Review Article

An essential role for *de novo* biosynthesis of L-serine in CNS development

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L-Serine plays a versatile role in intermediary metabolism in eukaryotic cells. The physiological significance of its *de novo* biosynthesis, however, remains largely unexplored. We demonstrated previously that neurons lose the ability to synthesize L-serine after their final differentiation and thus depend on astrocytes to supply this amino acid. This is due to a lack of neuronal expression of 3-phosphoglycerate dehydrogenase (Phgdh), which initiates *de novo* L-serine synthesis via the phosphorylated pathway from the glycolytic intermediate 3-phosphoglycerate. In rodent brain, Phgdh is expressed exclusively by the neuroepithelium/radial glia/astrocyte lineage. In humans, serine deficiency disorders can result from a deficiency of Phgdh or other enzymes involved in serine biosynthesis in the phosphorylated pathway. Patients with such disorders have lower serine levels in plasma and cerebrospinal fluid; they exhibit severe neurological symptoms including congenital microcephaly, feeding disabilities, and psychomotor retardation. L-Serine supplementation can attenuate developmental defects in these patients. To define the physiological importance of *de novo* L-serine production, we generated *Phgdh* knockout mice using targeted gene disruption technique. *Phgdh* deletion drastically reduced serine and glycine levels in the body. *Phgdh* knockout mice exhibited overall growth retardation with severe brain malformation, culminating in embryonic lethality. These observations highlight the vital role of *de novo* L-serine synthesis in the formation and function of the mammalian central nervous system. Furthermore, the embryonic lethal phenotype of *Phgdh* knockouts indicates that L-serine must be synthesized endogenously in mouse (and probably humans) during embryonic development.

Key Words: 3-phosphoglycerate dehydrogenase, human serine deficiency, knockout mouse, brain development

INTRODUCTION

The amino acid, L-serine is classified as nutritionally non-essential.[1] Besides its role in protein synthesis, L-serine serves as a metabolic precursor/intermediate necessary for various biosynthetic pathways. These include synthesis of glycine, L-cysteine, phosphatidylserine (PS), sphingolipids, and D-serine—an activator of the N-methyl-D-aspartate (NMDA)-selective glutamate receptor. Furthermore, L-serine participates indirectly in the biosynthesis of purines and pyrimidines by transferring a methylene group (*C*3-serine) to tetrahydrofolate (THF). Although the amount of L-serine synthesized *de novo* is estimated to be ~370 mg/100 g body weight/day in the rat,[2] the *in vivo* significance of its biosynthesis remains largely uncharacterized. During the past decade, however, accumulating evidence obtained from *in vitro* and *in vivo* studies has increasingly connected *de novo* synthesis of L-serine with development and function of the central nervous system (CNS) in mammals.

Here, I illustrate the physiological role of *de novo* synthesized L-serine in mammalian neurobiology as assessed in rodent models and humans. This article enhances our understanding of serine metabolism in health and disease and suggests that the established viewpoint of nonessential amino acids should be revaluated.

BIOSYNTHESIS AND METABOLIC FATE OF L-SERINE

L-Serine can be derived from diet, glycine, protein degradation, and *de novo* biosynthesis from the glycolytic intermediate 3-phosphoglycerate (3-PG). Although *de novo* biosynthesis was previously described by two different pathways—the phosphorylated pathway from 3-PG and the non-phosphorylated pathway from 2-phosphoglycerate — only the former appears to be operative for the synthesis.[2] In the phosphorylated pathway, Phgdh initiates the reaction by oxidizing 3-PG to 3-phospho-hydroxypyruvate, which then undergoes transamination and dephosphorylation to yield L-serine (Fig. 1). L-Serine is then utilized for protein synthesis but also contributes to non-protein-related metabolism (Fig. 1). In terms of metabolic fates of L-serine, it is of note that cytosolic and mitochondrial serine hydroxyl

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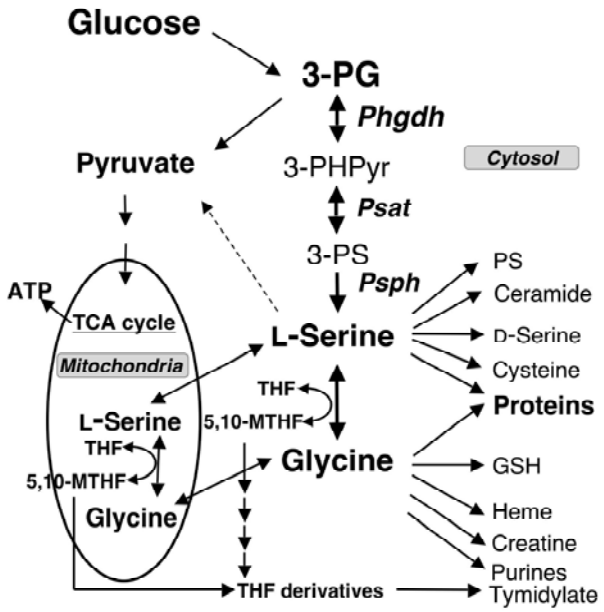

Figure 1. Pathway of *de novo* serine biosynthesis from 3-phosphoglycerate and its metabolic fates in animal cells. Abbreviations are: 3-PG, 3-phosphoglycerate; PHPyr; 3-phosphohydroxypyruvate; 3-PS, 3-phosphoserine; THF, tetrahydrofolate; 5,10-MTHF, 5,10-methylene-THF; Phgdh, 3-phosphoglycerate dehydrogenase; Psat, phosphoserine aminotransferase; Psph, phosphoserine phosphatase; GSH, glutathione; PS, phosphatidylserine. The dashed line indicates a major catabolic pathway of L-serine.

methyl-transferases (Shmt) catalyze interconversion between serine and glycine. There are substantial metabolic demands for glycine in healthy adults and infants, not only for protein synthesis but also as a precursor for biosynthetic pathways of bile acids, creatine, glutathione, porphyrins, and purines. Conversion of serine to glycine simultaneously results in the formation of 5,10-methylene-THF, which can be used for THF-dependent one-carbon metabolism. Supplies of glycine and THF-containing one-carbon units are particularly important for supporting purine synthesis in proliferating cells. Therefore, unlike other amino acids, L-serine has a unique role in various aspects of intracellular metabolism. However, a limited number of studies on the roles and regulation of have been conducted in experimental animals and humans.

L-SERINE AS A TROPHIC FACTOR FOR NEURONS

In 1998, we serendipitously discovered that L-serine could serve as a glia-derived trophic factor to enhance the survival and neuritogenesis of hippocampal neurons and cerebellar Purkinje neurons in dissociated monolayer cultures.[3,4] Before these findings, we were aware that DMEM/F-12, a culture medium rich in all nonessential amino acids, supported well the survival and neurite growth of these neurons in culture without addition of major known proteinaceous neurotrophic factors.[5] With regard to trophic support to neurons in the CNS, astrocytes, one of three major glial cell types, have long been known to provide structural and metabolic support that is necessary for neuronal survival, neuritogenesis, and synapse formation. Indeed, the survival of dissociated hippocampal neurons has been reported to be dependent on soluble factors released by cultured astrocytes.[6] Culture medium conditioned by astrocytes (CMCA) contains a set of survival/growth-promoting factors for central neurons and thus has been widely used as a supplement for *in vitro* cell culture of neurons. To our surprise, the survival of cultured neurons maintained in DMEM/F-12 was equivalent to that in CMCA.

Based on these findings, we analyzed amino acids released by astrocytes but not neurons and identified L-serine as an active molecule.[3,4] Exogenously supplied L-serine alone enhanced the survival and neurite growth of cultured primary neurons in a dose-dependent and saturable manner.[3,4] Trophic effects of L-serine alone on neurons appeared to be comparable to those obtained with CMCA. The potency of L-serine on neuronal survival is much greater than that of certain known polypeptide neurotrophic factors.[3,4] In addition to the enhancement of survival and morphological growth, L-serine significantly promotes maturation of membrane voltage responses of Purkinje neurons.[4]

LACK OF PHGDH EXPRESSION IN NEURONS

What is the mechanism underlying the trophic effects of L-serine on neurons? Our lipid analysis demonstrated that when neurons were maintained in the absence of L-serine, PS level diminished in neurons, whereas the unusual aminophospholipid phosphatidylthreonine (PT) appeared.[7] PT can be synthesized *in vitro* with much lower efficiency than PS by a base-exchange enzyme that catalyzes the synthesis of PS in the endoplasmic reticulum.[7] These results are indicative of a lowered capability of neurons to synthesize L-serine by themselves. L-Threonine was hence abnormally recruited to the base-exchange reaction instead of L-serine.

To verify this possibility at the molecular level, we performed northern blotting, *in situ* hybridization and immunohistochemical analyzes of enzymes involved in the phosphorylated pathway. Northern bolt analysis showed that *Phgdh* mRNA was expressed preferentially in cultured astrocytes, and was virtually absent in cultured neurons.[8] *Psat* and *Psph* mRNAs, encoding phosphoserine aminotransferase and phosphoserine phosphatase, respectively, were expressed in both cell types.

In situ hybridization and immunohistochemistry for Phgdh in rodent CNS further revealed that *Phgdh* mRNA and protein are highly enriched in neuroepithelium or radial glia cells, which serve as neural stem/progenitor cells, during embryogenesis and thereafter in astrocytes.[9] Neurons, on the other hand, constitutively lack *Phgdh* mRNA and protein after their final differentiation in the proliferating germinal zone. These observations led us to hypothesize that neurons, once differentiated, depend mainly on radial glia/astrocyte lineages for their supply of L-serine. Thus, L-serine appears to act as a strong trophic factor for neurons in culture. Furthermore, our histological examinations were the first to show radial glia/astrocyte lineage–specific intense expression of Phgdh in developing and mature brain. Altogether, these *in vitro* and *in situ* observations imply that *de novo* L-serine biosynthesis in the CNS plays an indispensable role in CNS development and the function of the mature CNS.

HUMAN SERINE DEFICIENCY DISORDER

In 1996 Jaeken and colleagues first reported human patients with defects in serine biosynthesis.[10] These defects were found to be a consequence of PHGDH deficiency (OMIM #601815) and PSPH deficiency (OMIM #172480), the former of which has been reported more frequently than the latter. Patients with PHGDH deficiency suffer from severe neurodevelopmental defects from infancy, which include growth and psychomotor retardation, congenital microcephaly, feeding difficulties and intractable seizures. Magnetic resonance imaging analysis revealed dysmyelination or white matter attenuation in brains of the patients with PHGDH deficiency.[10,11] Serine concentrations in plasma and cerebrospinal fluid (CSF) of these patients were markedly reduced, whereas glycine concentrations were decreased only moderately to mildly. On average, the decrease in serine concentration was noticeably more severe in CSF than in plasma of PHGDH deficiency patients. Mutation analysis demonstrated a 1468G to A substitution in the gene, resulting in a valine-to-methionine substitution at position 490.[12,13] Although this mutation appears to be a common cause of human PHGDH deficiency irrespective of ethnic background, the exact mechanism underlying reduced enzyme activity remains controversial.[12,13]

The only known case of PSPH deficiency was found in a Williams syndrome patient.[10] In addition to facial dysmorphism suggestive of Williams syndrome, this patient presented growth retardation and moderate psychomotor retardation, congenital microcephaly, and feeding difficulties, but not intractable seizures. The observed decrease in serine levels in CSF and plasma were less pronounced than in PHGDH deficiency patients.[10] These mild outcomes might be due to a considerable residual activity of this PSPH mutant enzyme. Recently, two siblings were identified with PSAT deficiency (OMIM #610992).[14] They had low serine and glycine concentrations in plasma and CSF, and presented microcephaly, hypertonia, psychomotor retardation, poor feeding and intractable seizures.[14] Thus, acquired microcephaly, feeding difficulties, and psychomotor retardation appear to be common symptoms in patients with serine deficiency syndrome.

Oral supplementation of relatively high doses of L-serine alone (200~500 mg/kg per day) or in combination with glycine (200~300 mg /kg per day) reportedly increases serine and glycine levels in plasma and CSF and ameliorates intractable seizures and white matter attenuation seen in serine deficiency patients.[11,14] However, no improvement in psychomotor development has been observed in patients treated after age one year. Notably, neurological symptoms can be prevented when L-serine supplementation is started during pregnancy. A prenatal diagnosis of PHGDH deficiency in an 11-week- old female fetus revealed that head circumference decreased gradually after gestation week 20. L-Serine treatment via maternal oral supplementation was started at gestation week 27 and continued until birth. The child then developed normally and was born without incident, after which she continued receiving L-serine supplementation (500 mg/kg per day). To date, her neurological status and psychomotor development have been normal.[11] Although based on only one case, this successful treatment suggests that there is a critical period (i.e., after gestation week 20) during which the embryonic CNS has a higher demand for L-serine.

GENERATION OF *PHGDH* KNOCKOUT MICE

To define the physiological role of *de novo* L-serine biosynthesis via the phosphorylated pathway in CNS development *in vivo*, and to better understand the pathobiology of serine deficiency disorder, we generated mice with targeted disruption of the gene *Phgdh*.[15] *Phgdh* heterozygotes ($Phgdh^{+/-}$) had normal development, gross anatomy, and fertility. In contrast, *Phgdh* knockouts ($Phgdh^{-/-}$) could not be detected after embryonic day 13.5 (Table 1).[15] Thus, *Phgdh* homozygous deficiency causes embryonic lethality.

Phgdh protein was not detected in tissue lysates prepared from *Phgdh* knockout embryos.[15] The absence of Phgdh protein correlated with markedly decreased free serine levels compared to wild type embryos (Furuya et al., unpublished observation); glycine content was also decreased but to a lesser extent. Consistent with these changes, levels of PS and sphingolipids, both of which utilize L-serine as an indispensable precursor, were drastically reduced in *Phgdh* knockout embryos.[15]

Table 1. Genotype analysis of litters of heterozygous *Phgdh* (+/–) intercrosses*

Stages	Genotype		
	Wild (+/+)	Heterozygote (+/–)	Knockout (–/–)
Embryonic day 9.5	6	17	8
Embryonic day 13.5	9	14	8
Postnatal day 0	10	22	0

* From reference 15.

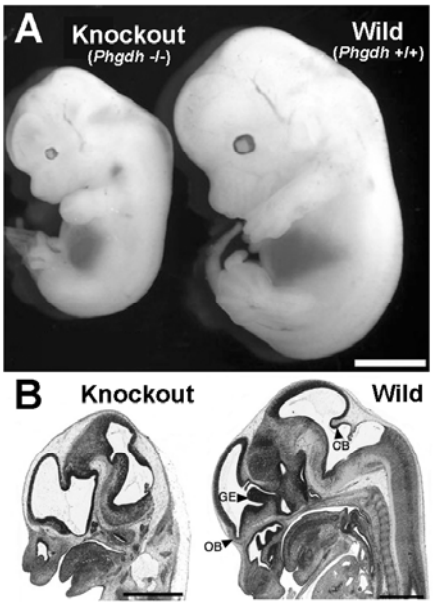

Figure 2. Gross morphology (A) and hematoxylin/eosin-stained sagittal sections (B) of *Phgdh* knockout (–/–) embryos and wild type littermates (Wild, +/+) at embryonic day 13.5.[15] Note that the olfactory bulb (OB), ganglionic eminence (GE), and cerebellum (CB) are missing in the *Phgdh* knockout. Scale bars: 2 mm for A, 1 mm for B.

In addition to a substantial decrease in L-serine, complete inactivation of *Phgdh* resulted in overall growth retardation and striking defects in CNS morphological development (Fig. 2).[15] The affected embryos exhibited hypoplasia of the telencephalon, diencephalon, and mesencephalon; in particular, the olfactory bulbs, ganglionic eminence, and cerebellum appeared as indistinct structures at embryonic day 13.5. Notably, *Phgdh* knockout embryos displayed microcephaly, which is the most conspicuous phenotype of serine deficiency patients. Although the molecular mechanism by which diminished L-serine levels affect development of CNS remains to be characterized, morphological and molecular analyses strongly suggest that cell proliferation of embryonic neural stem/progenitor cells is severely impaired or dysregulated.[15] This may lead to microcephaly in the knockout embryos. Altogether, these results show that the *Phgdh*-dependent pathway of *de novo* L-serine biosynthesis is the only source of the amino acid, and thus this pathway is essential for mouse embryonic development.

WHAT DO *PHGDH* KNOCKOUT MICE AND HUMAN SERINE DEFICIENCIES TELL US ABOUT L-SERINE?

The results from the *in vitro* and *in vivo* studies presented here definitively show that *de novo* L-serine biosynthesis is essential for human or mouse embryo viability and growth. Unlike essential amino acids, the maternal supply of L-serine under normal feeding condition is not sufficient to meet metabolic demand for L-serine in the CNS after mid-gestational stages. Hence, embryos cannot acquire an adequate supply of L-serine from maternal sources. Future studies using tissue-specific gene knockout mice will enhance our understanding of the physiology and pathology involved in *de novo* biosynthesis of L-serine.

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AUTHOR DISCLOSURES

Shigeki Furuya, no conflicts of interest.

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