on extrasynaptic NMDARs, which are mostly involved in neurodegenerative disorders and cell death [12, 13]. In addition, glycine acts as an inhibitory neurotransmitter via ionotropic glycine receptors transmission.

What is striking for brain L-serine, is the incapacity of neurons to produce it, at least after embryogenesis [14]. L-serine is a glia-specific amino acid whose production and release are tightly regulated to fulfil the need of neurons. Despite the potential importance of this amino acid for brain functioning, it is surprising how little we know about the mechanisms regulating L-serine production in the normal brain. We will therefore review available data concerning the biosynthesis of L-serine, the cellular localization and the regulation of the metabolic pathways leading to L-serine production in the brain. We will then summarize recent work that is beginning to unravel the roles of L-serine both in the healthy and diseased brain, leading to the notion that this amino acid is indispensable in the central nervous system (CNS). We propose that restoring astrocyte-mediated L-serine homeostasis may provide new therapeutic strategies for brain disorders.

Biosynthesis of L-serine

L-serine can be taken up from the extracellular milieu or synthesized endogenously. Although L-serine is present in the blood, L-serine diffuses poorly through the blood brain barrier (BBB), so that *de novo* synthesis represents a substantial contribution to serine availability in the brain. We will then summarize what we currently know about the metabolic pathways leading to L-serine production in the brain, their cellular localization and the mechanisms regulating the L-serine pool.

In 1965, Bridgers [15] was the first to demonstrate that L-serine can be produced via the phosphorylated pathway (PP) in the mammalian brain as it was originally described in bacteria in 1963. The PP starts from 3-phosphoglycerate (3PG), a glycolytic intermediate, and through the action of three enzymes, gives rise to L-serine (Figure 1). The first reaction is catalyzed by phosphoglycerate dehydrogenase (PHGDH), which oxidizes 3PG into 3-phosphohydroxypyruvate (3PHP) using NAD^+ as a cofactor. Then, 3PHP is transformed into 3-phosphoserine (3PS) by phosphoserine aminotransferase 1 (PSAT1) using L-glutamate as the amino group donor, which is converted into α-ketoglutarate (α-KG). The last step is catalyzed by phosphoserine phosphatase (PSPH) which hydrolyzes 3PS into L-serine and releases one inorganic phosphate. In the brain, the initial precursor 3PG probably comes from glucose (through glycolysis) and not from phosphoenolpyruvate (PEP) through gluconeogenesis. *Phgdh*-deficient mice have been produced using targeted gene disruption in embryonic stem cells [16]. *Phgdh*-null embryos have small bodies with abnormalities in particular in the CNS and die at E13.5. These results highlight the importance of L-serine production for proliferating cells, and especially for normal brain development, but they also suggest that the majority of L-serine production is due to *de novo* synthesis from the glycolytic intermediate 3PG through the PP.

The PP is of great importance in the brain not only because it contributes to the vast majority of L-serine production but also because it uses and produces cofactors ($NADH/NAD^+$, glutamate and α-KG) that play key role in brain functioning. Supplementation of serine is often insufficient to rescue the defects in cellular fitness observed upon PHDGH inhibition [4]. The PP is a significant contributor of α-KG at least in cancer cells with high levels of Phgdh expression, since approximately 50% of the conversion of glutamate into α-KG relies on PSAT1 activity [4]. PSAT1 activity also regulates α-KG production in mouse embryonic stem cells [17]. Considering the crucial role of glutamate, α-KG and $NADH/NAD^+$ ratio in the brain, it is important to take into account the activity of the enzymes of the PP as users/producers of these molecules.

Alternative pathways can also regenerate L-serine. They include the conversion of glycine to L-serine and protein breakdown. The interconversion between L-serine and glycine is coupled to the folate cycle [6] and is catalyzed by serine/glycine hydroxymethyltransferase (sgHMT). L-Serine conversion to glycine generates 5,10-methylenetetrahydrofolate (5,10-MTHF) from tetrahydrofolate (THF), making L-serine a one-carbon unit donor, an aspect that will be further discussed later. Finally, recycling of amino-acids may be especially important in the brain, as the uptake of some amino acids by this tissue is restricted.

Cellular localization of the PP in the brain

The first evidence suggesting that L-serine synthesis is mostly performed in glial cells comes from studies examining the cellular expression of *Phgdh* mRNA by *in situ* hybridization and of PHGDH protein by immunostaining [18, 19]. While Phgdh is strongly and homogeneously expressed amongst ventricular neuroepithelial stem cells at E13, its expression is lost during neuronal differentiation. Phgdh expression is then transmitted to the radial glia and later to astrocytes in the gray and white matters. In the adult rodent, PHGDH is found in astrocytes in many brain regions including the dentate gyrus, olfactory bulb, cerebellar Purkinje cell layer, corpus callosum, hippocampal fimbria and anterior commissure. Further evidence suggests L-serine synthesis is mostly performed in glial cells not only in mice but also in non-human primates and humans. The brain RNA-seq database from Ben Barres Lab [20] shows an enrichment of the transcripts of the three enzymes of the PP (PHGDH, PSAT1 and PSPH) in astrocytes and oligodendrocytes, but not in neurons from P7-mouse cortex. Similar results were found in purified cells from the human temporal lobe cortex, obtained from surgeries for treating epilepsy and tumors [21]. Another recent database, obtained using single-cell transcriptional profiling of the adult mouse nervous system allows searching for cell types expressing a combination of genes [22]. The combination of the three mRNAs is found in mature oligodendrocytes, fibrous astrocytes, satellite and enteric glial cells (proliferating cells). We recently conducted visible immunostaining on mouse, non-human primate (NHP) and human brain tissues as well as fluorescent co-labelling on mouse brain tissues. Visible PHGDH and PSAT1 staining mostly showed typical astrocytic-shaped cells in mouse, NHP and human hippocampus. Fluorescent co-labelling showed PHGDH colocalizes with GFAP in rodent brains. Noteworthy, PHGDH antibody stands out as an excellent astrocyte marker in the brain; it is highly expressed in most astrocytes (not as GFAP) in the mouse and displays a prominent cytoplasmic staining (Figure 2).

Astrocyte-specific deletion of Phgdh using a conditional Cre/Lox strategy in adult mice was shown to decrease the levels of L-serine by more than 80% [23, 24]. Levels of D-serine and glycine were also reduced in those animals therefore suggesting that the astrocytic PP is crucial for L-, D-serine and glycine availability in the brain. Since the PP is branching from glycolysis, these results highlight a critical link between astrocyte glucose metabolism and L-serine availability (see below). The fact that L-serine biosynthesis is restricted to glial cells strongly suggests that specific transporters should be present to mediate shuttling of L-serine to neurons. The existence of such L-serine shuttle was first put forward by the group of Furuya [14]. The amino acid transporters (AATs) are part of the solute carrier (SLC) superfamily that comprises 65 families, 11 of which contain AATs [25]. Since the concentration of amino acids is usually higher inside than outside cells, ion-coupled transporters or amino acid exchangers are required to transport amino acids inside cells. Serine, a small neutral amino acid is transported by three systems; the sodium-dependent transporter system ASC (alanine, serine, and cysteine preferring) which includes two members, ASCT1 and ASCT2; the sodium-dependent transporter system A (alanine-preferring) which includes three members, SAT1 to 3 and the sodium independent transporter system asc which includes Asc-1. ASCT1 is the main transporter for serine in astrocytes [26, 27], as also evidenced by the fact that deficiency of ASCT1 induces neurological manifestations in children that overlap with those observed in serine biosynthesis defects [28], see paragraph on serine deficiency.

Regulation of the activity of the PP

Since the PP is of paramount importance to generate *de novo* L-serine in the brain, we will discuss the different mechanisms that could affect the activity of the three enzymes of the PP. It is worth to mention that the occurrence of most of them in the brain has not yet been reported.

Structural modifications

At least three types of PHGDH structures have been described [29]. Human PHGDH belongs to the type I, in which PHGDH proteins are composed of one substrate binding domain and one co-factor binding domain completed with two regulatory domains (ACT and ASB). Crystal structure of the human PHGDH is unknown, but the structure of PHGDH from *Mycobacterium tuberculosis*, which is close to the human form [30], has been solved. It reveals a tetramer structure with four similar active sites and diverse organization of the regulatory domains [31]. PSAT1 from *E. coli* and *A. thaliana* are the only PSAT1 proteins whose structure has been determined [32, 33]. The structure of the human form and its possible allosteric regulation remain unknown. The structure of crystal human PSPH has been determined in a complex with the substrate analog AP3. PSPH forms a dimer in physiological conditions. Each subunit is formed of a core domain and a dimerization domain, and the active site is found in the cleft between these two domains [34].

Mutation or phosphorylation leading to an alteration of the 3D structure of PHGDH affects its activity. Indeed, SIV490M mutation, which causes one form of inherited Neu-Laxova disease (see below) [35], impairs the folding and/or assembly of PHGDH [36]. Phosphorylation at residues Ser55, Thr57, and Thr78, catalyzed by PKCζ, reduces PHGDH activity during glucose deprivation by changing PHGDH stability and ligand binding efficiency [37]. PHGDH is a target for O-glycosylation [38] but the effect of such a modification on its structure remains unknown.

Competitive inhibition

PHGDH activity in mammals directly depends on the concentration of 3-PHP, which makes it very sensitive to competitive inhibition. No competitive effect has been observed with L-serine or any other amino acid that could have bound PHGDH at one of the active sites [39]. However, binding of ADP-Ribose on the cofactor binding site instead of NADH reduces PHGDH activity by ~50% [31].

Non-competitive inhibition

In bacteria and plants, it has been shown that L-serine can bind PHGDH on a regulation site and regulate L-serine synthesis through allosteric mechanisms [30]. Even if the mammalian forms of PHGDH display the same regulation site, there is no allosteric regulation of PHGDH through L-serine binding on mammalian PHGDH [39]. This means that L-serine production is not regulated by a direct feedback loop on PHGDH. However, L-serine may be an uncompetitive inhibitor of human PSPH [40].

Expression of the enzymes of the PP is regulated by several transcription factors

The main transcription factor that regulates the expression of the enzymes of the PP is ATF4 [41]. Its expression is increased when cells are deprived of amino acids or subjected to endoplasmic reticulum stress. ATF4 directly binds

to promoters of L-serine biosynthetic genes to activate their transcription [42, 43]. Both translation and transcription of ATF4 can be regulated by other transcription factors such as nuclear factor erythroid 2‑related factor 2 (NRF2) [42], but also via histone methylations [44, 45] and by phosphorylation of the alpha subunit of eukaryotic initiation factor 2 (eIF2α) [46]. In the brain, little is known about these transcriptional regulatory mechanisms that have mostly been studied in cancer cells (see [7] for review) but a recent work suggests that ATF4 is also operating in brain cancers [47]. It is worth to mention that ATF4 [48], NRF2 [49] and CREB1 [48] are highly expressed in glial cells. These regulatory pathways are crucial for synaptic activity [50-52] and neuron-glia metabolic interactions [49, 53] which definitely makes them potential targets as serine synthesis regulators in astrocytes in the adult brain.

Regulation of PP by glycolysis and vice versa

The majority of L-serine production comes from *de novo* catabolism of the glycolytic intermediate 3PG. It follows that the glycolytic flux directly controls serine production in the brain. Using metabolomics approach with [U-^{13}C]-glucose, it was found that a substantial fraction of glycolytic carbon is indeed transferred to serine [54]. The fraction of 3PG diverted toward serine synthesis remains unknown in the brain (~10% in cancer cells) and we are far from a complete understanding of the coordination of glycolysis and serine biosynthetic pathway. Since L-serine does not exert any direct feedback regulation on PHGDH, other mechanisms may exist to ensure a tight production of L-serine with respect to its cellular needs.

One mechanism has been suggested from the observation that L-serine can bind and activate the glycolytic enzyme pyruvate kinase (PK) [55]. PK catalyzes the last step of glycolysis by converting phosphoenolpyruvate (PEP) into pyruvate. Mammals express four PK isoforms, namely PKM1 (M1-PK, muscle isoform), PKM2 (embryonic and tumor isoform), PKL (liver isoform) and PKR (red blood cell isoform). While PKM1 is the constitutively active form present in many differentiated cells, PKM2 is mainly expressed in proliferating and cancer cells, where it acts as a rheostat of glycolytic activity [56]. It has been described in human colon carcinoma cells that L-serine specifically binds PKM2 and induces the formation of an active PKM2-tetramer [57]. Therefore, when the level of L-serine is high, PKM2 is fully activated and the glycolytic flux is increased (Figure 3). Conversely, when the level of serine is low, glycolytic efflux to lactate is transiently reduced, leading to an accumulation of glycolytic intermediates like 3PG that can be rerouted toward L-serine production [46] or 2PG that directly increases PHGDH activity [58]. Another indirect mechanism by which PKM2 could control serine production is via MDM2 chromatin binding, which was recently shown to increase the expression of *Phgdh*, *Psat1* and *Psph* [59]. Several reports also demonstrated that some factors regulating PKM2 do alter serine production [60, 61]. All these results show that the flux of the PP controls glycolysis by modulating PKM2 activity that, in turn, controls serine production through substrate availability.

Conversely, there is evidence that experimental conditions altering the L-serine flux also affect glucose metabolism. Thus, genetic silencing of *Phgdh* leads to the alteration of the levels of many glycolytic intermediates [54]. In addition, ablation of PHGDH or PSPH increases lactate production suggesting an increase of aerobic glycolysis that has been predicted by computational modelling [62]. During serine starvation, cells are unable to regulate their

glycolytic flux also suggesting a direct link between glycolysis and serine production [63, 64]. Finally, pharmacological inhibition of PHGDH alters the pentose phosphate pathway and the tricarboxylic acid cycle [65].

How can we integrate this information in the context of the brain? Astrocytes share many metabolic similarities with cancer cells [66]. A recent study showed that astrocytes express PKM2 instead of the usual expression of PKM1 in differentiated cells [21]. These observations suggest that the regulatory loop between glycolytic activity and serine production through PKM2 activity could occur in astrocytes and not in neurons. By activating PKM2, serine supports aerobic glycolysis and lactate production, a cardinal feature of astrocytes [67]. Moreover, in astrocytes, mitochondrial complex I is loosely assembled into supercomplexes, which explains the high generation of mitochondrial reactive oxygen species (mROS) by these cells [68]. Recently, such a naturally-occurring high mROS production by astrocytes was unveiled to up-regulate glycolysis *via* a redox mechanism involving HDAC4-mediated control of the pentose phosphate pathway [69].

What could L-serine serve for in the brain?

Providing D-serine

In the early 1990s, a group in Japan used gas chromatography (GC) and GC with mass spectrometry (GC–MS) to show that free D-serine is present and enriched in the brain at a high concentration [70]. The detection of D-serine in mice bred in germ-free conditions [71] confirmed that D-amino-acids are not produced only in bacteria. Radiolabeled molecules of glucose, L-serine and glycine were used to investigate the origin of this endogenous mammalian D-serine [72] and it was found that L-serine is the direct precursor of D-serine. The conversion of L- into D-serine is catalyzed by serine racemase (Sr), a pyridoxal 5'-phosphate dependent enzyme that was purified in 1999 from glial cultures of rat cerebral cortex [73]. Transfection of Sr in HEK293 cultured cells promotes D-serine production and decreases L-serine content, validating Sr function *in vitro* [74]. Mice with a targeted deletion of Sr display a large decrease of D-serine levels in the brain (80-90%), thereby confirming the function of Sr *in vivo* [75, 76]. It is now commonly accepted that Sr is the main enzyme that catalyzes the racemization of L-serine to D-serine, but debates are still raging to determine (1) in which cell type this racemization takes place and (2) which cell type is releasing it for neurotransmission [77, 78]. The aforementioned authors all agree with the astrocytic production of L-serine, however, some claim it is racemized into D-serine and released by astrocytes as a gliotransmitter whereas others claim L-serine is shuttled to neurons where it is racemized and thereafter released. In addition to ASCT1, asc-1 has been more recently reported to be expressed by neurons and to play a significant role in regulating extracellular concentration of D-serine in the brain [79-81]. Therefore, the existence of a serine shuttle between astrocytes and neurons for synthesis, release and degradation is likely, but its regulation still needs to be fully characterized (Figure 3).

The critical role that D-serine plays in the brain has been extensively reviewed [82, 83] and D-serine is now recognized as the main endogenous ligand of the strychnine insensitive glycine-binding site of synaptic NMDA receptors (Figure 3) [13, 84]. The co-agonist binding site of NMDA-R is not saturated *in vivo* [85], minor alterations of

D-serine concentration at the synapse can therefore dynamically modulate NMDA-R responses [86]. D-serine regulates many functions of these receptors including synaptic transmission and plasticity [87, 88]. Accordingly, genetically modified mice lacking the ability to produce D-serine endogenously display altered glutamatergic neurotransmission and impaired spatial memory [75, 89]. Conversely, mice deficient for DAAO (the enzyme that selectively degrades D-serine) show facilitation in LTP induction and an increase in memory abilities [90, 91]. Recent experiments performed *in vitro* and in slices suggest that the activation of NMDA-Rs by D-serine and glycine may be regulated by the PP [92].

The role of D-serine is not limited to synaptic plasticity (see references in [93]), as it is well recognized that NMDA-Rs play very important roles in physiological and pathological processes in the brain. For example, D-serine binding on NMDA-Rs affects neuronal proliferation in the dentate gyrus [94], neuronal migration during cerebellum development [95], synaptogenesis [96], dendritic morphology [97, 98], spine density [98, 99], spine stability and synapse integration [98, 100].

Therefore, concentration of D-serine needs to be tightly regulated. Degradation of D-serine is performed by D-amino acid oxidase (DAAO), a flavoenzyme [101]. Its expression in the brain is highly heterogeneous, being most abundant in the cerebellum and the brainstem with respect to the forebrain. Serine racemase can also catalyze α,β-elimination of water from L- or D-serine [102]. The elimination was shown to compete with the isomerization for regulating intracellular D-serine levels, especially in forebrain areas that have low DAAO activity [102]. It is worth mentioning that α,β-elimination of water from L- or D-serine yields pyruvate, a major substrate for oxidative metabolism. Whether this pathway may serve to fuel mitochondria in neurons for ATP production or may participate in the production of lactate in astrocytes for signaling purposes, needs to be further tested.

L-serine and lipids

L-serine has long been described as a neurotrophic factor necessary for axonal guidance, dendrite branching and neuritogenesis. The presence of L-serine significantly increases the size of neurons from chicken embryonic dorsal root ganglion [10], while addition of glycine, ethanolamine or D-serine failed to do so, suggesting that L-serine itself can drive processes important for brain development and activity. Lipids are the most abundant organic compounds found in the brain, accounting for up to 50% of its dry weight with a specific enrichment in sphingolipids and cholesterol, particularly in the form of myelin. Interestingly, L-serine is the head group of the phospholipid phosphatidylserine and a mandatory substrate for ceramide synthesis, the common backbone of sphingolipids. However, whether and how these components of L-serine metabolism participate in cellular physiology is not well understood (for review see [103]). A recent study has provided evidence for a novel role of L-serine in supporting mitochondrial function, morphology and membrane potential through ceramide metabolism [104]. During development, astrocytes promote the formation of synapses in distinct neuronal populations via the release of many molecules including membrane lipids such as cholesterol [105]. Moreover, sphingolipids are enriched in lipid rafts, membrane micro-domains that contribute to a wide variety of biological functions including protein trafficking/exocytosis, membrane transport, cell adhesion, and cell survival.

Besides development and cell proliferation, whether L-serine-derived lipids are involved in the dynamic processes underlying dendritic growth and spines formation [106] deserves further investigation (Figure 3).

L-serine and reactive oxygen species (ROS)

Reactive oxygen species (ROS) are an intricate part of normal cellular physiology but, when produced in excess, ROS lead to widespread oxidative damage of lipids, protein and nucleic acid, and ultimately to cell death [107]. L-Serine plays a vital role in the antioxidant defense system because it is a precursor for the synthesis of glutathione (GSH). GSH is a tripeptide (γ-L-glutamyl-L-cysteinyl-glycine) whose biosynthesis requires L-serine as the precursor of glycine and cysteine (Figure 3). Glutathione exists as the thiol-reduced (GSH, predominant) and disulfide-oxidized (GSSG) forms. GSSG is reduced by glutathione reductase, which uses NADPH as cofactor to regenerate the pool of GSH and hence to restore the reducing power of cells [108]. The most direct route to produce NADPH in the cytosol is the oxidative branch of the pentose phosphate pathway (oxPPP). However, as recently observed [109], the folate cycle fueled by L-serine is another significant provider of NADPH. Since astrocytes do not preferentially consume glucose *via* the oxPPP [69], as neurons do [110], the folate cycle may represent an alternative source of NADPH to reduce GSSG in these cells. The conversion of serine to glycine could have the combined advantage of providing GSH synthesis and the NADPH-reducing power to maintain GSH in its reduced form.

Recent findings indicate serine biosynthesis is critically involved in the defense mechanisms against oxidative stress in the retina [111, 112]. The authors found that Müller cells (the major glial cells of retina) from the macula display greater rate of *de novo* serine synthesis as well as increased levels of reduced GSH and ROS compared to those present in the peripheral retina. Therefore, an impaired *de novo* serine synthesis would affect ROS balance in macular Müller cells more than in the peripheral retina. These results suggest that dysregulation of this pathway may be a potential cause of the high susceptibility of the human macula to developing blinding conditions.

ROS can also directly influence serine production. In cancer cells, elevated levels of ROS inhibit Kelch-like ECH-associated protein 1 (Keap1) which in turn activate NRF2 and promote the expression of almost 200 genes involved in the antioxidant responses, including the 3 genes of the PP [42, 107].

L-serine and folate, methionine, H2S

Conversion of L-serine to glycine by serine/glycine hydroxymethyl transferase (s/gHMT) is feeding folate and methionine cycles, two metabolic pathways that are tightly imbricated in what is called one-carbon (1C) metabolism (Figure 3) [6, 113]. This complex metabolic process transfers one-carbon units for biosynthetic processes including purine and thymidine synthesis and homocysteine remethylation. It is therefore critical for cellular proliferation and potentially also for epigenetics. Homocysteine concentrations are regulated by the activity of another pathway, the transsulfuration pathway, along which the homocysteine sulfur atom is transferred to serine to make cysteine thanks to the action of cystathione β-synthase (CBS). In the brain, the transsulfuration pathway is required to support glutathione synthesis [114] and may contribute to cellular redox homeostasis. CBS can also perform desulfhydratation of cysteine and/or homocysteine, which leads to the production of hydrogen sulfide (H_2S). Three different pathways can produce H_2S, however, among the enzymes involved in H_2S synthesis, CBS is the only one found at high levels in

the brain [115]. L-serine metabolism is then intimately linked with H_2S synthesis through CBS activity. In the brain, H_2S has been described as a gasotransmitter that can participate in synaptic transmission via multiple pathways. H_2S can alter glutamatergic transmission via sulfhydration of NR2A-containing NMDARs (Figure 3), a post-translational modification that prevents the inhibitory action of synaptically released Zn^{2+} [116]. It can also promote surface expression of the AMPAR subunit GluR1/GluA1 involved in regulation of synaptic plasticity [117] and finally it has been shown to facilitate LTP via sulfhydration of Sr, a modification that increases its activity (Figure 3) [118]. H_2S may also indirectly increase the serine biosynthesis via the sulfhydration of Keap1, a modification that will increase the nuclear translocation of NRF2 and therefore the transcription of its target genes, including the 3 genes of the PP (see above).

In the brain, CBS is mainly, if not only, located in astrocytes [119], which makes astrocytes the major site of free H_2S production. H_2S can be stored in three different ways in brain cells: acid-labile sulfurs are found in mitochondria and release H_2S under acidic conditions, bound sulfurs are found in the cytoplasm and release H_2S under alkaline conditions and finally free H_2S is thought to exist in the cytoplasm but at so low concentrations (<10 µM) that it cannot be easily measured [120].

H_2S used for synaptic transmission is likely to be released by astrocytes from bound sulfurs only during alkaline conditions, that is when these glial cells take up K^+ that are released by nearby active neurons [120]. Neuronal K^+ can also exert a strong stimulatory effect on astrocytic glycolysis [121], therefore suggesting an integrated and coordinated response of astrocytes during neuronal activity [53] with respect to ROS maintenance, serine production and glycolytic flux.

Epigenetic regulation

As mentioned above, serine metabolism is influenced by epigenetic modifiers such as histone lysine methyltransferases (KMTs) and demethylases (KDMs) that play a key role in regulating transcription by controlling the state of histone lysine methylation. KMTs use S-adenosylmethionine (SAM) as the methyl group donor while KDMs need flavin adenine dinucleotide (FAD) and α-KG for demethylation, strongly suggesting that their activities are sensitive to changes in cell metabolism (see references in [45]). Therefore, metabolism can also influence epigenetics by regulating the availability of cofactors required for key epigenetic enzymes [122]. This is true for L-serine metabolism since it is linked to one-carbon metabolism (Figure 3), providing methionine as a precursor for SAM, the major methyl donor regulating methylation status of nucleic acids and histone proteins [123]. Using stable isotope tracing, mass spectrometry, and nutrient modulation in cancer cells, the role of L-serine in supporting methylation through maintenance of nucleotide levels has been recently highlighted [124]. This crosstalk between cellular metabolism and the epigenome probably represents a key regulatory pathway to study further. No information is yet available on the interplay between L-serine metabolism and epigenetics in the brain, despite increasing evidence that (1) epigenetic mechanisms are critical for the maintenance of a healthy brain and are dysregulated in the course of many neurodegenerative diseases [125] and (2) peripheral metabolism can impact brain histone acetylation and behavior [126].

Serine deficiency

Genetic defects

Very interesting lessons regarding L-serine biosynthesis in the CNS can be drawn from clinical observations. Serine synthesis disorders were first reported by Jaeken and colleagues in 1996 [127, 128] in patients with neurological symptoms in which amino acid analysis revealed low values of serine in plasma and CSF. They found that the symptoms were caused by mutations in either PHGDH or PSPH. These patients displayed severe neurological impairments including congenital microcephaly, psychomotor retardation, and seizures, which suggests an important role for PHGDH activity and L-serine biosynthesis in the metabolism, development, and function of the central nervous system. In 2014, more than 40 years after its initial description by Neu and Laxova [129, 130], Neu–Laxova syndrome (NLS [MIM 256520]) was found to represent the severe end of serine biosynthesis defects [131]. NLS is genetically heterogeneous because it can be caused by mutations in each of the 3 enzymes of the L-serine biosynthesis pathway (PHGDH, PSAT1 and PSPH) [132]. The hallmark clinical features of this rare syndrome are a characteristic facies, microcephaly, seizures, intra-uterine growth restriction (IUGR) and skin abnormalities. A serine transport defect resulting from mutations of ASCT1, the main transporter for serine in the CNS, has been recently described in children with neurological manifestations similar to those observed in NLS [133]. The phenotype caused by all these genetic defects strongly suggests that the PP is the major route for serine production in the CNS.

Other useful information concerns the use of L-serine as a potential therapeutic solution. Since serine deficiency is the main etiological factor in serine biosynthesis defects, chronic treatment with serine has already been performed in patients. L-serine therapy (recommended dose of L-serine is 500-700mg/kg/day for infants with severe infantile form) has proven to be beneficial in preventing or ameliorating symptoms in serine biosynthesis and transport defects, if started before neurological damages occur [28]. In one patient with a prenatal diagnosis of L-serine deficiency, L-serine treatment was administered to the mother starting from week 27 and was continued after birth [134] and completely prevented the onset of neurological symptoms for more than 10 years. When given too late, L-serine failed to substantially improve neurocognitive impairments [135]. These disappointing results of serine supplementation are likely because *in utero* serine deficiency has already resulted in neurological damages that cannot be reversed by postnatal supplementation. Contrarily to D-serine that may induce nephrotoxicity [136], there is no toxicity associated with chronic L-serine since some patients with PHGDH deficiency have taken their daily amino acid supplements for more than a decade without any side-effects. These data support the contention that L-serine production is critical for CNS development and function and that its dietary supplementation can at least partly bypass the endogenous synthesis.

Alteration of L-serine biosynthesis in neurodegenerative diseases?

One important process that becomes dysfunctional early in the course of many neurodegenerative diseases (NDs) such as Alzheimer's (AD), Parkinson's (PD) and Huntington's diseases (HD) is the metabolism of glucose [137]. Reduced glucose consumption has been widely reported in the caudate/putamen of HD patients [138] and in specific

cortical areas in AD patients [139]. Most of these diseases are associated with impaired mitochondrial function leading to a disruption of ATP production [140], so it is commonly admitted that the loss of ATP bioavailability is instrumental in precipitating dysfunction and neurodegeneration in NDs [141]. However, more recent data support the idea that a selective defect of glycolysis occurs early in the course of the disease, as shown in HD [142] and AD patients [143, 144]. Since glycolysis is a source of carbon for L-serine, any slowing of the glycolytic flux may lead to its reduced synthesis. However, old as well as more recent biochemical analyzes have not produced conclusive results on the evolution of L/D-serine levels in the brain of AD patients [145-150]. Possible explanations for these contradicting results include analytical methods, the fact that AD patients were not at the same stage of the disease progression, the very low number of samples and the fact that CSF samples were obtained by lumbar puncture (far from the affected regions) or even during autopsies, following opening of the cranial cavity. Additional studies are required to obtain a clearer picture of the time-course of both L- and D-serine levels in the brain of AD patients. This is important because any significant alteration of the biosynthesis of L-serine may potentially contribute to the pathogenesis of NDs. We recently found that the extracellular levels of both L- and D-serine were reduced in the hippocampus of 3xTg-AD, a mouse model of AD that displays lower glycolytic flux in hippocampal astrocytes [24]. Such impairment of glycolysis-derived L-serine production in astrocytes contributes to early synaptic and behavioral deficits since a chronic supplementation of L-serine given in the food was able to restore those deficits [24]. Our findings highlight oral L-serine as a potential therapy for AD. Such a supplementation with L-serine was recently shown to ameliorate motor and cognitive performance in a rare NMDA-related severe encephalopathy leading to glutamatergic signaling deficiency [151]. Besides AD, L-serine is currently in a phase I clinical trial as a treatment for hereditary sensory autonomic neuropathy type 1 [152] and amyotrophic lateral sclerosis [153]. Taken together, these data illustrate the need to better understand how L-serine production is regulated in the brain in order to define whether and how L-serine can be considered a ready-to-use therapeutic option for treating potentially numerous brain diseases as well as normal aging, a condition where NMDA/D-serine activity is also known to be impaired [154]. It is noteworthy that people living in Okinawa, a Japanese island known for the overall longevity of its population, have a diet characterized by an L-serine content that is four times higher than that of the average American [155].

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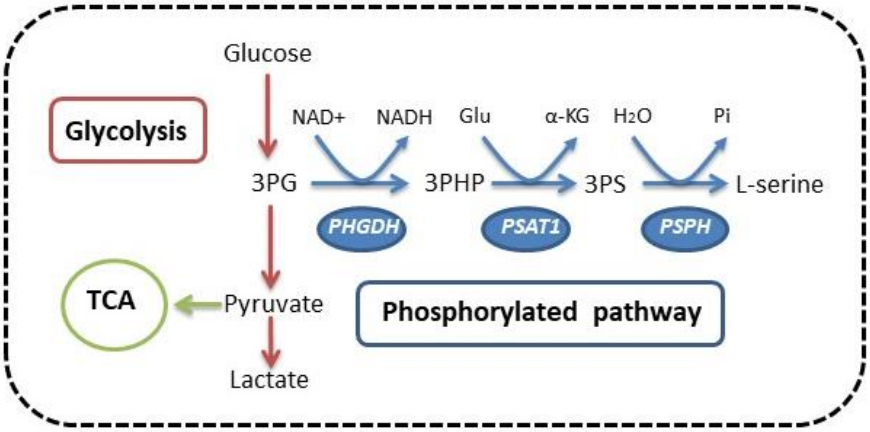

Figure 1. The phosphorylated pathway of L-serine biosynthesis is a short metabolic sequence branching from glycolysis. The glycolytic intermediate 3-phosphoglycerate (3PG) is converted into 3-phosphohydroxypyruvate (3PHP), in a reaction catalyzed by 3-phosphoglycerate dehydrogenase (PHGDH) and using NAD+ as a cofactor. 3PHP is metabolized into 3-phosphoserine (3PS) by phosphohydroxypyruvate aminotransferase (PSAT1) using L-glutamate (Glu) as the amino group donor, which is converted into α-ketoglutarate (α-KG). Finally, phosphoserine phosphatase (PSP) catalyzes the final and irreversible step of L-serine synthesis by hydrolyzing 3PS to produce L-serine and inorganic phosphate (Pi).

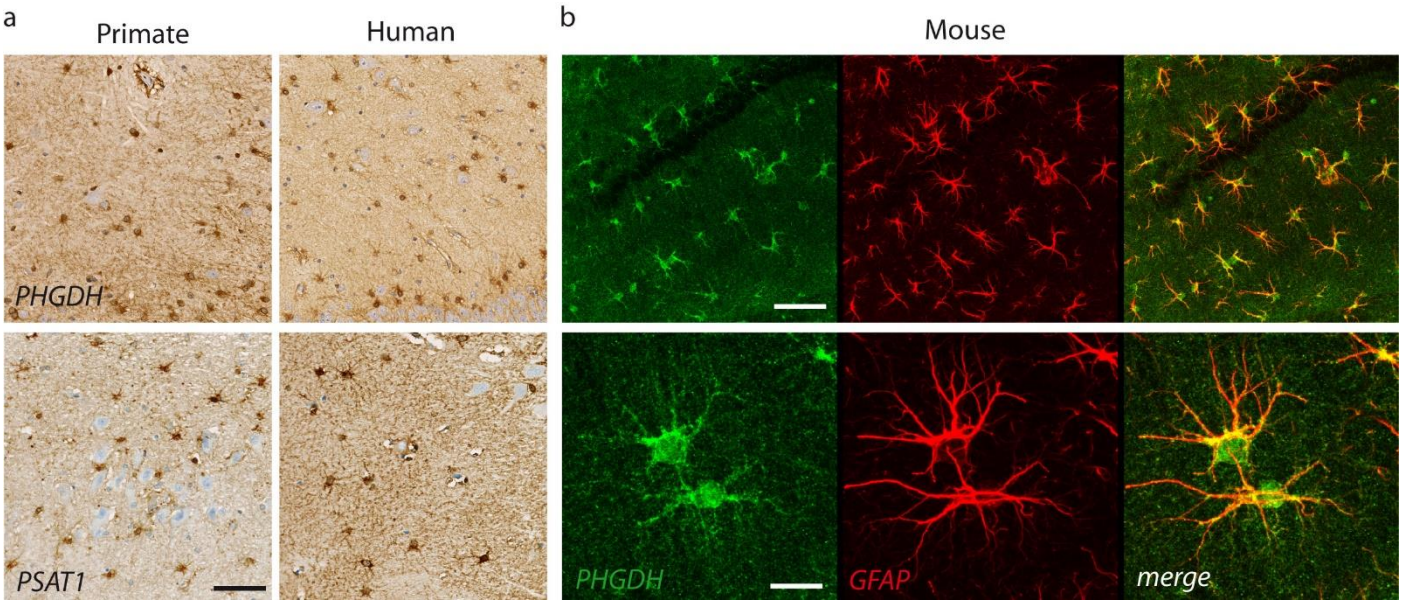

Figure 2. (a) Immunostaining of PHGDH and of PSAT1 in the hippocampus of a non-human primate and a human brain. Both enzymes of the phosphorylated pathway are mainly expressed in astrocytes. **(b)** Double immunofluorescent staining of brain sections from 6-month old mice using PHGDH (green) and an astrocyte marker (GFAP, red) indicates that the biosynthesis of L-serine is mostly performed into adult astrocytes. Bar = 50 µm (a), 50 µm (b, up) or 10 µm (b, down).

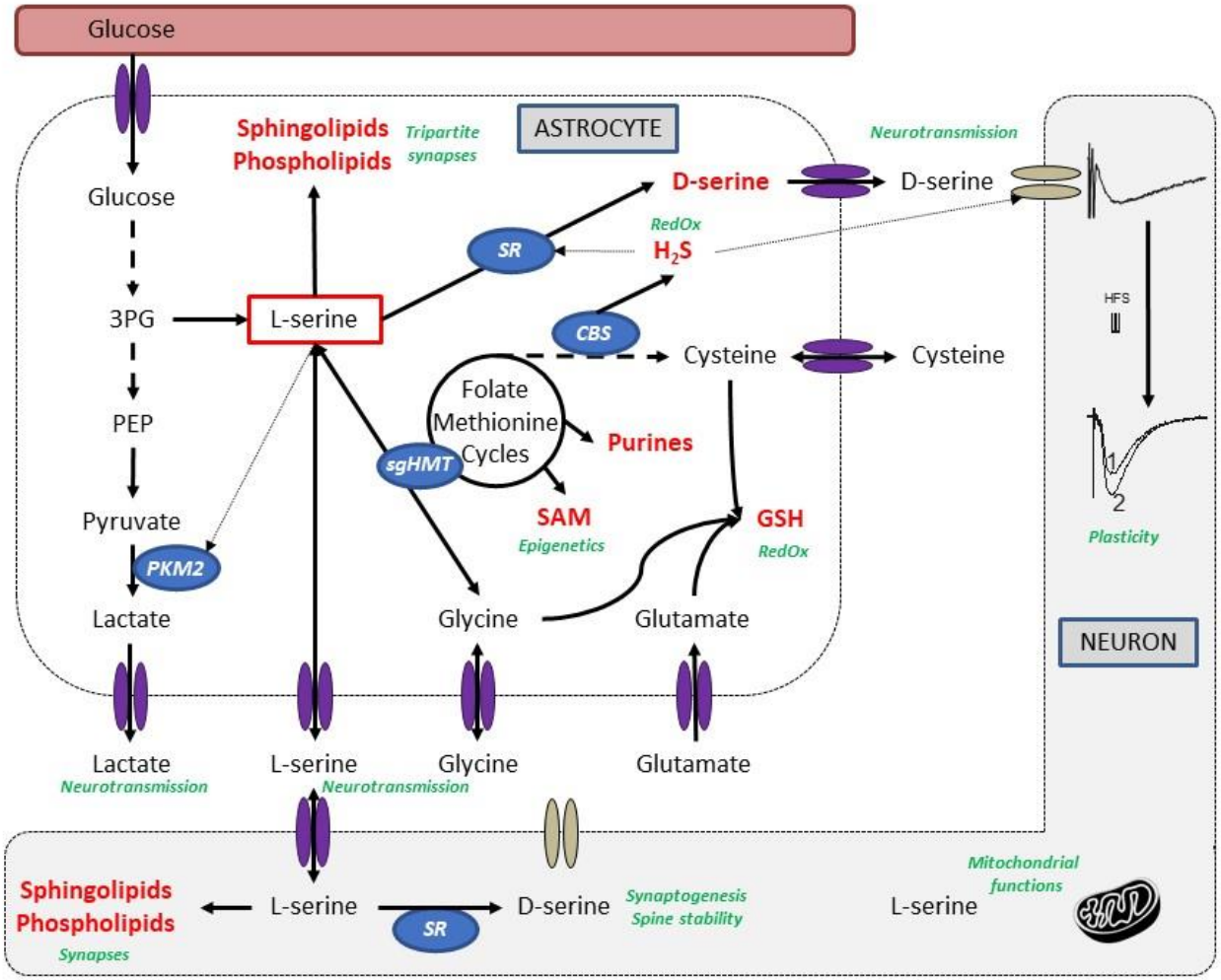

Figure 3. In the brain, astrocytic L-serine plays pivotal roles (all indicated in green). L-serine is critical for neurotransmission and synaptic plasticity, being the main precursor of glycine and D-serine, two co-agonists of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor, required for long-term potentiation of post-synaptic responses during high frequency stimulation (HFS). By regulating the glycolytic flux via its binding on PKM2, L-serine can also alter the production of lactate, a positive modulator of NMDAR-mediated signaling. Via its link to one-carbon metabolism, L-serine contributes to the regulation of redox status (via synthesis of glutathione (GSH), epigenetics (through synthesis of S-adenosylmethionine, SAM), generation of nucleotide pools (purines) and production of hydrogen sulfide, a gasotransmitter that can directly affect NMDA activity. L-serine may also significantly contribute to the homeostasis of tripartite synapses and of mitochondrial function by providing sphingolipids, phospholipids and ceramide.

Abbreviations : 3PG, 3-phosphoglycerate ; SR, serine racemase ; CBS, cystathione β-synthase; PKM2, pyruvate kinase isoform 2; sgHMT, serine/glycine hydroxymethyltransferase; H_2S, hydrogen sulfide ; GSH, glutathione ; HFS, high frequency stimulation, SAM, S-adenosylmethionine