Brain-specific Phgdh Deletion Reveals a Pivotal Role for L-Serine Biosynthesis in Controlling the Level of D-Serine, an N-methyl-d-aspartate Receptor Co-agonist, in Adult Brain

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In mammalian brain, D-serine is synthesized from L-serine by serine racemase, and it functions as an obligatory co-agonist at the glycine modulatory site of N-methyl-D-aspartate (NMDA)-selective glutamate receptors. Although diminution in D-serine level has been implicated in NMDA receptor hypofunction, which is thought to occur in schizophrenia, the source of the precursor L-serine and its role in D-serine metabolism in adult brain have yet to be determined. We investigated whether L-serine synthesized in brain via the phosphorylated pathway is essential for D-serine synthesis by generating mice with a conditional deletion of D-3-phosphoglycerate dehydrogenase (Phgdh; EC 1.1.1.95). This enzyme catalyzes the first step in L-serine synthesis via the phosphorylated pathway. HPLC analysis of serine enantiomers demonstrated that both L- and D-serine levels were markedly decreased in the cerebral cortex and hippocampus of conditional knock-out mice, whereas the serine deficiency did not alter protein expression levels of serine racemase and NMDA receptor subunits in these regions. The present study provides definitive proof that L-serine-synthesized endogenously via the phosphorylated pathway is a key rate-limiting factor for maintaining steady-state levels of D-serine in adult brain. Furthermore, NMDA-evoked transcription of Arc, an immediate early gene, was diminished in the hippocampus of conditional knock-out mice. Thus, this study demonstrates that in mature neuronal circuits L-serine availability determines the rate of D-serine synthesis in the forebrain and controls NMDA receptor function at least in the hippocampus.

Glutamate is the principal excitatory neurotransmitter in mammalian brain, acting on ionotropic and metabotropic glutamate receptors. Ionotropic glutamate receptors can be divided into three classes based on their preference for the ligands N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid, and kainate. Among these receptors, NMDA receptors have been implicated in synapse refinement, synaptic plasticity, and learning/memory as well as brain pathologies including excitotoxicity and psychiatric diseases (for review, see Refs. 1–3). Two NMDA receptor subunits, NR1 and NR2, form tetramers that comprise the functional receptors. For the NMDA receptor to function as a ligand-gated ion channel, a co-agonist must occupy the glycine modulatory site on NR1 coincident with glutamate binding to the transmitter recognition site on NR2 (4–6).

D-Serine occurs naturally in the adult brain of higher vertebrates and is particularly enriched in forebrain regions (7). This D-amino acid acts as an endogenous co-agonist at the glycine modulatory site of NMDA receptors (for review, see Refs. 8–11). In the telencephalon, where D-serine is abundant (12), its distribution pattern resembles that of NR2A/B subunits (13). In contrast, immunoreactivity for free glycine is very low in telencephalon and is detected primarily in the hindbrain and hypothalamus, where NR2A/B expression is weaker than in other regions (13). In addition to these contrasting localization patterns, the potency of D-serine for NMDA receptors is comparable with, or greater than, that of glycine (5, 14). These findings provided a basis for the idea that D-serine is the primary NMDA receptor co-agonist, at least in the telencephalon of mature brain. Degradation of endogenous D-serine by exogenously applied D-amino acid

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3 The abbreviations used are: NMDA, N-methyl-D-aspartate; GFAP, glial fibrillary acidic protein; Phgdh, D-3-phosphoglycerate dehydrogenase; Srr, Serine racemase; NSE, neuron-specific enolase.
oxidase attenuates NMDA receptor-mediated neuronal transmission in cerebellar, hippocampal, and hypothalamic slices (15, 16). Furthermore, when d-serine is injected into the brain of live animals, it positively modulates NMDA receptor function (17) and ameliorates stereotypical behavioral alterations and ataxia caused by administration of the NMDA antagonist phencyclidine (18, 19). These results suggest that d-serine may mitigate nervous system symptoms related to NMDA receptor hypofunction, including schizophrenia. Several clinical trials indeed demonstrated significant improvement in negative and cognitive symptoms of schizophrenia patients when combined with antipsychotics (20–22).

Serine racemase (Srr), which catalyzes the racemization between L- and d-serine, was first purified from mammalian brain by Wolosker et al. (23). Maximum enzyme activity requires pyridoxal 5'-phosphate as well as divalent cations complexed with ATP, GTP, or ADP (24–26). The enzyme also has α,β-eliminase activity, which forms pyruvate from L-serine and water (26–28). Synthesis of d-serine from L-serine is restricted to the brain of adult rats and mice; other sources of d-serine via blood circulation seem not to play a major role in establishing steady-state levels of this amino acid (29). Genetic deletion of Srr in mice results in a marked decrease in brain d-serine content (30–32), providing evidence that d-serine is synthesized from L-serine by Srr in adult brain. However, the origin of the L-serine precursor in mature brain remains unknown.

L-Serine can be derived from the diet, glycine, protein degradation, and/or de novo biosynthesis from the glycolytic intermediate 3-phosphoglycerate via the phosphorylated pathway (33, 34). In the first step of L-serine biosynthesis, D-3-phosphoglycerate dehydrogenase (Phgdh; EC 1.1.1.95) catalyzes the formation of 3-phosphohydroxyruvurate from 3-phosphoglycerate. In the rodent nervous system Phgdh is expressed specifically in a glial cell lineage throughout ontogeny and is enriched mainly in embryonic neuroepithelium and radial glia and thereafter in astrocytes (35, 36).

Neurons, in contrast, lack Phgdh mRNA and protein after their final differentiation in the proliferating germinal zone (36). Thus, neurons seem to have a diminished capacity for L-serine synthesis. In mature brain, L-serine appears to be supplied to neurons from external sources, such as astrocytes and/or the circulation. PHGDH-deficient patients have lower free serine levels in plasma as well as in cerebrospinal fluid, which is most likely a consequence of their reduced capacity for L-serine synthesis. These patients exhibit severe neurological symptoms including congenital microcephaly, psychomotor retardation, and intractable seizures (37). Cerebrospinal fluid d-serine concentrations in two patients with PHGDH deficiency (ages 2–3 months and 6–7 years) were markedly decreased before serine supplementation. Concentrations of L- and D-serine increased to a normal healthy range after daily oral administration of a high dose of L-serine (38), suggesting that PHGDH deficiency decreases the overall capacity for L-serine synthesis, which affects cerebrospinal fluid d-serine levels in infants and children. However, whether and to what extent brain-synthesized L-serine contributes to maintaining D-serine levels in mature brain tissues remains unknown.

To ascertain whether L-serine synthesized in the brain impacts d-serine levels in mature brain, we conditionally inactivated Phgdh in the nervous system using human glial fibrillary acidic protein (hGFAP)-Cre transgenic mice (39). Using this approach, we bypassed the embryonic lethal phenotype observed after systemic deletion of Phgdh (40). We found that intrabrain L-serine availability determines the d-serine level in the cerebral cortex and hippocampus. The compensatory supply of L-serine from the circulation to the brain is insufficient to maintain normal levels of L- or D-serine in mature brain.

**EXPERIMENTAL PROCEDURES**

**Conditional Phgdh Knock-out Mice**—A targeting vector for mouse Phgdh was constructed (40) as shown in Fig. 1A. To produce the conditional Phgdh allele, Cre-mediated deletion of the targeted allele (Phgdh<sup>−/−</sup>neo) was carried out by crossing Phgdh<sup>−/−</sup>neo heterozygous F1 mice with Ella Cre deleter mice. The F2 offspring showed a variable degree of mosaicism for partially Cre-recombined Phgdh alleles in various tissues, as described (41). F3 mice carrying a conditional Phgdh allele (Phgdh<sup>Phgdh/Phgdh</sup>+/floxed) were obtained by crossing F2 female mosaic mice with wild-type C57BL/6J males. The heterozygous mice were thereafter backcrossed to the C57BL/6J strain for more than 10 generations.

Mice with homozygous conditional Phgdh alleles (Phgdh<sup>Phgdh/Phgdh</sup>+/floxed), hereafter called Floxed, were obtained by intercrossing heterozygous mice (Phgdh<sup>Phgdh/Phgdh</sup>+/floxed). The presence of a conditional allele (Phgdh<sup>floxed</sup>) was identified by PCR using tail DNA with a primer pair directed against the third intron (forward primer, 5′-CATGAGGAA CTGAACTGAAGGTTATTCAACTTGCACCATGC-3′; reverse primer, 5′-CAAGGAGGCTCACACATCCAGAAC-3′), which generated a 310-bp amplicon (Fig. 1B). Mice conditionally lacking Phgdh in astrocytes (hGFAP<sup>−/−</sup>Cre::Phgdh<sup>Phgdh/Phgdh</sup>+/floxed), hereafter called KO, were obtained by interbreeding female Floxed mice with male Floxed mice carrying the hGFAP-Cre transgene, which were generated by crossing Floxed mice with hGFAP-Cre transgenic mice (39). To detect the Cre transgene used in this study, PCR was carried out with the primers 5′-AATTTCCTGACATCCATGGTTATTCAACTTGCACCATGC-3′ and 5′-CCATTTCCGGTTATTCAACTTGCACCATGC-3′, which generated a 190-bp amplicon of part of the Cre-coding region (Fig. 1B).

Mice were maintained in a 12-h light/dark cycle with unlimited access to water and laboratory chow containing 20% casein. The animal experiment protocols for this study were approved by the Animal Ethics Committees of RIKEN Brain Science Institute and Kyushu University.

**Amino Acid Analysis**—The content of glycine and other amino acids in serum, the cerebral cortex, and hippocampus, pancreas, kidney, liver, and muscle of Floxed and CKO mice (males, 10 weeks old) was determined as described (42, 43). Briefly, tissues were weighed and then homogenized individually in 5 volumes of water and centrifuged at 20,000 × g for 30 min at 4 °C. Proteins in the supernatants were removed by adding 5% perchloric acid as described (40), and the amino acid composition of the supernatants was determined with an amino acid analyzer (L-8500; Hitachi).
Enantiomers of serine were determined using a two-dimensional HPLC system as described (44) with slight modifications. Briefly, amino acids in the cerebral cortex, hippocampus, and serum of 10-week-old mice were deproteinized as described above and were derivatized with a fluorescent labeling reagent, 4-fluoro-7-nitro-2,1,3-benzoxadiazole. The reaction mixture was subjected to a combined HPLC system with a micro-octadecylsilane column and an enantioselective column. The micro-octadecylsilane column isolated the fraction of 7-nitro-2,1,3-benzoxadiazole-Ser, and the enantiomers were separated and determined with the enantioselective column. Fluorescence of the derivatized amino acids was detected at 530 nm with excitation at 470 nm.

Some Phgdh$^{+/+}$ and hGFAP$^{+/+}$/Phgdh$^{+/+}$ mice (females, 8 months old) were administered l-serine (150 mM in saline; 5 mmol/kg; 105–131 μmol/mouse) intraperitoneally. As a control, saline alone was injected intraperitoneally. Mice were decapitated 3 h after the injection because radiolabeled l-serine is degraded promptly within the body but can be detected in adult mouse brain 2–4 h after intraperitoneal administration (29).

**Immunoblot Analysis**—The cerebral cortex and hippocampus were dissected from adult brain of Floxed and CKO mice (male, 7–11 weeks old) and were then homogenized in a buffer containing 10 mM Tris-HCl (pH 8.0), 8 M urea, 4% CHAPS, protease inhibitor mixture (Nacalai Tesque), and phosphatase inhibitor mixture (Nacalai Tesque). Homogenates were centrifuged at 20,000 × g for 10 min to obtain total protein extracts, and concentrations were determined using a Protein Assay Bicinchoninic acid kit (Nacalai Tesque). Protein samples were fractionated by 10% SDS-polyacrylamide gel electrophoresis and electroblotted onto a PVDF membrane (Bio-Rad). Blotted proteins were probed with the following primary antibodies: anti-Phgdh (rabbit, 0.3 μg/ml) (36), anti-serine racemase (clone 29, mouse monoclonal, BD Biosciences, 1:1000), anti-NMDA receptor subunit NR1 (also known as GluR1; rabbit, termed GluR1 C2 antibody, 1:1000) (45), anti-NR2B (also known as GluRe2; rabbit, termed GluRe2N antibody, 1:1000) (46), anti-neuron-specific enolase (NSE) (rabbit, Polysciences, Inc., 1:45000), anti-SNAP 25 (clone BR05, mouse monoclonal, Wako Pure Chemical Industries, 1:500), anti-glial fibrillary acidic protein (Gfap) (rabbit, DAKO, 1:500), and anti-glyceraldehyde-3-phosphate dehydrogenase (Gapdh) (mouse monoclonal, Chemicon, 1:50000). Bound antibodies were visualized with a chemiluminescence detection system (SuperSignal® West Pico, Pierce) after incubating with the appropriate secondary antibodies conjugated with horseradish peroxidase (Cell Signaling Technology).

**Histological Analysis**—Floxed and CKO mice (males, 7–8 weeks old) were anesthetized with diethyl ether and perfused transcardially with 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.2). Brains were removed and postfixed overnight in the same fixative followed by washing in phosphate-buffered saline (pH 7.4, phosphate-buffered saline (PBS)). For hematoxylin and eosin staining, brains were embedded in paraffin wax and sectioned at 5 μm using a microtome.

**Quantitative Analysis of Arc mRNA Expression**—Wild-type (male, 12 weeks old), Floxed (female, 12–18 weeks old), and CKO (female, 12–18 weeks old) mice were injected intraperitoneally with a subconvulsant dose of NMDA (Sigma, 75 mg/kg body weight in 0.9% saline) (47) or saline. Floxed and CKO mice used in this experiment were littermates. Mice were anesthetized 3 h after injection and euthanized by decapitation followed by rapid excision of brain from the skull. Each individual hippocampus was quickly isolated from brain on ice, immersed into RNAlater reagent (Ambion) according to the manufacturer’s instructions, and then stored at −80 °C until used. Total RNA of the hippocampus was prepared using RiboPure kit (Ambion). The quality of total RNA samples was assessed by electrophoresis on a 1% denaturing agarose gels, and samples with A$_{260}$/A$_{280}$ of >1.8 were used for subsequent experiments. After removing contaminating DNA in RNA samples with TURBO DNA-free™ (Ambion), 1 μg of DNase-treated RNA was used to generate cDNA by reverse transcription using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems). Quantitative real-time PCR was performed with a Model 7500 Real Time PCR system (Applied Biosystems) in 20-μl reactions containing SYBR Premix Ex Taq (Takara Bio) and the reference dye ROX according to the manufacturer’s recommendations. Primer sequences used were: Arc forward, 5′-GGCCAGTCCTTGAGG-AGCATAG-3′; Arc reverse, 5′-ATGGCTGAGTCACGGAGCTG-3′. All reactions were performed in triplicate. Data analysis was carried out using the cycle threshold values of target gene expression normalized by Gapdh as the internal control. Our DNA microarray analysis demonstrated that Gapdh was not differentially expressed in the adult hippocampus of Floxed and CKO brains.4

**Statistical Analyses**—Differences between two groups were examined with the Student’s t test. Differences among more than two groups were analyzed with a one-way analysis of variance followed by Dunnett’s post-hoc test. p values ≤0.05 were considered significantly different. All statistics were performed using KaleidaGraph 4.0 (Synergy Software).

**RESULTS**

**Generation of Brain-specific Phgdh Knock-out Mice**—We generated a conditional allele for mouse Phgdh by introducing two loxP sites flanking exons 4 and 5 (Fig. 1A) (40). Removing these exons by crossing mice having the conditional allele with Ella-Cre deleter mice produces a frameshift mutation, Phgdh protein expression in Phgdh null embryonic tissues becomes virtually undetectable by Western blotting (40). Mice with the homozygous conditional Phgdh alleles, Floxed Phgdh mice (Phgdh$^{lox/lox}$), developed normally (data not shown).

To inactivate serine biosynthesis via the brain phosphorylated pathway, the Phgdh allele was disrupted by crossing female Floxed mice with male mice expressing Cre under the control of the human GFAP promoter (referred to as hGFAP-Cre mice). In hGFAP-Cre mice, it was observed that Cre was first detected in the telencephalon at embryonic day 13.5, and

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4 A. Wada and S. Furuya, unpublished observations.
thereafter its expression increased gradually and occurred mainly in astrocytes but also in certain neurons, oligodendrocytes in the corpus callosum, and ependymal cells in the lateral ventricle of the mature brain (39). We obtained mice in subsequent generations with the hGFAP/H11001/Cre::Phgdhflox/flox genotype, referred to as CKO (Fig. 1B). Unlike Phgdh null mice (40), CKO mice were born at the expected Mendelian ratio when female Floxed mice were crossed with male CKO mice (data not shown). Littermates with the Floxed genotype were used as normal controls.

The efficacy of Cre-mediated deletion of Phgdh was assessed by Western blotting. CKO brain exhibited 20 and 5% levels of Phgdh protein expression in the cerebral cortex and hippocampus, respectively, compared with Floxed mice (Fig. 1C). These results indicate that the efficacy of Cre-mediated recombination by the hGFAP-Cre transgene differs among brain regions. We did not observe significant changes in the Phgdh protein content of the kidney, liver, muscle, and pancreas of CKO mice relative to controls (data not shown).

We then compared brain morphology between the two genotypes. At postnatal day 0, gross appearance of CKO brain was similar to that of Floxed mice (Fig. 2A). Histological examination of hematoxylin and eosin-stained coronal sections demonstrated no overt alterations in overall brain morphology between the two genotypes (Fig. 2B); moreover, there was no significant difference in brain weight (Fig. 2C). At postnatal day 42, however, CKO mice had mild microcephaly with remarkable atrophy of the forebrain (Fig. 2D). Although structural organization of both the cortex and hippocampus was maintained, these regions in CKO appeared smaller than in Floxed controls (Fig. 2E). Consistent with this finding, mean brain weight was subtly yet significantly decreased in CKO mice compared with age-matched Floxed controls (Fig. 2F). Immunohistochemical staining revealed that the CKO...
cortex largely lacked immunoreactivity against Phgdh, but some Gfap-positive and -negative cells were Phgdh positive, whereas Phgdh immunoreactivity was present throughout the gray and white matter and colocalized well with Gfap, an astrocyte-specific marker, in the cerebral cortex of Floxed brain (supplemental Fig. 1), as seen in wild-type mice (36). Thus, the vast majority of astrocytes showed Cre-mediated recombination, which leads to inactivation of Phgdh expression. The residual expression may be a compensatory response of Cre-negative cells to brain serine deficiency. Detailed analysis of alterations in brain morphology and associated behavior phenotypes will be published elsewhere.

Functional Phgdh Regulates L- and D-Serine Levels in Brain—
We quantified serine enantiomers and other amino acids in serum, the cerebral cortex, and the hippocampus of CKO and Floxed mice. Serum L-serine concentrations did not differ significantly between the two genotypes (Fig. 3A; Student’s t test, p = 0.147), whereas the serum D-serine concentration tended to be less in CKO mice (Fig. 3B; Student’s t test, p = 0.071). Glycine concentration subtly increased in CKO serum (Fig. 3C; Student’s t test, p = 0.071) compared with that of Floxed mice. Serum concentrations of other amino acids did not differ between Floxed and CKO mice. Also, there were no significant differences in serum concentrations of free amino acids between wild-type and Floxed mice except for isoleucine, which was significantly higher in Floxed mice (66.9 ± 3.0 μM) than in wild type (54.2 ± 2.9 μM) (Student’s t test, p < 0.05). Free serine levels did not differ between CKO and Floxed mice in liver, pancreas, and kidney (data not shown), where Phgdh mRNA was reported to occur at high levels (48–50).

The levels of free L- and D-serine in the cerebral cortex of adult CKO mice were markedly decreased compared with Floxed cortex (Fig. 3D). Relative to Floxed cortex, L- and D-serine levels in CKO cortex were 17.7% (p < 0.0001) and 9.1% (p < 0.0001), respectively. The free glycine content also was significantly lower in CKO cerebral cortex (p < 0.01), although the extent of the decrease was less than that of L- and D-serine (Fig. 3E). Likewise, CKO hippocampus exhibited striking 84.5% (p < 0.0001) and 92.7% (p < 0.0001) decreases in the levels of free L- and D-serine, respectively, compared with Floxed mice (Fig. 3F). The glycine level was also reduced by 60% relative to Floxed controls (Fig. 3G), suggesting that L-serine contributes to glycine synthesis, as seen in embryonic brain tissue (43). The levels of L-serine and D-serine in the cerebral cortex and hippocampus did not differ between Floxed and wild-type mice. Other amino acids whose levels appeared to be altered in CKO cortex and/or hippocampus are shown in Table 1. These include increases in the arginine and phenylalanine levels measured in the cerebral cortex and a decrease in cystathionine. In addition, a significant increase in glutamine level in both the CKO and Floxed hippocampus was observed compared with wild type. Furthermore, a subtle decrease (6%) in glutamic acid content and an obvious decrease (27–38%) in threonine content were detected.

To gain insight into D-serine formation in CKO brain, we investigated the effects of intraperitoneal L-serine administration on serine metabolism in the cerebral cortex. A single injection of excess L-serine (5 mmol/kg; 105–131 μmol/mouse) caused a significant increase in the L-serine content of CKO cortex 3 h after injection; that is, 2.7-fold higher than in saline-injected or untreated animals (Fig. 4A). In parallel, the D-serine content in

FIGURE 2. Brain-specific deletion of Phgdh causes postnatal microcephaly. A, shown is the dorsal view of CKO (left) and Floxed (right) brain at postnatal day 0. Bar, 1 mm. B, coronal sections are shown of CKO (left) and Floxed (right) forebrain region with hematoxylin and eosin staining at postnatal day 0. Bar, 1 mm. C, shown is quantification of brain weight. Mean brain weight of newborn male pups (n = 5) is shown. Note that the gross morphology and weight of brain of CKO mice are indistinguishable from Floxed littermates (right). D, shown is a dorsal view of CKO (left) and Floxed (right) brain at postnatal day 24. Bar, 1 mm. The brain of a Phgdh CKO mouse is consistently smaller than its Floxed littermate. The arrows indicate the length of forebrain. E, shown are coronal sections of CKO (left) and Floxed (right) forebrain region with hematoxylin and eosin staining at postnatal day 42. Bar, 1 mm. The brain of a Phgdh CKO mouse is consistently smaller than its Floxed littermate. The arrows indicate the length of forebrain. F, quantification of brain weight at age 6 weeks (male, n = 6) is shown.
CKO cortex increased 2.6-fold (Fig. 4B). L-Serine levels increased slightly (1.3-fold) in the cortex of Floxed mice, whereas D-serine levels were unaffected 3 h after injection (Fig. 4, C and D). The glycine content was not significantly affected in either genotype (data not shown). These observations indicate that D-serine can be synthesized from L-serine that originates outside the brain. At 3 h post-injection, the net increases in L-serine and D-serine in CKO cortex were 99.95 and 25.95 nmol/g wet tissue, respectively (Fig. 4, A and B). Given that each mouse received 105–131 μmol of L-serine, it seems likely that L-serine does not pass efficiently.
Brain L-Serine Is Essential for Controlling D-Serine Level

Table 1

Amino acid composition of Phgdh KO cerebral cortex and hippocampus

Data are depicted as the mean ± S.E. (wild n = 4; Floxed n = 7; CKO n = 6).

| Amino acid (nmol/g tissue) | Wild type | Phgdh+/-/floxed (floxed) | hGFAP+/Cre+/Phgdh+/-/floxed (CKO) | Ratio |
|---------------------------|-----------|-------------------------|----------------------------------|-------|
|                           |           |                         |                                  | CKO/floxed | CKO/wild | Floxed/wild |
| Cerebral cortex: increased |           |                         |                                  |       |         |             |
| Arg                       | 46.15 ± 1.97 | 48.69 ± 1.83            | 57.23 ± 3.49                  | 1.18  | 1.24    | 1.01        |
| Phe                       | 43.50 ± 1.00 | 47.17 ± 1.12            | 53.40 ± 1.99                  | 1.13  | 1.23    | 1.08        |
|                           |           |                         |                                  |       |         |             |
| Cerebral cortex: decreased |           |                         |                                  |       |         |             |
| Cystathionine             | 5.75 ± 2.08  | 9.46 ± 3.06             | 3.01 ± 0.86                   | 0.32  | 0.52    | 1.65        |
|                           |           |                         |                                  |       |         |             |
| Hippocampus: increased    |           |                         |                                  |       |         |             |
| Arg                       | 47.40 ± 1.96  | 52.36 ± 1.52            | 58.18 ± 1.98                   | 1.11  | 1.23    | 1.11        |
| Glu                       | 2569.5 ± 58.6 | 2796.0 ± 45.6           | 2858.8 ± 60.6                 | 1.02  | 1.11    | 1.09        |
| Phe                       | 47.95 ± 1.40  | 47.57 ± 2.36            | 56.18 ± 2.07                   | 1.18  | 1.17    | 0.99        |
|                           |           |                         |                                  |       |         |             |
| Hippocampus: decreased    |           |                         |                                  |       |         |             |
| Cystathionine             | 13.94 ± 3.43  | 14.41 ± 4.14            | 3.90 ± 2.24                   | 0.27  | 0.28    | 1.03        |
| Glu                       | 4383.3 ± 40.5 | 4378.2 ± 66.0           | 4113.5 ± 53.5                 | 0.94  | 0.94    | 1.00        |
| Thr                       | 242.84 ± 53.20 | 205.79 ± 33.72         | 150.67 ± 22.74                | 0.73  | 0.62    | 0.85        |

* Statistical significance versus Phgdh+/-/floxed (Student’s unpaired t test): p < 0.05.
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Figure 4

Changes in L- and D-serine levels in the cerebral cortex after L-serine administration. A–D, shown is the L-serine (A and C) and D-serine (B and D) content in the cerebral cortex of CKO (A and B) and Floxed (C and D) mice 3 h after intraperitoneal injection of excess L-serine. Data represent the mean ± S.E. E, shown is the ratio of D-serine to total serine in Floxed and CKO cortex after serine administration. Data represent the mean ± S.E. NT, no treatment, n = 7 for Floxed, n = 6 for CKO; Saline, saline-injected, n = 3; L-Ser, L-serine-injected, n = 3), *, p < 0.05; **, p < 0.01; ***, p < 0.005 versus saline-injected mice (one-way analysis of variance, Dunnett’s post-hoc test). The L- and D-serine data shown in Fig. 3D were used as no treatment in A–D. In E, ***, p < 0.005 versus saline-injected Floxed mice (one-way analysis of variance, Dunnett’s post-hoc test).

Expression of Serine Racemase and NMDA Receptor Subunits—The marked decreases in L- and D-serine content prompted us to hypothesize that expression of Srr and/or NMDA receptor subunits could be dysregulated in CKO

Through the blood-brain barrier even when there is a serine deficiency in the brain. Notably, the D-serine to total serine ratio of CKO cortex appeared constant but was significantly less than that of Floxed cortex after L-serine administration (Fig. 4E).
Brain L-Serine Is Essential for Controlling D-Serine Level

Western blot analysis demonstrated that normalized levels of Srr protein in the cerebral cortex and hippocampus did not differ significantly between the two genotypes (Fig. 5, A and B), suggesting that Srr expression was not influenced by diminished substrate level. Immunohistochemistry of cortical sections demonstrated that the Srr expression level in CKO cortex was similar to that of Floxed controls. The cellular distribution of Srr immunoreactivity (green in supplemental Fig. 2, A and B) was mutually Exclusive with that of S100 αβ, an astroglial marker (red in supplemental Fig. 2). Srr protein co-localized mainly with a neuronal marker, NSE, in both genotypes (supplemental Fig. 2, C and D). The neuron-enriched localization of Srr is in agreement with recent immunohistological studies (51, 52).

We then examined the expression of the major types of NMDA receptor subunits, NR1 (also known as GluRζ1) and NR2B (also known as GluRe2) in the cerebral cortex and hippocampus. The normalized levels in these regions were comparable between Floxed and CKO mice (Fig. 5, C and D), indicating that the availabilities of L-serine and D-serine did not play a regulatory role in maintaining steady-state levels of NR1 and NR2B subunits in these two regions, as seen in for Srr (Fig. 5, A and B).

In addition, our Western blot analysis demonstrated that normalized expression levels of neuronal markers SNAP-25 and NSE as well as an astrocytes maker Gfap did not differ between CKO and Floxed mice in the cortex and hippocampus (Fig. 6, A–D). Thus, it seems unlikely that these two regions of CKO brain undergo degeneration of neuronal and/or astroglial populations.

NMDA-induced Arc Transcription—Although NR1 and NR2B subunit levels did not differ between the two genotypes, we examined whether NMDA receptor function was altered in CKO brain. To monitor NMDA receptor activation, we quantified expression of the gene encoding activity-regulated cytoskeleton-associated protein (Arc), which was identified as a brain-specific immediate early gene whose mRNA level is elevated by synaptic activity in an NMDA receptor activation-dependent manner (53–55). Our preliminary gene expression profiling of the hippocampus via DNA microarrays demonstrated that basal expression levels of Arc mRNA did not differ between Floxed and CKO mice in the absence of stimulation by NMDA.5 Administration of a subconvulsant dose of NMDA (75 mg/kg body weight) (47) caused a significant increase in Arc mRNA level in the hippocampus of wild-type mice 3 h after injection (Fig. 7A). In the hippocampus of Floxed mice, a similar 1.8-fold increase in Arc mRNA level was detected, whereas no increase in Arc mRNA level after NMDA administration was observed in CKO hippocampus (Fig. 7B). The attenuation of NMDA-evoked Arc transcription is indicative of impaired NMDA receptor function in CKO hippocampus.

DISCUSSION

A Critical Role for Endogenous L-Serine in Maintaining Brain D-Serine Level—The aim of the present study was to elucidate the role of brain L-serine synthesis in modulating D-serine level, a co-agonist of NMDA receptors, in vivo. We developed conditional Phgdh mutant mice by loxP-Cre re-
Brain l-Serine Is Essential for Controlling d-Serine Level

**FIGURE 6. Expression of neuronal and astroglial marker proteins.** A and B, neuronal markers SNAP-25 and NSE were detected in protein lysates (10 μg) of the cerebral cortex (A) and hippocampus (B) by Western blotting. Expression levels were normalized to Gapdh levels. Data represent the mean ± S.E. (cerebral cortex, n = 5; hippocampus, n = 4). C and D, Gfap was detected in protein lysates (10 μg) of the cerebral cortex (C) and hippocampus (D) by Western blotting. Expression levels were normalized to Gapdh levels. Data represent the mean ± S.E. (cerebral cortex, n = 5; hippocampus: n = 4).

**FIGURE 7. Altered Arc induction in hippocampus evoked by NMDA administration.** Arc mRNA levels 3 h after administration of NMDA or PBS in the hippocampus of wild-type (A), Floxed (B), and CKO (B) mice were measured with quantitative real-time PCR. Expression levels of Arc mRNA are shown as arbitrary units normalized to Gapdh levels. Data represent the mean ± S.E. (wild type, n = 4 for each treatment; Floxed, n = 5 for PBS treatment, n = 6 for NMDA treatment; CKO, n = 3 for PBS treatment, n = 5 for NMDA treatment). Differences between treatments were analyzed by Student’s t test (A) or one-way analysis of variance, Dunnett’s post-hoc test (B) (*, p < 0.05; **, p < 0.005).

combination because systemic deletion of the gene results in embryonic lethality (40). The hGFAP promoter-Cre transgenic mouse (39) was used to generate brain-specific Phgdh mutant mice for the first time. Brain-specific Phgdh deletion resulted in marked simultaneous decreases in the l- and d-serine content of the cerebral cortex and hippocampus (Fig. 3, D and F), demonstrating unequivocally that in the adult brain l-serine synthesized endogenously via the phosphorylated pathway is the principal precursor of d-serine. Under our experimental conditions, deletion of Phgdh via the hGFAP-Cre construct resulted in marked decreases in Phgdh protein expression in the cerebral cortex and hippocampus (Fig. 1C), whereas up-regulation of Phgdh protein was seen in the choroid plexus of CKO brain.6 This may be a compensatory response to brain serine deficiency and may underlie residual l- and d-serine content in CKO cortex. Notably, the glycine contents in CKO cortex and hippocampus were moderately decreased (Fig. 3, E and G), which is in sharp contrast to the marked reduction (70%) in embryonic systemic Phgdh KO tissues, including the central nervous system (42, 43). This difference suggests that, unlike the embryonic central nervous system, l-serine does not serve as a major glycine precursor in adult cerebral cortex and hippocampus. Thus, brain glycine may be supplied by other means, such as circulating blood.

l-Serine and d-serine were not significantly increased in CKO serum (Fig. 3, A and B). Intrapertitoneal l-serine administration resulted in a comparable transient net l-serine increase in the cortex of CKO and Floxed mice at 3 h post-injection (Fig. 4, A and C). The net increase in d-serine content, however, was seen only in CKO cortex (25.95 nmol/g wet tissue in CKO versus 1.35 nmol/g wet tissue in Floxed; Fig. 4, B and D), suggesting that conversion of l-serine to d-serine in the cortex occurs more efficiently under serine-deficient conditions than normal conditions. Bulk transfer of l-serine from the periphery to brain across the blood-brain barrier seems inefficient for maintaining normal levels of l- and d-serine in adult cerebral cortex (Fig. 4). However, the serine supplementation experiment suggests that a transient increase in blood l-serine concentration (e.g. after eating) may contribute to brain d-serine synthesis under serine-deficient conditions.

Cultured young neurons undergo neurite degeneration and cell death when maintained in medium lacking l-serine under glial cell-free conditions (35, 56). However, CKO cortex showed no morphological signs of degeneration or neuronal cell death.6 Western blotting demonstrated that normalized expression levels of neuron-specific proteins SNAP25 and NSE did not differ between Floxed and CKO cortex (Fig. 6). Indeed, it has been reported that hGFAP-directed Cre expression in brain begins at mid-embryonic stages and then reaches a maximum after birth (39). Hence, the l-serine content in developing CKO brain may be above the threshold needed to support neuronal survival during the embryonic and early postnatal periods.

**Altered NMDA Receptor Function**—In mouse cerebral cortex, Srr protein expression is first detected at postnatal day 7 and is up-regulated gradually thereafter, at least until postnatal day 56 (52). Morphine administration increases steady-state levels of Srr mRNA and protein in rat brain (57), but the regulatory mechanism governing Srr expression remains largely unexplored. The present biochemical and histological experiments demonstrate that marked reductions of serine

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6 J. H. Yang and S. Furuya, unpublished observation.
enantiomers do not affect the expression levels of Srr (Fig. 5, A and B) and NMDA receptor subunits (Fig. 5C and D) in the cortex and hippocampus of CKO mice. Thus, it is unlikely that L- or D-serine availability regulates Srr and NMDA receptor subunit levels, at least in the cerebral cortex and hippocampus. Despite the lack of significant alterations in the expression levels of Srr and NMDA receptor subunits (Fig. 5), we demonstrated that NMDA-evoked Arc transcriptional activation was diminished in CKO hippocampus (Fig. 7). Recent studies using knock-out/mutant mice demonstrated that Srr deletion resulted in marked decreases in brain D-serine, which was associated with attenuation of NMDA receptor-mediated neurotransmission (31) and neurotoxicity (30, 58).

In addition, behavioral deficits were evident in two strains of these mutant mice (31, 32), although the detailed behavioral phenotypes did not coincide with each other. Based on the present study together with phenotypes of these Srr knock-out/mutant mice, we propose that the availability of l-serine synthesized via the phosphorylated pathway in brain is a key determinant for normal NMDA receptor function, which depends on D-serine being maintained within a certain concentration range. Notably, deletion of functional Phgdh culminates in mild postnatal microcephaly (Fig. 2), which is not seen in Srr knock-out/mutant mice. Further studies aimed at elucidating the correlation between serine deficiency and morphological and behavioral aberrations are necessary to define a role for endogenously synthesized L- and D-serine in brain physiology and pathology.

Implications for Human Serine Deficiency Disorder—Our results reveal that Phgdh-dependent biosynthesis of brain L-serine is essential for establishing D-serine in adult cerebral cortex and hippocampus. Conditional ablation of Phgdh in brain using hGFAP-Cre deleter mice resulted in marked decreases in the tissue content of both L- and D-serine (Fig. 3, D and E) even though their serum concentrations were comparable in Floxed and CKO mice (Fig. 3, A and B) fed normal chow, which contains 20% milk casein as a source of amino acids. Furthermore, single-dose supplementation of excess L-serine via an intraperitoneal injection did not fully restore the content of L- or D-serine in CKO cortex (Fig. 4). These observations are in agreement with the finding that L-serine uptake is extremely inefficient in adult brain (59, 60), implying that L-serine supplementation therapy would be less effective in adult patients with serine deficiency disorder than in infant/child patients. Therefore, CKO mice may serve as a useful animal model for studying the pathophysiology of serine deficiency disorder, including PHGDH deficiency (37, 38), and developing efficacious treatments for patients with reduced brain L- and/or D-serine levels, such as serine deficiency disorder and schizophrenia patients (61, 62). To our surprise, conditional ablation of Phgdh in brain using hGFAP-Cre deleter mice did not cause any widespread alterations in the layer structure of the cerebral cortex (supplemental Fig. 1). Ongoing investigations are aimed at determining whether serine deficiency in adult CKO brain causes region-specific morphological and/or functional alterations.

REFERENCES

1. Cull-Candy, S., Brickley, S., and Farrant, M. (2001) Curr. Opin. Neurobiol. 11, 327–335
2. Scheetz, A. J., and Constantine-Paton, M. (1994) FASEB J. 8, 745–752
3. Nakazawa, K., McHugh, T. J., Wilson, M. A., and Tonegawa, S. (2004) Nat. Rev. Neurosci. 5, 361–372
4. Johnson, J. W., and Ascher, P. (1987) Nature 325, 529–531
5. Kleckner, N. W., and Dingledine, R. (1988) Science 241, 835–837
6. Danyusz, W., and Parsons, C. G. (1998) Pharmacol. Rev. 50, 597–664
7. Hashimoto, A., Nishikawa, T., Oka, T., and Takahashi, K. (1992) FEBS Lett. 296, 33–36
8. Hashimoto, A., Yamashita, K., Fujii, N., Harada, K., Oka, T., and Takahashi, K. (2005) Pharm. Bull. 28, 1561–1565
9. Wolosker, H., Dumin, E., Balan, L., and Foltyn, V. N. (2008) FEBS J. 275, 3514–3526
10. Hashimoto, A., Nishikawa, T., Oka, T., and Takahashi, K. (1993) J. Neurochem. 60, 783–786
11. Wolosker, H., Dumin, E., Balan, L., and Foltyn, V. N. (2008) FEBS J. 275, 3514–3526
12. Schell, M. J., Brady, R. O., Jr., Molliver, M. E., and Snyder, S. H. (1997) J. Neurochem. 17, 1604–1615
13. Matsui, T., Sekiguchi, M., Hashimoto, A., Tomita, U., Nishikawa, T., and Wada, K. (1995) J. Neurochem. 65, 454–458
14. Mothet, J. P., Parent, A. T., Wolosker, H., Brady, R. O., Jr., Linden, D. J., Ferris, C. D., Rogawski, M. A., and Snyder, S. H. (2000) Proc. Natl. Acad. Sci. U.S.A. 97, 4926–4931
15. Panatier, A., Theodosius, D. T., Mothet, J. P., Touquet, B., Pollegioni, L., Poulain, D. A., and Oliet, S. H. (2006) Cell 125, 775–784
16. Wood, P. L., Emmett, M. R., Ruo, T. S., Mick, S., Cler, J., and Iyengar, S. (1989) J. Neurochem. 53, 979–981
17. Contreras, P. C. (1990) Neuropharmacology 29, 291–293
18. Tanii, Y., Nishikawa, T., Hashimoto, A., and Takahashi, K. (1994) J. Pharmacol. Exp. Ther. 269, 1040–1048
19. Tsai, G., Yang, P., Chung, L. C., Lange, N., and Coyle, J. T. (1998) Biol. Psychiatry 44, 1081–1089
20. Heresco-Levy, U., Javitt, D. C., Ebstein, R., Vass, A., Lichtenberg, P., Bar, G., Catini, S., and Ermilov, M. (2005) Biol. Psychiatry 57, 577–585
21. Javitt, D. C. (2004) Mol. Psychiatry 9, 984–997
22. Wolosker, H., Sheth, K. N., Takahashi, M., Mothet, J. P., Brady, R. O., Jr., Ferris, C. D., and Snyder, S. H. (1999) Proc. Natl. Acad. Sci. U.S.A. 96, 721–725
23. Cook, S. P., Galve-Roperh, I., Martinez del Pozo, A., and Rodriguez-Crespo, I. (2002) J. Biol. Chem. 277, 27782–27792
24. De Miranda, J., Panizzutti, R., Foltyn, V. N., and Wolosker, H. (2002) Proc. Natl. Acad. Sci. U.S.A. 99, 14542–14547
25. Needle, A., and Dunlop, D. S. (2002) Neurochem. Res. 27, 1719–1724
26. Strisovský, J., Jurásková, J., Barinka, C., Majer, P., Rojas, C., Clusser, B. S., and Konvalinka, J. (2003) FEBS Lett. 535, 44–48
27. Foltyn, V. N., Bendikov, I., De Miranda, J., Panizzutti, R., Dumin, E., Shleper, M., Li, P., Toney, M. D., Kartvelishvily, E., and Wolosker, H. (2005) J. Biol. Chem. 280, 1754–1763
28. Dunlop, D. S., and Needle, A. (1997) Biochem. Biophys. Res. Commun. 235, 26–30
29. Inoue, R., Hashimoto, K., Harai, T., and Mori, H. (2008) J. Neurosci. 28, 14486–14491
30. Basu, A. C., Tsai, G. E., Ma, C. L., Ehrens, J. T., Mustafa, A. K., Han, L., Jiang, Z. L., Benneyworth, M. A., Froimowitch, M. P., Lange, N., Snyder, S. H., Bergeron, R., and Coyle, J. T. (2009) Mol. Psychiatry 14, 719–727
31. Labrie, V., Fukumura, R., Rastogi, A., Fick, L. I., Wang, W., Boutros, P. C., Kennedy, J. L., Semerulal, M. O., Lee, F. H., Baker, G. B., Belsham, D. D., Barger, S. W., Gondo, Y., Wong, A. H., and Roder, J. C. (2009) Hum Mol. Genet. 18, 3227–3234
32. Snell, K. (1984) Adv. Enzyme Regul. 22, 325–400
33. Hirabayashi, Y., and Furuya, S. (2008) Prog. Lipid Res. 47, 188–203
34. Furuya, S., Tabata, T., Mitoma, J., Yamada, K., Yamasaki, M., Makino, A., Yamamoto, T., Watanabe, M., Kano, M., and Hirabayashi, Y. (2000) Proc. Natl. Acad. Sci. U.S.A. 97, 11258–11253
35. Yamazaki, M., Yamada, K., Furuya, S., Mitoma, J., Hirabayashi, Y., and
Brain l-Serine Is Essential for Controlling d-Serine Level

Watanabe, M. (2001) J. Neurosci. 21, 7691–7704
37. de Koning, T. J. (2006) J. Inherit. Metab. Dis. 29, 347–351
38. Fuchs, S. A., Dorland, L., de Sain-van der Velden, M. G., Hendriks, M., Klomp, I. W., Berger, R., and de Koning, T. J. (2006) Ann. Neurol. 60, 476–480
39. Zhuo, L., Theis, M., Alvarez-Mayà, I., Brenner, M., Willecke, K., and Messing, A. (2001) Genesis 31, 85–94
40. Yoshida, K., Furuya, S., Yoshida, K., Kawakami, Y., Yang, J. H., Sayano, T., Azuma, N., Tanaka, H., Kuhara, S., and Hirabayashi, Y. (2008) Funct. Integr. Genomics 8, 235–249
41. Kawakami, Y., Yoshida, K., Yang, J. H., Suzuki, T., Azuma, N., Sakai, K., Hashikawa, T., Watanabe, M., Yasuda, K., Kuhara, S., Hirabayashi, Y., and Furuya, S. (2009) Neurosci. Res. 63, 184–193
42. Morikawa, A., Hamase, K., and Zaitsu, K. (2003) Anal. Biochem. 312, 66–72
43. Abe, M., Fukaya, M., Yagi, T., Mishina, M., Watanabe, M., and Sakimura, K. (2004) J. Neurosci. 24, 7292–7304
44. Watanabe, M., Fukaya, M., Sakimura, K., Manabe, T., Mishina, M., and Inoue, Y. (1998) Eur. J. Neurosci. 10, 478–487
45. Menniti, F. S., Pagnozzi, M. J., Butler, P., Chenard, B. L., Jaw-Tsai, S. S., and Frost White, W. (2000) Neuropharmacology 39, 1147–1155
46. Cho, H. M., Jun, D. Y., Bae, M. A., Ahn, J. D., and Kim, Y. H. (2000) Gene 245, 193–201
47. Klomp, I. W., de Koning, T. J., Malingré, H. E., van Beurden, E. A., Brink, M., Opdam, F. L., Duran, M., Jaeken, J., Pineda, M., Van Maldergem, L., Poll-The, B. T., van den Berg, I. E., and Berger, R. (2000) Am. J. Hum. Genet. 67, 1389–1399
48. Takasaki, C., Miura, E., and Watanabe, M. (2007) Biomed. Res. 28, 61–69
49. Kartvelishvily, E., Shleper, M., Balan, L., Dumin, E., and Wolosker, H. (2006) J. Biol. Chem. 281, 14151–14162
50. Miya, K., Inoue, R., Takata, Y., Abe, M., Natsume, R., Sakimura, K., Hongou, K., Miyawaki, T., and Mori, H. (2008) J. Comp. Neurol. 510, 641–654
51. Link, W., Konietzko, U., Kauselmann, G., Krug, M., Schwanke, B., Frey, U., and Kuhl, D. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 5734–5738
52. Lyford, G. L., Yamagata, K., Kaufmann, W. E., Barnes, C. A., Sanders, L. K., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., Lanahan, A. A., and Worley, P. F. (1995) Neuron. 14, 433–445
53. Coba, M. P., Valor, L. M., Kopanitsa, M. V., Afinowi, N. O., and Grant, S. G. (2008) J. Biol. Chem. 283, 34101–34107
54. Mitoma, J., Furuya, S., and Hirabayashi, Y. (1998) Neurosci. Res. 30, 195–199
55. Yoshikawa, M., Shinomiya, T., Takayasu, N., Tsukamoto, H., Kawaguchi, M., Kobayashi, H., Oka, T., and Hashimoto, A. (2008) J. Pharmacol. Sci. 107, 270–276
56. Mustafa, A. K., Ahmad, A. S., Zeynalov, E., Gazi, S. K., Sikka, G., Ehmen, J. T., Barrow, R. K., Coyle, J. T., Snyder, S. H., and Doré, S. (2010) J. Neurosci. 30, 1413–1416
57. Oldendorf, W. H. (1971) Am. J. Physiol. 221, 1629–1639
58. Smith, Q. R., Momma, S., Aoyagi, M., and Rapoport, S. I. (1987) J. Neurochem. 49, 1651–1658
59. Hashimoto, K., Engberg, G., Shimizu, E., Nordin, C., Lindström, L. H., and Iyo, M. (2005) Prog. Neuropsychopharmacol Biol. Psychiatry 29, 767–769
60. Bendikov, I., Nadri, C., Amar, S., Panizzutti, R., De Miranda, J., Wolosker, H., and Agam, G. (2007) Schizophr. Res. 90, 41–51