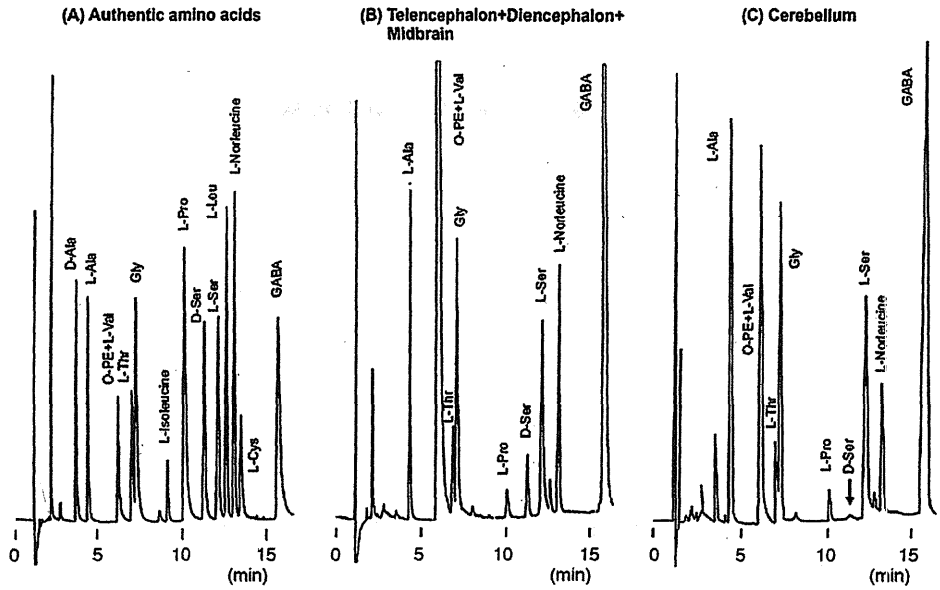

Fig. 1. Gas chromatographic detection of endogenous D-serine in the mammalian brain. The present schemes show representative gas chromatograms of *N,O*-pentafluoropropionyl isopropyl derivatives of the authentic standard amino acids (A), the free amino acids in the extracts of the brain tissues consisting of the telencephalon, diencephalon and midbrain (B) and in those of the cerebellum (C) (unpublished data) in the young adult rats. These gas chromatography assays were performed on a capilally column of Chirasil-L-Val (25 m × 0.25 mm, film thickness 0.12 μm, Gaskuro Kogyo, Japan) as described in Ref. [12]. Note the marked differences in the heights of the D-serine (D-Ser) peaks between the rostral brain regions (B) and the cerebellum (C). L-Norleusine was added as the internal standard. The panels (A) and (B) have been reorganized from the figure published in Ref. [12]. Abbreviations: D-Ala, D-alanine; L-Ala, L-alanine; L-Cys, L-cystein; Gly, glycine; L-Leu, L-leucine; O-PE, O-phosphoethanolamine; L-Pro, L-proline; D-Ser, D-serine; L-Ser, L-serine; L-Thr, L-threonine; L-Val, L-valine.

[20]. This anti-PCP action was barely observed with the corresponding L-forms [21] and attenuated by a selective antagonist for the glycine site [20], supporting the potentiality of an agonist for the NMDA receptor glycine regulatory site as a new antipsychotic.

Furthermore, the present author planned and initiated a study on the possible presence of free D-serine or D-alanine in the brain of rats treated with their myristoylated compounds by GC–MS and HPLC in collaboration with the late Tokishi Hayashi, Ph.D. at the National Institute of Neuroscience to verify the mechanisms of the anti-PCP action of these compounds. By the additional collaboration with Noriko Fujii, Ph.D. at Tsukuba University, we provided the first evidence using GC and GC–MS for the constant presence of D-serine at a substantial content in the rat brain [12]. As this author predicted on the basis of the brain-preferring distribution of D-serine, brain endogenous D-serine was shown to exhibit an NMDA receptor-related distribution [15,22]. From the neuroanatomical and functional correlates of D-serine with the NMDA receptor [15,22] and the presumed anti-schizophrenia action [20,21,23], it was proposed by the present author that brain D-serine might play an important role as an endogenous NMDA receptor allosteric agonist in the regulation of higher brain functions [12,15,22]. After early confirmations of our initial findings were obtained by our group [24–26] and others [27–29], subsequent studies of endogenous D-serine in the central nervous system have expanded to include a wide variety of topics [30].

This review article summarizes the findings regarding the metabolism and function of endogenous D-serine as primarily related to the mammalian brain. The significance of these findings will be discussed in terms of the pathophysiology and the development of novel therapies for neuropsychiatric disorders.

2. Distribution and metabolism of D-serine

Recent advances in the separation and detection techniques for the chiral amino acids have enabled us (the investigators) to perform the qualitative and quantitative examinations of the free or protein-conjugated D-amino acids in the biological samples. Detection of endogenous free D-serine indeed appears to be one of their important products that open a novel path to understand, at least, the complex regulatory systems for the mammalian brain.

2.1. Analysis of free D-serine in mammals

Presently, D-serine can be identified or quantitatively assayed by GC [12] (Fig. 1), GC–MS [12,15], HPLC with fluorometric detection [22,24,31,32] (Fig. 2), thin-layer chromatography in combination with HPLC [14], enzymatic assay using D-serine dehydratase [33], online microdialysis–capillary electrophoresis [34], and immunohistochemical staining by using anti-D-serine antibody raised against protein-conjugated D-serine by glutaraldehyde [29,35–37]. By means of these various methodologies, tissue and cellular localizations and their dynamics of D-serine have extensively been examined (Figs. 3 and 4). Although most investigations on mammalian intrinsic D-serine have focused on the brain, it is also intensively studied in other organs such as spinal cord [26,38], retina [39,40], kidney [14], bone [41], and urine [42]. In these studies, biochemical phenomena that suggest the existence of metabolic and functional processes of D-serine, including biosynthesis, storage, release into extracellular fluid, interaction with the receptor site, uptake, and degradation, are observed (Fig. 5).

2.2. Distribution (Figs. 3 and 4)

In the maturation period of mammals, including rats, mice, and humans, D-serine shows a selective distribution in the brain and retina; its concentration is very low in the spinal cord, peripheral tissues such as kidney and liver, and blood (Fig. 3), but relatively high in the urine (contents in the human urine (18–55 years old), 0.08–0.246 μmol/ml; D-serine to total serine ratios, 18–53%: rat, ratios, 13%) [12,15,22,42]. The distribution in the brain is also uneven; the concentrations of D-serine and

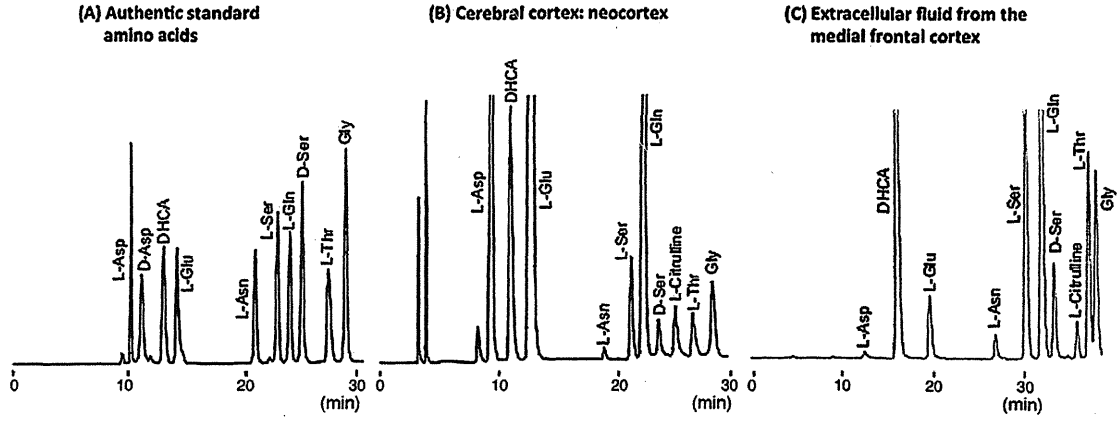

Fig. 2. Detection of brain D-serine by high-performance liquid chromatography with fluorometric detection. The present schemes show representative chromatograms of *N*-tertiary-butyloxycarbonyl-L-cysteine-*o*-phthaldialdehyde derivatives of the authentic standard amino acids (A), the tissues of the cerebral cortex (the neocortical portions) (B) and in the extracellular fluid collected from the medial frontal cortex (C) in the young adult rats obtained by a reversed-phase high-performance liquid chromatographic system with a fluorescence detector [22,24,66]. Routinely, D-homocysteic acid (DHCA) was added as an internal standard in the analysis of the brain tissues (panels B) and of the extracellular fluid (panels C). The panel (C) has been reorganized from the figure published in Ref. [66]. Abbreviations: L-Asn, L-asparagine; D-Asp, D-aspartate; L-Asp, L-aspartate; L-Cys, L-cystein; L-Gln, L-glutamine; L-Glu, L-glutamate; Gly, glycine; DHCA, D-homocysteic acid; D-Ser, D-serine; L-Ser, L-serine; L-Thr, L-threonine.

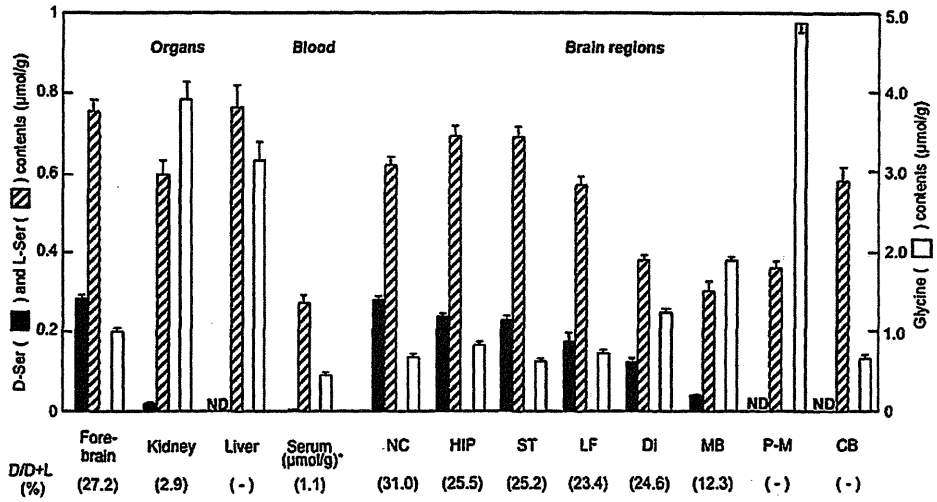

Fig. 3. Contents of D-serine, L-serine and glycine in the various organs, serum and brain regions of the rat. The distribution pattern of D-serine differs markedly from those of L-serine and glycine in the body and brain of the rat [12,22]. The present scheme has been reorganized from the figures published in Refs. [12,22]. Abbreviations: CB, cerebellum; DI, diencephalon (thalamus and hypothalamus); Forebrain (neocortex, paleocortex, striatum, hippocampus and the tissues among them); HIP, hippocampus; MB, midbrain; NC, neocortex; ND, not detectable; P-M, pons–medulla; ST striatum. *Note the unit.

their ratios of D- to total serine are high in regions of the forebrain (contents, 0.35–0.25 μmol/g tissue at the highest level in the cerebral cortex, hippocampus, striatum, etc.; ratios, 31–25%), medium to low in the diencephalon and midbrain (0.19–0.9 μmol/g tissue; 24–12%), and trace in the brainstem and cerebellum [15,22]. The forebrain-preferred distribution pattern of D-serine [15,22,29] has been shown to be strongly correlated with that of the density of the binding sites for glutamate, PCP, and glycine of the NMDA receptor [43,44] and closely resembles the mRNA distribution of the NMDA receptor GRIN2B (NR2B) subunit in particular [45].

The D-serine distribution in the brain changes markedly along with development. Although the distribution in the rodent brain immediately after birth is almost uniform (approximately 0.1 μmol/g), the pattern comes close to that of the maturation period at about three weeks after birth [22,46]. The changes after birth vary with brain regions; the D-serine concentration in the cerebral neocortex reaches the level observed in the maturation period (approximately 0.3 μmol/g) on the postnatal days 21, but, in the cerebellum, it becomes comparable with that of the mature neocortex on the 7th day after birth and then decreases quickly. These changes also correspond with the postnatal development of distribution pattern of GRIN2B mRNA (NR2B) in the brain [22,46,47].

At the cellular level of the mammalian brains, D-serine-like immunoreactivity has been observed in astrocytes in the gray and white matter [29], neuronal cell bodies [36], dendrites [36], axons [36], and microglia [37], and very recently in a small population of oligodendrocytes [48]. These observations are consistent with the quantitative data obtained by HPLC that D-serine contents did not differ between the white and gray matter in the human cerebral neocortex [26]. In addition, Müller cells, astrocytes, and neuronal ganglion cells of the retina has been reported to be positive to the D-serine immunostaining [40].

Phylogenetically, the concentrations of D-serine and the ratios of D- to L-serine contents are extremely low in the brains of fish,

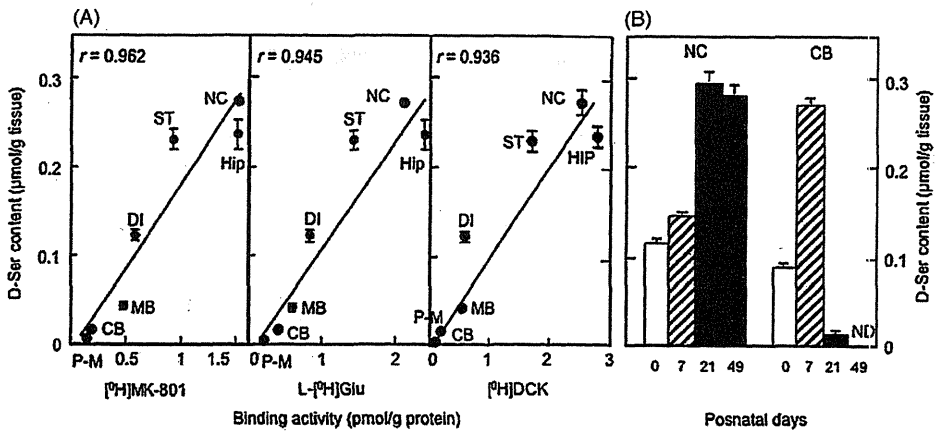

Fig. 4. NMDA receptor-like regional distribution of D-serine in the adult and developing rats. The regional distribution of D-serine contents is closely correlated with that of the densities of the phencyclidine, glycine and glutamate binding sites of the NMDA receptor (panel A) [12,22,43,44] and with the expression levels of its GRIN2B subunit. D-Serine concentrations in the neocortex and cerebellum dramatically change during the postnatal development in a manner observed for the GRIN2B subunit mRNA expression (panel B) [12,22,45,46]. These data indicate that D-serine and various NMDA binding sites and/or the GRIN2B subunit may exhibit similar regional distribution patterns during postnatal development. The limbic forebrain ("LF" in this figure) contains nucleus accumbens, septum, olfactory tubercle and their interstitial tissues. The present schemes have been reorganized from the figures and tables published in Refs. [12,22,43,44,46]. Abbreviations: CB, cerebellum; DI, diencephalon (thalamus and hypothalamus); HIP, hippocampus; LF, limbic forebrain; MB, midbrain; NC, neocortex; ND, not detectable; P–M, pons–medulla; ST striatum.

amphibians, and birds (0.001–0.018 μmol/g tissue; 0.2–1.4%), suggesting that endogenous D-serine is specifically maintained at a high level in the mammalian brain among the vertebrates [49]. It, however, should be noted that the blood of an invertebrate, silkworm, has been reported to exhibit high ratios of D- to total serine contents up to 59% during development [6].

2.3. Synthesis (Fig. 5)

In the rat brain, the existence of serine racemase that synthesizes D- from L-serine was presumed based on the following phenomena: (a) the D-serine concentration is increased when the concentration of L-serine (or glycine) is increased [50]; (b) [^{3}H] L-serine is converted to [^{3}H] D-serine [51]. In fact, pyridoxal 5′-phosphoric acid-dependent serine racemases of rats, mice, and humans, which are activated by ATP or Mg^{2+}, have been isolated [52,53]. The important role of this enzyme in D-serine biosynthesis has been supported by the experiments indicating that (a) immunoreactivity of this enzyme shows a D-serine-like distribution [54] and is detected in both astrocytes and neurons that contain D-serine in the brain [54,55], and (b) the concentrations of D-serine is dramatically decreased by 90% in the brain of its gene knockout mice [56–58] and of the mice with ENU-induced mutation that results in a complete loss of the racemase activity [59]. Moreover, the putative physiological role of L-serine as a precursor for D-serine has indeed been supported by the results that forebrain-specific deletion of the phosphorylation pathway enzyme for L-serine synthesis, D-3-phosphoglycerate dehydrogenase (Phgdh), produced a significant diminution in the cerebral cortical and hippocampal D-serine contents in the mouse [60], suggesting the conversion of L-serine to D-serine mediated by serine racemase. It is also reported that serine racemase is activated by glutamate neurotransmission involving AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors via GRIP (glutamate receptor interacting protein) binding to the enzyme [61].

The physiological functioning condition of the mammalian serine rasemase, however, remains unclear because this enzyme exhibits a serine dehydratase activity higher than racemase activity [55] and shows a serine racemase activity about 1/1000 as low as the alanine racemase of yeast [62] *in vitro*. Moreover, the incomplete depletion of D-serine in the serine racemase gene knockout animals suggests another pathway for D-serine synthesis. From this viewpoint, it is of interest to note that D-serine contents in the postmortem cerebral cortex tissues are markedly decreased in the patients with non-ketotic hyperglycinemia that is caused by the lack of activity of the glycine cleavage system [63] and in the rats treated with an inhibitor of this enzyme system [63]. The possible involvement of the glycine cleavage enzyme system and other serine-related enzymes, such as serine hydroxymethyl transferase and phosphoserine phosphatase, in D-serine biosynthesis is also being studied [63,64].

2.4. Storage

Histochemical studies using a specific anti-D-serine antibody have revealed that D-serine-like immunoreactivity is observed in the astrocytes [29,55], neurons [36,48,55], microglia [37] and oligodendrocytes [48]. The storage sites of D-serine may vary with cell types of the brain.

In the astrocytes of the brain sections, both D-serine antibodies requiring glutaraldehyde-fixation [29,36,48,54] and those optimized for formaldehyde-fixation [37] produce D-serine immunostaining in the vesicular-like structure. In agreement with these observations, the C6 glioma cells and cultured astrocytes obtained from the brain tissue of infant animals seem to have an exocytotic vesicle-like structure to store D-serine, because the immunoreactions of at least D-serine and the synaptic vesicle marker VAMP2 coexist, and because D-serine release is no longer seen with the presence of tetanus toxin that degrades VAMP2 [65].

In contrast, D-serine release in the cultured neurons of the cerebral cortex is not significantly influenced by bafilomycin A1 that inhibits amino acid uptake into the vesicles [55], although D-serine-like immunoreactivity is reported in the vesicular-like structures in the neurons located in the hindbrain regions [37]. Together with the observations that D-serine is not transported into purified synaptic vesicles under conditions optimal for the uptake of known transmitters, these results suggest that the neuronal D-serine may be mainly stored in the cytoplasm [55].

Further investigation is needed to clarify the exact storage structures for endogenous D-serine and their molecular machineries in the astrocytes, neurons, and also in the other cells that exhibit the immunoreactivity to D-serine.

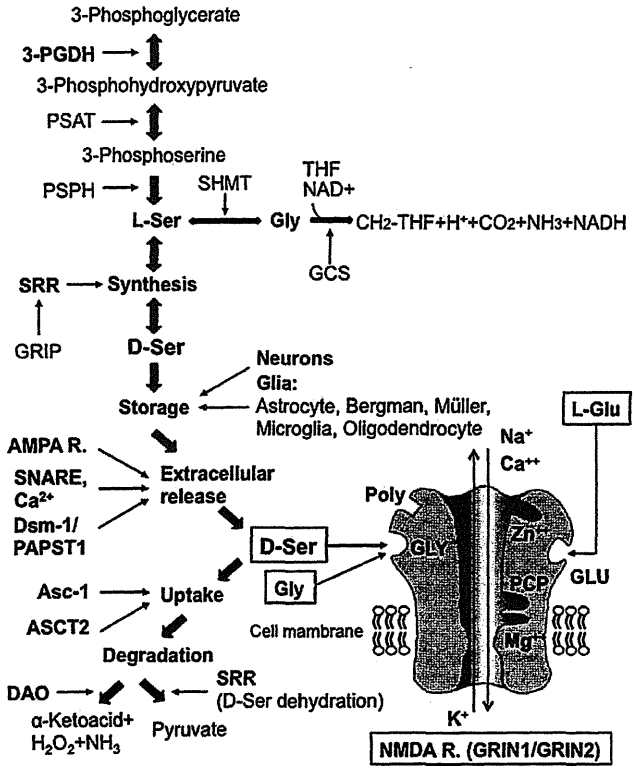

Fig. 5. Schematic representation of the presumed metabolic pathways for D-serine in the mammalian nervous systems. The candidate molecules or cells for the respective metabolic or functional processes of intrinsic D-serine in the mammalian nervous systems including the retina are summarized. However, most of their precise roles and cellular localization still await further elucidation. It is well established that D-serine acts on the glycine site of the *N*-methyl-D-aspartate type glutamate receptor (NMDA R.) consisting of GRIN1 (NR1) and GRIN2 (NR2) subunits. The NMDA receptor complex has the multiple regulatory binding sites for glutamate (Glu), glycine and D-serine (Gly), magnesium ions (Mg^{++}), phencyclidine (PCP), and polyamine (Poly). The oligomeric NMDA receptors form tetrameric channels comprising two copies each of GRIN1and GRIN2 subunit. D-Serine has also been indicated to interact with the NMDA receptor containing GRIN1 and GRIN3 subunits and δ2 glutamate receptor that are not shown in this scheme. Abbreviations: AMPA R., α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; Asc-1, Na^+-independent alanine–serine–cysteine transporter 1; ASCT2, Na^+-dependent broad-spectrum neutral amino acid transporter 2; DAO, D-amino acid oxidase; D-Ser, D-serine; Dsm-1/PAPST-1, D-serine modulator-1/3′-phosphoadenocine 5′-phosphosulfate transporter-1; GCS, glycine cleavage system; Gly, glycine; GRIP, glutamate receptor interacting protein; L-Glu, L-glutamate; NMDA R., *N*-methyl-D-aspartate type glutamate receptor; 3-PGDH, 3-phosphoglycerate dehydrogenase; PSAT, phosphoserine aminotransferase; PSPH, phosphoserine phosphatase; SHMT, serine hydroxymethyltransferase: SNARE, soluble *N*-methylmaleimide susceptibility factor attachment protein receptor; SRR, serine racemase; THF, tetrahydrofolate.

2.5. Extracellular release (Fig. 5)

As predicted by characteristics of endogenous D-serine that are high affinity and selective ligand properties for the NMDA receptor glycine site and a NMDA receptor-like tissue distribution in the brain, D-serine has been demonstrated to be present in the extracellular fluid of the brain regions of a freely moving animal by using an *in vivo* microdialysis technique [22,66]. The extracellular concentrations, like tissue contents, show a strong correlation with the densities of the NMDA receptor (approximately 5×10^{-6} M in the frontal cortex one of the portions that contain the highest levels) [66]. For the investigations of the control mechanisms underlying the extracellular D-serine dynamics, *in vitro* methods including brain slice and homogenate, primary culture and cell line preparations, retina and the Xenopus oocytes expression system have been successfully applied as well.

The concentrations of brain extracellular D-serine appears to be under a distinct regulation from those of classical neurotransmitters based upon the data obtained from *in vivo* dialysis experiments. Thus, unlike glutamate, glycine and dopamine, the amount of D-serine in the frontal extracellular fluid was not increased, but rather decreased, by depolarization caused by veratrine or high concentrations of potassium ion [66]. Although a recent *in vivo* extracellular amino acid monitoring revealed a small and transient enhancement of D-serine levels in the striatum after a veratridine and high potassium application, this change is dissimilar to those observed in classical neurotransmitters in the magnitude and the feature followed by an immediate and lasting reduction [67]. Furthermore, the interruption of the nerve impulse flow or removal of extracellular calcium ions failed to reduce the extracellular D-serine levels [66]. The differential effects of the altered neuronal activity support the idea that not only neurons but also certain glial cells could participate in the regulation of the extracellular D-serine [66]. A reversible glia-selective toxin, fluorocitrate, indeed attenuated significantly the extracellular contents of D-serine with complete loss of extracellular glutamine that is derived from the glial cells but not neurons [68]. Furthermore, retinal release of D-serine has been found to be contingent upon glial cell activity [69].

In vitro studies using astrocytes [29,61,65] or neurons [55] in primary culture, C6 glioma cells [65,67] and retina [69] indicate that the extracellular release of D-serine is increased by glutamate, AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), or kainic acid whereas NMDA and kainic acid have been observed to down-regulate the D-serine release in an *in vivo* system [70]. Glutamate-induced D-serine release in cultured astrocytes and C6 cells depends on intra- and extracellular Ca^{2+} and SNARE (soluble *N*-methylmaleimide susceptibility factor attachment protein receptor) and is suppressed by an inhibitor of the uptake of amino acids into the synaptic vesicles; therefore, it is presumed to be mediated by the exocytotic processes of synaptic vesicle-like structures [65]. In the astrocyte in primary culture, AMPA-induced release of D-serine is attenuated by volume-regulated anion channel blockers [67]. It is also reported that addition of micromolar concentrations of L-alanine or L-serine induces robust release of D-serine from astrocytes, but not from neurons [71]. In this releasing effect, L-serine is more effective than kainite [71]. Together with the fact that the efflux of D-serine via ASCT2 by ASCT2 substrates can also be demonstrated using the *Xenopus laevis* oocyte expression system [72], these phenomena suggests that D-serine release from astrocytes may be mediated by amino acid heteroexchange catalyzed by the neutral amino acid transporter ASCT rather than stimulation of the non-NMDA receptor [71]. Retinal D-serine efflux could similarly be originated from the Müller glial cells through the ASCT2 because (a) AMPA-evoked D-serine release from the retina persisted in the presence of neural inhibitors, tetrodotoxin and cadmium chloride, but was abolished after application of a glial toxin, α-aminoadipic acid, [69] and (b) a retinal Müller cell line and primary cultures of mouse Müller cells express serine racemase and ASCT2 [72].

In primary neurons, D-serine release evoked by glutamate receptor agonists depends on extracellular Ca^{2+}, but it is not decreased by the removal of intracellular Ca^{2+} or a potent inhibitor of vesicular neurotransmitter uptake [55], and depolarization (veratridine)-evoked release of D-serine does not require internal or external Ca^{2+}, suggesting an unknown nonvesicular release mechanism in a cytosolic route [55]. Finally, a depolarizing stimulus, veratridine or high potassium, increases the release of D-serine in primary neurons, but not in primary astrocytes [55].

The above findings indicate that the molecular mechanisms of D-serine release differ between glial cells and neurons, and could be diverse in the respective cell types. The inconsistent results

between *in vivo* and *in vitro* studies may, at least in part, be due to their distinct conditions in the cellular interactions and neuronal circuits. Therefore, the exact molecular and cellular setups of the release machinery for D-serine await further elucidation. In this point, it is proposed that rat dsm-1 gene [73], an ortholog of human 3′-phosphoadenosine-5′-phosphosulphate transporter-1 gene and its protein product could be candidates for the members of the molecular cascade regulating intra- and extra-cellular contents of brain D-serine, because (a) dsm-1-expressing oocytes diminishes the sodium-dependent and -independent accumulation of D-serine and the basal levels of the intrinsic D-serine and increases the rate of release of the pre-loaded D-serine [73] and (b) dsm-1 mRNA has a D-serine-like distribution in the rat brain [73]. It is also noteworthy that, in the hippocampus, ephrinBs that are transmembrane proteins and known to regulate synaptic transmission and plasticity have been shown to regulate D-serine synthesis and release in astrocytes through activating EphB3 and EphA4 receptors that interact with PICK1 (protein interacting with C-kinase) and protein kinase Cα [74].

2.6. Receptor (Fig. 5)

D-Serine was initially described as a selective ligand for the strychnine-insensitive glycine binding site of the *N*-methyl-D-aspartate (NMDA) type glutamate receptor in the 1980s [19]. Because (a) D-serine mimic the agonistic effects of glycine on the NMDA receptor, (b) it displays a low affinity (high μM order) for the strychnine-sensitive inhibitory glycine receptor and a negligible binding capacity for the other glutamate receptors, and (c) its high binding affinity for and stimulating effects on the glycine site of the NMDA receptor are highly stereoselective, the D-amino acid has been used as an excellent tool for the functional study of the NMDA receptor in the mammalian central nervous system [19]. Cristal structural analysis has revealed that D-serine binds to the GRIN1 (NR1) subunit S1S2 ligand-binding core like other glycine site full and partial agonists and antagonists such as glycine, D-cycloserine and 5,7-dichlorokynurenic acid [75,76]. Further, using ligand binding assay, crystallographic analysis and all-atom molecular dynamics simulations, the GRIN3 (NR3) subunit that is a more recently identified class of the NMDA receptor subunits family composing a functional receptor with the GRIN1 subunit has been revealed to have the ligand biding domain for D-serine and glycine with higher affinity than GRIN1 [77]

The δ2 type glutamate receptor, which is predominantly expressed in the cerebellum, has recently been found to bind D-serine with K_d values of approximately 1 mM [78]. There are so far no other established receptor sites that have a relatively high affinity for D-serine. However, a NMDA glycine site antagonist-insensitive binding site that is detected in the brain synaptosomes and show the affinity for D-serine at high nanomolar range and brain-preferring distribution could compose a novel target molecule for D-serine [79].

2.7. Uptake (Fig. 5)

The substantial uptake of radioactive D-serine is observed in the homogenates of the rodent cerebral cortex and cerebellum [80,81], primary astrocytes and neurons obtained from the cerebral cortex of rodents [29,82], and C6 cells of rat glioma origin [83,84]. By contrast, there is a very low level of tritiated D-serine uptake activity in the homogenates of the peripheral tissues such as liver and kidney [80]. D-Serine was not transported into purified synaptic vesicles under conditions optimal for the uptake of known transmitters [55], and the pharmacological properties of uptake are different from those of previously reported transporters [55] [88], indicating the existence of a novel carrier that transports D-serine into cells in the central nervous system. Already-described molecules expressed in the brain, having the ability to take up D-serine at the μM order, include sodium-dependent neutral amino acid transporters ASCT1 and ASCT2 [72], the sodium-independent neutral amino acid transporter Asc-1 [85,86], and the proton-dependent amino acid transporter PAT1 [87]. Among these, Asc-1 [85,86] has the highest affinity for D-serine (IC_{50} of approximately 10–50 μM), is distributed widely in the brain, and occurs mainly in presynaptic nerve terminals. In addition, D-serine incorporation in the cerebral cortex and cerebellum of Asc-1 knockout mice is decreased to about 1/3 or below, suggesting the possible involvement of Asc-1 in physiological D-serine transport [86]. ASCT2 is immunohistochemically demonstrated in the dendrites of neurons of the cortex, hippocampus and striatum, but not in their cell bodies and astrocytes, in the brain sections [81]. This observation is supported by the data that low affinity (K_T > 1 mM) sodium-dependent D-serine and L-glutamine uptake characteristic of ASCT2-mediated transport was observed in the cortical P2 synaptosomal preparations [81]. In cortical neuron and astrocyte primary cultures, ASCT2-like uptake activity and immunoreactivity has also been found, noting the differential contribution of ASCT2 to the low affinity uptake of D-serine in physiological [81] and cultured astrocytes [82].

D-Serine uptake in an intact retina tissue, a Müller cell line or primary cultures of mouse Müller cells has been shown to be sodium-dependent and blocked by L-alanine, L-threonine, L-cysteine, L-serine, glutamine, or asparagine, but not anionic amino acids or cationic amino acids, suggesting that D-serine transport in Müller cells occurs via ASCT2 rather than ASCT1 or $ATB^{0,+}$ [72]. Indeed, RT-PCR assay confirmed the expression of ASCT2, but not $ATB^{0,+}$, mRNA in Müller cells, and immunoblotting and immunohistochemistry also detected ASCT2 in retinal sections and in primary cultures of mouse Müller cells [72].

2.8. Degradation (Fig. 5)

D-Amino acid oxidase (DAO) is an only mammalian enzyme that is proven *in vivo* to degrade D-amino acids containing D-serine [88]. In fact, a mutant mouse strain lacking DAO activity contains much higher contents in the brain of D-serine and D-alanine, which are selective substrates for DAO, than the corresponding normal mouse strain [27,89]. D-Serine deaminase [9], D-serine dehydratase [8] and serine rasemase [90] are also able to break down D-serine. However, absence of the two former enzymes in the mammalian tissues and no increase in the D-serine contents in the serine rasemase deficient mice [56,57,59] are argued against their physiolocal roles as D-serine degrading enzymes.

The distribution pattern of DAO gene and protein expression in the brain is inversely correlated with that of D-serine contents [91]. Brain DAO activity in the rodents is detectable and increased rapidly in the cerebellum, pons, and medulla oblongata around the postnatal days 10 [88] when the D-serine concentration starts to decrease in these brain areas [46]. Expression of the DAO gene is noted in cultured astrocytes from the rat cerebral cortex [92] although the activity and gene or protein expression of DAO in the forebrain are very low or undetectable. The D-serine content of mutant mice lacking this enzyme activity is minimally and dramatically increased in the cerebral cortex and the cerebellum, respectively [27,89]. Therefore, DAO is presumed to degrade D-serine at least in the hindbrain and it cannot be denied that the molecules other than DAO might be involved in the physiological elimination of the D-serine in the forebrain. It is also possible that DAO could play a significant role in forming a characteristic concentration gradient of D-serine in the brain [30].

2.9. D-Serine-responsive molecules

The genes or their protein products whose expressions are selectively induced by D-serine, but not by L-serine seem to be candidates for the molecules related to the receptor-intracellular signal transduction systems or metabolic pathways for D-serine. For instance, previously unreported transcripts that have a stereoselective response to D-serine, dsr-1 (D-serine-responsive transcript-1) [93] and dsr-2 [94] were isolated from the rat cerebral neocortex. A part of dsr-1 is homologous to the M9.2 gene encoding the proton ATPase subunit, which may be involved in the incorporation and release of D-serine [93]. dsr-2 [94] shows a similar distribution in the brain to those of D-serine and NR2B during postnatal development, and the gene has been mapped to the opposite strand of the neurexin-3α gene that has been indicated to be associated with NMDA receptor regulation, suggesting a functional correlation with D-serine or the GRIN2B (NR2B) subunit.

3. Physiological functions of D-serine

Pharmacological, molecular and cellular biological, electrophysiological, and histochemical analyses have suggested that, in the mammalian central nervous system, endogenous free D-serine plays a major role in the regulation of the NMDA type glutamate receptor and of glia–neuron interaction and may, at least in part, participate in the control over the δ2 type glutamate receptor and the neural development.

3.1. Regulation of the glutamate receptors

3.1.1. NMDA receptor

3.1.1.1. GRIN1/GRIN2 (NR1/NR2)-type NMDA receptors (Fig. 5). D-Serine selectively stimulates the glycine-binding site of the NMDA receptor, consisting of GRIN1 and GRIN2A-D subunits, to facilitate the following actions of glutamate via this receptor [19]: (a) depolarization, (b) inward electric current, (c) Ca^{2+} influx, (d) cGMP production, (e) release of various neurotransmitters, and (f) neuronal cell death. These actions are shared with glycine and D-alanine, characterized by stereospecificity that is barely seen in L-serine and L-alanine. The facilitating influence is considered to result from the increased frequency of channel opening, based on the phenomena that D-serine and other glycine site agonists augment the radiolabelled ligand binding to the phencyclidine regulatory site within the ionic channel [19].

Stimulating the glycine-binding site alone cannot produce an excitatory postsynaptic membrane potential; however, it is obligatory required for the generation of sufficient neurotransmission by glutamate. Therefore, the glycine site agonists such as glycine, D-serine, and D-alanine are referred to as coagonists of the NMDA receptor [19,30]. Together with the strong neuroanatomical correlates between D-serine and NMDA receptor, especially GRIN2B subunit, in the brain, the aforementioned characteristic actions of D-serine on the NMDA receptor suggest that D-serine in the brain is a physiological coagonist of the GRIN1/GRIN2 (NR1/NR2) type receptor [12,15,22,30]. In consonant with this idea, the selective elimination of endogenous D-serine by means of application of D-amino acid oxidase or D-serine deaminase without affecting the glycine contents in the brain slice, the primary neurons or the mixed primary culture system of glial cells and neurons causes a marked attenuation in the following functions of the GRIN1/GRIN2 type NMDA receptor *in vitro*: the nitric oxide synthetic-enzyme activation [95], the induction of NMDA receptor-dependent long-term potentiation (LTP) [96], and the NMDA-elicited excitotoxicity [55]. It is also well accepted that the NMDA type glutamate receptors play pivotal roles in the control of the neural plasticity, learning and memory, behavioral responses, neuronal cell death *in vivo* at the individual levels. The facts that the various serine racemase deficiency mice displaying a marked loss of D-serine contents in the brain show the disturbances or changes in the above NMDA receptor-mediated functions such as spatial memory [57,59], memory for order [58] and NMDA neurotoxicity [56] add a further support the physiological coagonist actions of D-serine on the NMDA receptor.

The extracellular contents of D-serine and another intrinsic NMDA receptor coagonist glycine exceed those fully activating the glycine site of the NMDA receptor. However, the glycine site may not always be saturated under physiological conditions [97] because the NMDA receptor currents in the rat prefrontal area are enhanced when a selective inhibitor of the glial glycine transporter GLYT1 (N [3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl]sarcosine (NFPS)), which elevate the synaptic glycine levels, or D-serine (50–100 mg/kg iv) is administered systemically [97].

Then, the important question arises as to the possible differences in the physiological roles of D-serine and glycine as the coagonists for the NMDA receptor (Table 1). It can be presumed from the divergent distribution patterns of D-serine, glycine and four types of NR2 subunits that D-serine and glycine could have the distinct modes of actions to the four sorts of heteromeric NMDA receptors comprising GRIN1 and any one of GRIN2A to GRIN2D subunit. In each type of the heteromeric receptors expressed on the Xenopus oocyte, D-serine exhibits a several-fold stronger reinforcing effect on the glutamate-induced inward current than glycine [98]. Although there is no obvious discriminations in the influences of each amino acid on the four kinds of the heteromeric receptors [98], the differential regulations of extracellular signals between these two amino acids are pointed out by the *in vivo* dialysis experiments showing that a glia toxin, fluorocitrate [68], diminishes the frontal extracellular contents of D-serine with an increase in those of glycine, and D-cycloserine [99] produced an elevation and no alteration in the frontal D-serine and glycine contents in the dialysates, respectively. To clarify the exact cellular and molecular mechanisms underlying the release of D-serine and glycine would provide a critical clue for the biological significance of the dual coagonists for the NMDA receptor.

3.1.1.2. GRIN1/GRIN3-type NMDA receptors. GRIN3 subunits of the glutamate receptors are the lease characterized members of the NMDA receptor gene family and found to show the distinct ligand responses from GRIN2 subunits [100]. Neuroanatomical analyses have revealed in the rat that expression of the GRIN3A subunit is widely observed through the brain and spinal cord with peaking around early postnatal stage while GRIN3B is expressed predominantly in motor neurons in the brainstem and spinal cord.

Unlike GRIN1/GRIN2-type NMDA receptors, the GRIN1/GRIN3A or GRIN1/GRIN3B heteromeric NMDA receptors are excited by glycine, but not glutamate or NMDA (inward electric current) [100] in the Xenopus oocytes expression system or cerebral cortical neurons. An antagonist to the glutamate-binding site or PCP-binding site does not affect this glycine-induced electric current; however, it is suppressed by a selective agonist and antagonist for the glycine-binding site, D-serine and 5,7-dichlorokynurenic acid, respectively [100]. In contrast to glycine, D-serine alone has been reported to fail to or partially activate the GRIN1/GRIN3A or GRIN1/GRIN3B-type NMDA receptors expressed in the Xenopus oocytes or the HEK 293 cells [100]. Further, D-serine as well as glycine, in the absence of glutamate agonists induce excitatory responses in a NMDA glycine site antagonist-reversible and a glutamate-site antagonist-insensitive fashion in optic nerve myelin, and no longer produce this response in GRIN3A-deficient mice [101]. These data are consistent with

Table 1
Biological characteristics of D-serine and glycine in mammalian nervous systems.

	D-Serine	Glycine
Binding selectivity		
GRIN1/GRIN2 NR-associated glycine site	High affinity	High affinity
Inhibitory glycine receptor	Low affinity	High affinity
Effects on GRIN1/GRIN2 NR receptor	Facilitatory	Facilitatory
Effects on GRIN1/GRIN3 NR receptor	Inhibitory, excitatory or no effect	Excitatory
Effects on δ2 glutamate receptor	Inactivation	Inactivation
Distribution: adult period	Brain-enriched	Ubiquitous in CNS and periphery
Adult brain	Forebrain-dominant and GRIN2B-related	Hindbrain-preferential and NR-unrelated
Postnatal development	GRIN2B-related	NR-unrelated
Immunoreactivity	Neuron < glia	Neuron > glia
Synthesis	Serine racemase	Serine hydroxymethyl transferase
Precursor	L-Serine	L-Serine
Effects of L-serine synthesis deficit[a]	Marked loss	Little or moderate reduction
Extracellular release	Depolarization-induced increase (−)	Depolarization-induced increase (+)
Uptake	Na-dependent and -independent	Na-dependent
Transporter	Asc-1, ASCT2	GLYT1, GLYT2
Degradation	D-amino acid oxidase	Glycine cleavage system
Effects of depletion on NR	NR hypofunction	?
Roles in neurotransmission	Neuromodulator	Inhibitory transmitter/neuromodulator

CNS, central nervous system; GLYT, glycine transporter; NR, NMDA receptor; NR-associated glycine site, strychnine-insensitive glycine receptor (cf. inhibitory glycine receptor, strychnine-sensitive glycine receptor).
[a] Mice lacking 3-PGDH (3-phosphoglycerate dehydrogenase).

the view that D-serine is a candidate endogenous regulator of the GRIN1/GRIN3-type NMDA receptor but cannot be a coagonist like for the GRIN1/GRIN2-type.

3.1.2. δ2 glutamate receptor

Orphan receptor δ2 expressed mainly in Purkinje cells of the cerebellum is classified in the ionotropic glutamate receptor family based on its amino acid sequence, and is reported to be involved in the formation of long-term depression (LTD), motor learning, motor coordination, and synaptogenesis [78]. However, it does not form a glutamate-gated channel and its endogenous ligand is not known [78]. By the experiments using the mutated δ2 receptors at the ligand-binding core [102,103], D-serine and glycine have been recognized as potential endogenous regulators because they were demonstrated to bind to the δ2 receptor to cause the conformational changes, thereby inactivating the receptor with ED50 values, 182 and 507 μM, respectively. It is also suggested that the conformational modifications by D-serine and extracellular Ca^{2+} may be conserved between δ1 and δ2 glutamate receptors [104]. Because D-serine is concentrated in the Bergman glia cells in the cerebellum, and because its postnatal cerebellar concentrations are high and low before and after early postnatal days, respectively, the possible functional link between the glial D-serine and δ2 receptors during development has been an important issue to be solved. Very recently, this question has been answered by the observations that D-serine derived from the Bergman glia serves as an endogenous ligand for glutamate δ2 receptors to regulate LTD at synapses between parallel fibers and Purkinje cells in the immature (postnatal days 11–17), but not mature (older than postnatal days 30) cerebellum [105].

3.2. Glia–neuron interaction

Several lines of evidence have been accumulated indicating that D-serine may be involved in the communication between glutamate synapse and glial cells. Firstly, D-serine has a NMDA receptor-like distribution and is noted immunocytochemically in the neurons and glial cells including astrocytes, microglia and oligodendrocytes in the mammalian brains [29,36,37,48] and retinas [40,72]. The contact between the neurons and astrocytes seems to be essential for preserving the cellular D-serine signals, because the D-serine contents are significantly reduced in the cultured astrocytes in the absence of neurons as compared to those mixed with neurons [96]. Moreover, the extracellular D-serine concentrations are altered by modifications of not only neuronal but glial cell activities [66,67]. The D-serine-mediated synapse–glia interaction has indeed been reported in the hypothalamus [106] and hippocampus [107].

In the nucleus supraopticus hypothalami of female rats [106], the degree of astrocytic coverage of neurons governs the level of the NMDA receptor glycine site occupancy by D-serine, thereby affecting the activity dependence of long-term synaptic changes, LTP and LTD. The astrocytes in the hypothalamic area that display the D-serine-like immunoreactivity surround glutamate synapses tightly and loosely during the virgin and lactating period, respectively, in the female rats [106]. In the former period, the increased D-serine release has been found to diminish the threshold for the NMDA receptor-mediated induction of LTP and LTD, and vice versa in the latter period, in the hypothalamic slices with the selective absence of endogenous D-serine, but not glycine, by D-amino acid oxidase application [106].

Furthermore, NMDA receptor-dependent LTP in hippocampal area CA1 is blocked by clamping internal Ca^{2+} in individual CA1 astrocytes in the vicinity of which there is a reduction in the occupancy of the NMDA receptor-glycine sites [107]. This blockade can be reversed by exogenous D-serine or glycine, and mimicked by a selective antagonist for the glycine site and depletion of D-serine by high frequent astrocyte stimulation in combination with a serine racemase inhibitor or disruption of exocytosis by the light-chain tetanus toxin in an individual astrocyte [107]. Ca^{2+}-dependent release of D-serine from an astrocyte, therefore, might regulate NMDA receptor-mediated neuroplasticity in a group of hippocampal excitatory synapses [107].

D-Serine biosynthesis appears to occur predominantly in the neurons in the brain because the immunoreactivity [54] and mRNA [48] of the putative D-serine synthesizing enzyme, serine racemase, has mainly been detected in the neurons although the earlier histochemical studies described the exclusive distribution of serine racemase-like immunoreactivity in astrocytes of the rat brain [108]. In contrast, the synthetic pathway for a major D-serine precursor, L-serine, has been demonstrated in the astrocytes [60]. Together with the foregoing glial origin of extracellular D-serine, these observations indicate that the transfer systems of D-serine and/or its precursor between neurons and glia appear to be present in the brain.

3.3. Neural circuit formation

Because disruption of serine racemase gene that causes a marked loss of brain D-serine leads to alterations in cortical dendritic morphology in the mouse [58], D-serine could directly or indirectly participate in the wiring of certain neuron circuits in mammalian cortical tissues. In the cerebellum, the D-serine concentrations are very low in the adult period but increase transiently to the comparable levels in the adult cerebral cortex around postnatal week 1–2 [46]. This increase is prominent in the Bergmann glia (radial glial cells) that extend their protrusions around the granule cells during the NMDA receptor-mediated migration and synaptic formation of the granule cells [109]. The migration of granule cells is suppressed by the selective degradation of D-serine or inhibition of serine racemase while D-serine rescues this suppression [61]. Further, GRIP (glutamate receptor interacting protein) has been reported to augment serine racemase and D-serine release by binding serine racemase and the AMPA glutamate receptor, and overexpression of GRIP by means of its adenoviral infection in neonatal mouse cerebellum facilitates the above cell migration process [61]. These phenomena suggest an important role of D-serine in neural circuit formation of the developing cerebellum [61].

4. Pathophysiology of neuropsychiatric disorders and D-serine dysfunction

The characteristic metabolism, functions, and developmental changes of mammalian endogenous D-serine suggest the presence of a unique cellular and molecular mechanism, also called the D-serine system, in the central nervous system. The requirement of D-serine in physiological activation of the NMDA receptor indicates that the D-amino acid may play a pivotal role of in the expression and regulation of various higher brain functions related to the major glutamate receptor, and that abnormalities of the D-serine system may be implicated in the pathophysiology of various neuropsychiatric disorders.

4.1. Schizophrenia

As described at the beginning of this review, it has now been widely accepted that the reduced neurotransmission via the NMDA receptor may be involved in the pathophysiology of schizophrenia. This so-called "glutamate hypothesis" is chiefly based upon the following pharmacological observations: (a) antagonists for the NMDA receptor represented by phencyclidine (1(1-phenylcyclohexyl)-piperidine: PCP) and a chemically PCP-related and non-competitive antagonist for the NMDA receptor, ketamine, produce positive and negative symptoms and cognitive disturbances which are indistinguishable from those of schizophrenia [110], (b) a group of schizophrenic patients have been reported to be more sensitive to PCP and ketamine in their psychotomimetic actions than healthy controls because the schizophrenic patients in remission suffer exacerbation of their psychotic symptoms by the challenge doses of these drugs that fail to cause apparent schizophrenia-like symptoms in the controls [110–112], and (c) the psychotomimetic effects of the ketamine stereoisomers are closely correlated with their affinities for the NMDA receptor [113,114]. Because the NMDA receptor hypofunction have been shown to lead to the hyperdopaminergic activity in the brain in experimental animals [13,15,115,116] and humans [117,118], the "glutamate hypothesis" is not conflict with the "dopmanine (DA) hypothesis" of schizophrenia in that the excessive dopaminergic transmission following the reduced NMDA receptor function would produce positive symptoms of schizophrenia in a potent dopamine receptor antagonist antipsychotic-sensitive manner [13,15].

In consistent with these data, the facilitation of the NMDA receptor function by its glycine site agonists including glycine, D-serine, D-alanine, D-cycloserine and a glycine transporter inhibitor have been reported not only to improve conventional animal models of schizophrenia that are abnormal behaviors induced by NMDA receptor antagonists and DA agonists, but also to attenuate an NMDA antagonist-induced enhancement of DA transmission in the brain. Furthermore, these NMDA coagonists in combination with various antipsychotics except clozapine have been shown to produce a significant amelioration in currently used antipsychotic-resistant negative symptoms and/or cognitive dysfunctions of schizophrenic patients [119] when compared with conventional antipsychotics alone [119]. It is also worthwhile to note that D-alanine suppresses enhanced dopamine transmission in the frontal cortex of the animals acutely injected with a schizophrennomimetic NMDA receptor blocker [115], and that the repeated co-administration of glycine with PCP eliminates the augmented DA release in response to a challenge dose of amphetamine in the frontal cortex or striatum of the animals repeatedly treated with PCP alone [116]. The hyper-responsiveness of DA neurotransmission to amphetamines has also been observed in a group of schizophrenic patients [120,121], acutely ketamine-administered healthy volunteers [122] and an animal methamphetamine model of schizophrenia [123]. These findings agree with the ideas that the stimulation of the NMDA glycine site is expected to have a therapeutic efficacy on the antipsychotic-resistant symptoms of schizophrenia and that overall abnormalities, including positive symptoms, may be improved with a coagonist alone without the concomitant use of an antipsychotic drug.

The causative mechanisms whereby the NMDA receptor-mediated glutamate transmission is diminished in schizophrenic brains have intensively been investigated from a wide variety of aspects of the regulatory systems of the glutamate receptor. These standpoints contain the possibility that the insufficient D-serine signals could result in the hypofunction of the NMDA receptor because D-serine is an intrinsic coagonist for the glutamate receptor. In fact, the mice lacking serine racemase activity displayed behavioral abnormalities relevant to schizophrenia, including hyperactivity [57] and impairments in prepulse inhibition and sociability, the latter of which were shown to be exacerbated by an NMDA receptor antagonist and ameliorated by D-serine or the atypical antipsychotic clozapine [59].

There are so far no studies to show statistically significant differences in the D-serine contents in the postmortem brain tissues between the patients with schizophrenia and without neuropsychiatric diseases [26,124]. However, an increase in the densities of the glycine-regulatory sites of the NMDA receptor, which are the binding targets for endogenous D-serine, was observed in cerebral neocortical areas of the postmortem schizophrenic brains, such as the angular convolution, supramarginal gyrus, somatosensory area, and premotor area [125]. This increase could be considered as a compensatory change due to the decreased extracellular release of D-serine or glycine in a specific neural circuit. The decreased *in vivo* radioligand binding to the PCP site within the ion channel of the NMDA receptor of the hippocampus of schizophrenic patients could be a consequence of the reduced open frequency of the ion channel due to diminution of D-serine or glycine signaling [126]. Moreover, significant association between schizophrenia and single nucleotide polymorphisms (SNPs) and/or haplotypes of DAO [127] and serine racemase [128,129], and the expressional changes in the protein products or mRNAs of these genes in the postmortem brains of patients with schizophrenia, have been reported [130–133], although other studies fail to replicate these data [134–136].

Interestingly, a primate-specific gene encoding a candidate for a DAO modulator, G72, was isolated from chromosome 13q34 that

has been genetically linked to schizophrenia, and has been found to be associated with schizophrenia [127]. The genetic association was verified by several meta-analyses [137]. Because G72 was originally reported to be an interacting partner and an activator of DAO (DAOA/G72) expressed in the human brain [127] and because the possible physiological interaction has been supported by the observations that DAO- and G72-like immunoreactivity are both detected in astrocytes of the human cortex [138], an anomalous overexpression of DAOA/G72 that might reduce the brain D-serine contents by increasing activity of DAO could have a relation to schizophrenia susceptibility. However, this hypothesis is still debatable in terms of the subsequent studies that suggest alternative roles of G72 in the nervous system such as an inhibitor of DAO [138] and a regulator of mitochondrial functions [139].

Some studies pointed out a decrease in D-serine contents and the ratios of D-serine to the total serine concentrations in the blood and cerebrospinal fluid [124,134,140,141]. Further evaluation, however, is needed to clarify the significance of these results because (a) the results of the quantitative analysis of D-serine in the blood and cerebrospinal fluid of schizophrenic patients are controversial [142–144] and (b) the effects of a variety of factors that may influence the blood D-serine concentrations such as the medication, D-serine-containing foods and diurnal variation of D-serine metabolism cannot be excluded in these studies.

4.2. Bipolar disorder (Manic depressive psychosis)

Relationships with the SNPs and haplotypes of DAOA were noted not only in schizophrenia but also bipolar disorder; the changes in the D-serine system may occur in both psychiatric disorders [136]. This finding suggests the possibility that polymorphism of DAOA relates to common symptoms, such as agitation and delusion, but not to the disease. However, the association of G72 with bipolar disorder is still debatable [137], therefore requiring further investigation.

4.3. Anxiety

Anxiety-related actions emerge in animals receiving a local injection of D-serine in the dorsal periaqueductal gray matter (DPAG) [145]. This effect is mimicked by glycine in a NMDA receptor glycine site antagonist-reversible fashion and microinjections of these amino acids outside the DPAG, or of L-serine inside the DPAG, produced neither of these pro-anxiety effects [145]. These observations suggest that D-serine and/or glycine in specific brain regions may be associated with the manifestation of anxiety [145], and are in line with the results that mutant mice with reduced NMDA-GRIN1 glycine affinity or lack of DAO function exhibit an anxiolytic-like or anxiogenic-like behaviors, respectively [146]. Because DAO deficient mice show an increase in D-serine contents without affecting glycine levels in the brain [89], endogenous D-serine, but not glycine, could participate in the neural mechanisms underlying anxiogenic effects induced by the stimulation of the NMDA glycine site.

4.4. Non-ketotic hyperglycinemia: glycine cleavage enzyme deficiency

In the postmortem cerebral cortical tissues of patients with nonketotic hyperglycinemia, in which the glycine level in the blood is increased markedly because of lack or marked loss of the activity of the glycine cleavage enzyme system, D-serine and glycine concentrations have been found to be decreased to about one-third and increased up to approximately 7 times as compared with those in the patients without neuropsychiatric disorders as the control group, respectively, but no statistically significant changes in the contents of L-serine are observed [63]. The D-serine concentration of the rat brain is decreased after the administration of cysteamine with an inhibitory action on the glycine cleavage enzyme system [63], while it is increased by the administration of a large amount of glycine [50]; therefore, the above decrease is considered to result from inhibited activity of the glycine cleavage enzyme system, but not due to the secondary effects of the increased glycine levels or of the possible marked loss of L-serine that is supposed to be a major precursor for D-serine, and may be relevant to the various central nervous symptoms observed in the disease, such as mental retardation, convulsive seizure, apneic events, and lethargy. These data indicate that D-serine could have a synthetic pathway unrelated to serine racemase but related to the glycine cleavage enzyme system and/or its associating molecules.

4.5. Serine deficiency syndrome

Cases with severe nervous system disturbances, such microcephaly, convulsive seizure, and psychomotor retardation, are occasionally noted along with a drastic decrease in the L-serine concentrations of the blood and cerebrospinal fluid, which is called serine deficiency syndrome [147]. L-Serine replacement therapy is effective; it has been demonstrated that the activity of 3-phosphoglycerate dehydrogenase (3-PGDH), 3-phosphoserine aminotransferase (3-PSAT) or 3-phosphoserine phosphatase (3-PSP) of the L-serine biosynthesis system is lacking in some serine deficient syndrome cases while no apparent abnormality can be detected in the known L-serine synthetic enzyme in other cases [147]. The D-serine concentration is decreased markedly in the cerebrospinal fluids of patients with this syndrome, suggesting an involvement of lowered D-serine signals in the central nervous system with neuropsychiatric symptoms [147]. These findings suggest that D-serine of the human central nervous system may be mainly derived from L-serine, agreeing with the dramatic reduction in the brain contents of D-serine as well as L-serine in the mice lacking 3-PGDH gene (see Section 2.3) [60].

4.6. Cerebral ischemia

The extracellular D-serine concentration is increased in the rabbit piriform cortex in transient ischemia [148], and ischema-induced neuronal cell death is suppressed in rat hippocampus slices when their D-serine levels are decreased selectively by DAO treatment in the presence of the normal contents of glycine, another coagonist of the NMDA receptor [149,150]. In the rat cerebrocortical slice cultures, sensitivity of NMDA cytotoxicity to an NMDA receptor glycine site antagonist, 5,7-dichlorokynurenic acid, was diminished by L-serine that increased the extracellular levels of D-serine [151]. These observations are consistent with the view that potentiation of the D-serine signal is involved in the neuronal damage caused by overstimulation of the NMDA receptor in cerebrovascular disorders. In support of this hypothesis, a marked reduction of brain D-serine contents led to a decreased neurotoxicity induced by NMDA injections into the right parietal cortex [56] and a diminution in infarct volume following middle cerebral artery occlusion in the serine racemase deficient mice [152].

4.7. Alzheimer's disease

In Alzheimer's disease, the possible involvement of D-serine in the neurodegeneration related to the excessively accumulated amyloid β peptide (Aβ) and NMDA receptor has been examined. Aβ stimulated the release of D-serine as well as glutamate from microglia [153,154] and enhanced the transcription of serine racemase mRNA in microglia *in vitro* [153]. The conditioned medium obtained from the primary microglia treated with Aβ markedly

reduced the viability of the primary hippocampal neuron cultures in a NMDA glycine site antagonist or DAO treatment-reversible manner [153]. Moreover, there was an increased expression of serine racemase mRNA in the postmortem hippocampus of the patients with Alzheimer's disease [153]. These observations seem to be consistent with the idea that the increased synthesis and extracellular release of D-serine by Aβ may promote neuronal cell death [153]. Indeed, serine racemase knock-out mice with an approximately 90% decrease in forebrain D-serine contents have been found to display a reduced neurotoxicity induced by Aβ (1–42)-peptide injections into the right hippocampus [56]. However, no significant change in the tissue concentrations of D-serine has so far been reported in the postmortem brains of patients with Alzheimer's disease [26,28].

4.8. Amyotrophic lateral sclerosis

The death of motor neurons in amyotrophic lateral sclerosis (ALS) has been shown to, at least in part, connect to the neuronal toxicity of glia-derived factors including excitatory amino acids. Sasabe et al. [38] recently found in ALS model mice (transgenic mice expressing a high copy number of mutant human superoxide dismutase 1 (SOD1) with a Gly-93-Ala substitution (G93A-SOD1)) that the spinal cord motor neurons, but not astrocytes and microglia, were more susceptible to NMDA toxicity than those of a control group, and that D-serine contents and serine racemase activities in the spinal cord were augmented with the progression of the pathology. In MG5 microglial cells, serine racemase expression was enhanced by transient and enforced expression of G93A-SOD1 [38]. Elevated D-serine-like immunoreactivity in the spinal cords of patients with familial or sporadic ALS were observed, although these data are preliminary due to the limited number of the tissues examined [38]. These results have led to the proposal of a mechanism whereby the overproduction of D-serine in glial cells damages the motor neurons of ALS patients [38].

The possible disturbances in D-serine metabolism has also been supported by the molecular genetic study indicating that familial ALS was associated with a mutation in D-amino acid oxidase and expression of this mutation in neuronal cell lines, primary motor neurons or astrocytes cocultured with motor neurons reduced viability of these neuronal cells [155]. The pathophysiological implication of D-serine in ALS therefore seems to be valuable for further evaluation.

4.9. Cerebellar ataxia

Competitive and non-competitive antagonists for the NMDA receptor, combined gene disruption of the NMDA receptor GRIN2A and GRIN2C subunits [156] and genetic deletion of δ2 glutamate receptor [157] have been demonstrated to produce ataxic movements related to the cerebellum in experimental animals. In humans, PCP-induced motor disturbances are similar to those in the patients with cerebellar ataxia [112]. The ability of a NMDA receptor antagonist, dizocilpine, to induce cerebellar ataxic movements has recently been shown to be attenuated in the mice lacking DAO activity with increased cerebellar D-serine contents [158]. These observations suggest that diminution in NMDA receptor function and/or D-serine signal may also be involved in the pathophysiology of the cerebellar ataxia, whereas there are no studies on the D-serine contents or D-serine- or NMDA receptor-related molecules in the postmortem brain tissues from patients showing cerebellar ataxia.

The plausible disturbances appear to be consonant with the amelioration of the abnormal movements seen in some kinds of the model mice of cerebellar ataxia by D-serine and D-cycloserine, which is a partial agonist for the glycine site of the NMDA receptor [159]. D-Cycloserine has also been reported to attenuate the scores of the International Cooperative Ataxia Rating Scale (ICARS) in a group of the patients with spino-cerebellar degeneration [160].

4.10. Neuropathic pain

Several lines of evidence suggest that endogenous D-serine may play an important role in the mechanisms of central sensitization in NMDA receptor-related neuropathic pain. Thus, (a) D-serine antagonizes the analgesic action of gabapentin and S(+)-3-butyl GABA (γ-aminobutyric acid) against pain produced by the stimuli of heat and formalin [161]; (b) the potentiation of the nociceptive reaction to and the NMDA receptor-mediated excitatory synaptic current by formalin-induced pain is noted in DAO activity-deficient mice [162]; and (c) a selective reduction of D-serine signals in the cingulate cortex by a local injection of a D-serine degrading enzyme DAO or a selective antagonist for the NMDA receptor glycine site suppresses the formation of avoidance behavior in response to the formalin-induced pain [163].

5. Relevance of D-serine system as a target for the development of the NMDA receptor tuning therapy for neuropsychiatric disorders

Based upon growing evidence that NMDA receptor dysfunction may be implicated in the pathophysiology of a variety of neuropsychiatric disorders, the development of a novel class of drugs that tune the glutamate receptor-mediated transmission has been attempted. Also, clinically approved therapeutic agents or the substances used as food additives with some action at the NMDA receptor are applied in the clinical trials for the treatment to the intractable neurologic or psychiatric disturbances or symptoms that are mimicked by the NMDA receptor agonists or antagonists.

Experimental results whereby an NMDA receptor antagonist attenuated neuronal cell death (see Section 4.6) [164,165] and neuropathic pain (see Section 4.10) [166] elicited by cerebral ischemia and an excitatory amino acid were consistently reported. Diminution of the NMDA receptor activity has also been considered to delay or protect the cell dysfunction and loss occurring in neurodegenerative diseases. For acceleration of the NMDA receptor, agonists for its glycine site have been chosen because direct agonists for its excitatory amino acid site, but not the glycine site, have been shown to often produce cell over-excitation or death in the nervous system or convulsion (see Sections 1 and 4.1). These allosteric agonists or coagonists for the NMDA receptor have been demonstrated to improve positive and negative symptoms and cognitive deficits of schizophrenia, and ataxic movements of the patients with spino-cerebellar degeneration (see Sections 4.1 and 4.9).

Recent animal experiments have further been extending the application of the substances acting at the coagonist site of the NMDA receptor for elimination of ill memories in various psychiatric disorders. It has well been established that conditioned fear or drug seeking behavior is eradicated by being replaced by subsequent learning of harmless or alternative cues. This extinction phenomena have been applied as the behavioral therapies to the treatment of phobias, obsessive-compulsive disorder, post-traumatic stress disorder (PTSD) or drug abuse, and have newly been shown to be facilitated by the NMDA receptor glycine site agonists in their animal models [167–172]. In several randomized placebo-controlled clinical trials for phobia [173], social anxiety disorder [174], obsessive-compulsive disorder [175], PTSD [176] or panic disorder [126], low dose of D-cycloserine, a partial agonist for the glycine site of the NMDA receptor, in combination with a conventional cognitive behavioral therapy (CBT) has been reported to be more effective in ameliorating fear or anxiety compared with CBT alone. These experimental and clinical observations suggest

that the enhanced NMDA receptor function may accelerate acquisition and overwriting new memories that lead to extinction of previous harmful memories.

However, modulation of the NMDA receptor functions has not yet been successfully achieved. In clinical trials of prophylactic medication for cerebral ischemia, the competitive and non-competitive NMDA receptor antagonists induced psychotic symptoms [164,165]. Moreover, current clinically applicable coagonists of the NMDA receptor have problems such as: (a) the low BBB permeability that requires a large dose to obtain a sufficient brain content and therapeutic efficacy (e.g., glycine, D-serine, D-alanine, and glycine transporter inhibitors) [30,119], (b) low selectivity for the NMDA receptor due to another potent action at the inhibitory glycine receptor (e.g., glycine and glycine transporter inhibitors) [19], (c) difficulty in dose selection due to a narrow range of therapeutic dosage because of a partial agonist property (i.e., D-cycloserine) [30,119], and (d) nephrotoxicity (i.e., D-serine) [177,178]. Finally, synthesizing a glycine site agonist that specifically binds to the glycine regulatory site with the high BBB permeability has long remained unsuccessful.

To avoid adverse effects by an excessive change in the activity of the NMDA receptor or by toxicity due to large doses from the aforementioned problems, manipulation of the endogenous D-serine signal in the brain seems to have some advantages for the fine-tuning of the NMDA receptor, because an incomplete loss of NMDA receptor currents under the depletion of D-serine by D-amino acid oxidase suggests that the moderate alterations of the NMDA receptor activity could be achieved by an indirect regulation of the NMDA glycine site, and because NMDA receptor-like distribution of endogenous D-serine may lead to the selective effects of the D-serine signal operation on the NMDA receptor. Alternatively, relatively hind brain-selective increase in D-serine contents might be effective on the cerebellar ataxia associated with NMDA receptor hypofunction, and performed by inhibition of D-amino acid oxidase, the distribution of which is inversely related to that of D-serine. Therefore, the molecules specifically participating in the different stages of D-serine metabolism may be suitable targets for the development of a novel class of the excellent therapeutic agents controlling the NMDA receptor functioning. Facilitation of D-serine synthesizing or release machinery members and blockade of transporters and degrading enzymes of D-serine would enhance NMDA receptor-mediated transmission, and vice versa. For this purpose, exact molecular and cellular mechanisms underlying each metabolic step and their localization should further be clarified.

6. Conclusion

In conclusion, a body of evidence has been accumulated indicating that endogenous D-serine differs in many ways, besides simply being a D-amino acid, from other signaling substances in the nervous system examined thus far. First, a peculiar mechanism is required to maintain the extracellular concentration of D-serine within an adequate range, in turn allowing D-serine to play a role as a coagonist for the GRIN1/GRIN1-type NMDA receptors by forming the tonic signaling in the synapse. By contrast, classical neurotransmitters exhibit their nerve impulse-dependent extracellular release and rapid elimination in the synaptic clefts to generate a phasic signaling. The particular type of regulatory system for D-serine remains to be fully elucidated. Furthermore, the exact differences in the physiological roles of glycine and D-serine as ligands at the NMDA receptor glycine site are still unclear. In addition, it is strongly suggested that, unlike most of classical neurotransmitters, D-serine is widely present in both glial cells and neurons and indispensable for their interaction. Certain D-serine-releasing glial cells could function as a fine-tuning station for the excitatory synapse in the nervous system. Such features reveal a host of differences between D-serine and glycine (Table 1), and these differences may not only substantially complicate the elucidation of the metabolism and dynamics of D-serine but also contribute to the elegant control of the NMDA receptor and related molecular cascades that are presumed to be dysfunctional in the various neuropsychiatric symptoms. Therefore, further elucidation of the cellular and molecular mechanisms underlying the metabolism and functions of D-serine in the brain will provide a clue as to unknown information processing systems that regulate higher brain functions, and is expected to markedly advance our understanding of the pathogenesis and pathophysiology of neuropsychiatric disorders: it is hoped that this series of investigations will facilitate the development of novel therapies for these disorders. Furthermore, additional D-serine research will shed light on the biological significance of D-serine in the molecules other than the NMDA receptor and the extra-brain organs, and its differences from glycine.

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No.12

統合失調症の病態メカニズム

上里彰仁
Akihito UEZATO
東京医科歯科大学大学院精神行動医科学分野助教

西川 徹
Toru NISHIKAWA
東京医科歯科大学大学院精神行動医科学分野教授

1 はじめに

統合失調症は世界人口の約0.8%に発症し，その多彩な症状によって苦悩するのみならず，社会的機能の障害による本人と社会の不利益が大きい疾患である．この疾患の克服は，多くの患者そして社会に多大な利益をもたらすと考えられ，その病態解明と治療法の開発が急務となっている．

本セミナーでは，現在までに考えられている統合失調症の病態メカニズムを紹介する．

2 統合失調症の症状

統合失調症の症状は陽性症状，陰性症状，および認知機能障害に分類される．陽性症状は正常な精神活動では本来現れないもの，すなわち幻覚・妄想などを示す．逆に陰性症状は正常な精神活動から欠落したもの，すなわち感情鈍磨・快楽消失・発動性低下などを示す．また認知機能障害は記憶力，注意・集中力，計画・判断・実行力などの障害を示し，患者の社会的機能を低下させる主な要因である．その意味では，認知機能障害は陰性症状の一部とも考えられる．人間の精神活動はいうまでもなく大脳により営まれているが，病的な精神症状は，何らかの理由で正常な大脳機能が破綻したことにより発現する．大脳機能は神経細胞およびグリア細胞を中心とする複雑なネットワークの躍動的な総体であるが，そのネットワークの担い手はドパミン，セロトニン，グルタミン酸，γ-アミノ酪酸(GABA)などの神経伝達物質であり，現在の統合失調症の病態研究はこれらの神経伝達物質に関する遺伝学的・分子生物学的アプローチを主体としている．

3 ドパミンと統合失調症

統合失調症における最も重要な病態仮説は，精神症状は脳内のドパミン神経伝達が過剰であることにより生じるというドパミン仮説である．[1,2] この仮説は，次のような複数の事実を根拠とする．すなわち，アンフェタミン，レボドパ，メチルフェニデートなどのドパミン神経伝達を賦活する薬剤は，健康な人に対して統合失調症の陽性症状に似た症状，特に被害妄想を引き起こす．さらに，統合失調症患者に対して投与すると症状を増悪させる．また逆に，ドパミン受容体を遮断する薬剤は統合失調症の症状を改善する．実際に現在の統合失調症の薬物治療の主流は，ドパミン受容体遮断薬である．

それでは，統合失調症では脳のどの部位でドパミンが過剰となっているのであろうか．中脳の黒質緻密部や腹側被蓋野をそれぞれ起始核とするドパミン神経の投射先は線条体および側坐核であるが，PET(positron emission tomography)を用いた画像研究では，これらの領域におけるドパミン代謝の増加が示されている．また，線条体・側坐核におけるドパミンD_2受容体の密度が増加していることが報告されている．さらに抗精神病薬は線条体のドパミンD_2受容体を遮断し，その占有率は統合失調症の陽性症状と相関がある．このようなことから，統合失調症の陽性症状は，これらの領域におけるドパミン伝達の過剰と関連していると考えられている．

一方で，陰性症状および認知機能障害は，中脳から前頭前野に投射するドパミン神経のD_1受容体を介した活動低下に起因するという考え方がある．統合失調症では，前頭葉の相対的血流低下がSPECT (single photon emission computed tomography)

などの画像研究により観察されており，これを"hypofrontality"と呼んでいる．前頭葉はヒトを人らしくする感情・意欲の表現や，計画・判断・実行などの認知機能を担う役割を持っているため，前頭葉におけるドパミン神経活動の低下とhypofrontalityおよび陰性症状・認知機能障害の関連は，一定の説得力を持つ．

動物実験では，前頭前野でドパミン神経を選択的に破壊すると，線条体においてドパミンやD_2受容体の密度が増加することが知られている．逆に，前頭前野へのドパミンアゴニストの投与は，線条体のドパミンレベルを減少させる．これらのことから，前頭前野のドパミン神経活動の低下は線条体のドパミン神経活動の過剰を引き起こし，陽性症状の原因となるという仮説が立てられている．[3] またリスペリドンなどの非定型抗精神病薬は，セロトニン5HT2受容体遮断作用によりドパミンD_1受容体活性を増加させ，症状を改善するとされている．しかし，統合失調症患者の前頭前野におけるドパミン伝達が低下していることを示すデータは乏しく，次に述べるように他の神経システムを介したメカニズムを検討する必要がある．

4 グルタミン酸と統合失調症

近年，統合失調症の分子生物学的病態仮説として注目されているのは，グルタミン酸仮説である．これは，統合失調症の脳においてグルタミン酸神経の伝達が何らかの理由で障害されることにより症状を発現するという仮説であるが，後述するようにこの仮説はドパミン仮説と対立するものではなく，むしろ相互に補完するものである．

グルタミン酸と統合失調症の関連を最初に示唆したのは，統合失調症患者の髄液中のグルタミン酸濃度が低下していることを示したKimらであった．[4] また，麻酔薬として開発されたフェンサイクリジン(phencyclidine；PCP)の乱用者が統合失調症と区別し難い症状を呈するという事実が観察されたが，後にPCPはグルタミン酸受容体の1つである*N*-methyl-D-aspartate(NMDA)受容体を遮断する作用があることが分かった．また同様に，NMDA受容体拮抗作用を持つケタミンも統合失調症に似た症状を引き起こすことが分かった．前述のように，アンフェタミンなどのドパミン神経伝達を賦活する薬剤が統合失調症の陽性症状に似た症状のみを引き起こすのに対し，これらのグルタミン酸受容体遮断薬は陽性症状のみならず，陰性症状や認知機能障害も引き起こすことが特徴であり，グルタミン酸仮説は統合失調症の病態により近く迫ると考えられる．

図1に示すように，NMDA受容体はNR1サブユニットと4種のNR2サブユニットA～Dの少なくとも1種が組み合わさったヘテロメリック集合体であると考えられている．これらのユニット上にグルタミン酸結合部位(Glu)，グリシン結合部位(Gly)，マグネシウムイオン結合部位(Mg^{2+})，フェンサイクリジン結合部位(PCP)，ポリアミン結合部位(Poly)などの種々の調節部位を持つ．GlyはNR1上に，GluはNR2上にあると考えられている．これらにより構成されるNMDA受容体は，細胞外からNa^+やCa^{2+}を流入させ，細胞内からK^+を透過させるイオンチャネルとして機能する．

PCPはイオンチャネル内にあるフェンサイクリジン結合部位に結合し，チャネル遮断作用をもたらすと考えられる．PCPを急性投与された動物モデルでは，移所運動量の増加，常同行動の増加，認知機能障害，社会的行動障害などが認められる．NR1サブユニットノックアウトマウスでも，同様の症状が見られる．ヒトにおいてはケタミンを健常

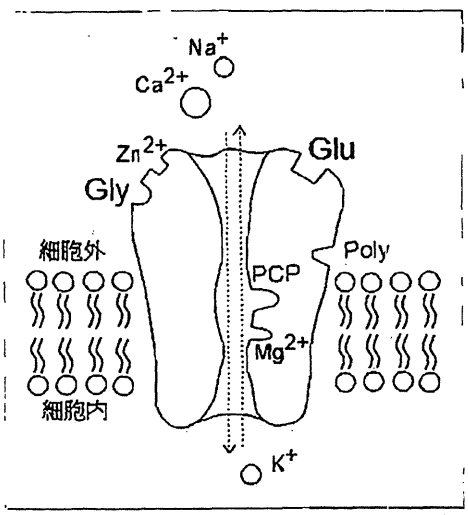

図1 NMDA受容体

者に投与する研究が行われており，陽性症状，陰性症状および認知機能障害を引き起こすことが示されているほか，[5] 統合失調症患者の死後脳において，NMDA受容体の結合能の低下や各NRサブユニットの遺伝子発現の低下が報告されている．

5 ドパミンとグルタミン酸

以下に述べるように，主に陽性症状を説明するドパミン仮説と，陽性症状，陰性症状および認知機能障害を説明するグルタミン酸仮説は互いに矛盾する仮説ではない．Laruelleらは SPECT を用いて，統合失調症患者にアンフェタミンを投与すると，健常対照者に比べ線条体でのドパミン放出が有意に亢進していることを示した．[6] またBreierらは PET を用いて，健常者に NMDA 受容体遮断薬であるケタミンを投与すると，線条体でのドパミン放出が亢進することを見いだした．[7] このドパミン放出の増加は，アンフェタミン負荷によるドパミン放出の増加と同程度のものであった．これらの事実により，線条体でのドパミン放出には NMDA 受容体を介する系があり，統合失調症患者におけるアンフェタミン刺激性のドパミン放出の亢進の機序に，NMDA 受容体機能の低下が関与していることが示唆された．

グルタミン酸神経伝達の障害がドパミン過剰を引き起こすのは，どのような機序によるものだろうか．中枢神経はグルタミン酸を介する興奮性神経システムに，抑制性神経である GABA を介するシステムが介在することで成り立っているといえる．統合失調症におけるドパミン神経活動の増加は，抑制性神経システムの破綻によりドパミン神経が脱抑制されるために生じると考えられる．すなわちラットを用いた研究を根拠とする理解として，前頭前野のグルタミン酸神経は側坐核の GABA 神経に投射しているが，GABA 神経上の NMDA 受容体が障害されているため GABA 神経の活動が低下し，GABA 神経により抑制されていたこれらの領域におけるドパミン神経前終末が脱抑制され，結果的にドパミン放出が増加するものと考えられる(図 2)．[8,9] また，線条体に投射する黒質緻密部のドパミン神経は視床下核のグルタミン酸神経の投射を受け，視床下核の

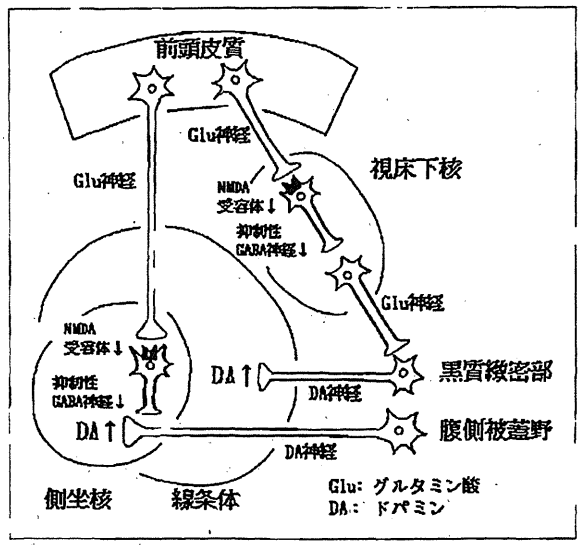

図2 前頭皮質，側坐核・線条体のグルタミン酸，GABA，ドパミン神経

電気刺激はドパミン放出を増加させる．GABA 受容体拮抗薬を視床下核に投与すると線条体においてドパミン放出が増加し，ここでもグルタミン酸や GABA の関連が示唆される．[10] これらを支持するように，統合失調症患者の死後脳研究では，前頭皮質や線条体・視床を含む脳の各領域において，GABA 神経の活動性の低下を示す所見が報告されている．[11,12]

6 分子生物学から精神症状論へ

これまで統合失調症の病態メカニズムについて，分子生物学的な説明を行ってきた．それではこのような分子生物学的な変化は，どのように統合失調症の陽性症状，陰性症状および認知機能障害といった症状に結び付くのだろうか(図 3)．

前述したように，統合失調症では前頭葉活動の低下(hypofrontality)が特徴的である．これにより感情や意欲の表現が障害され，陰性症状として現れると考えられる．情報を一時的に保ちながら他の情報を同時に操作するためのシステムをワーキングメモリ(作業記憶)というが，前頭葉ではこのワーキングメモリをはじめとした認知機能により，情報を統合調整し合理化するなどの高度な処理が行われている．統合失調症においては，ウィスコンシン・カー

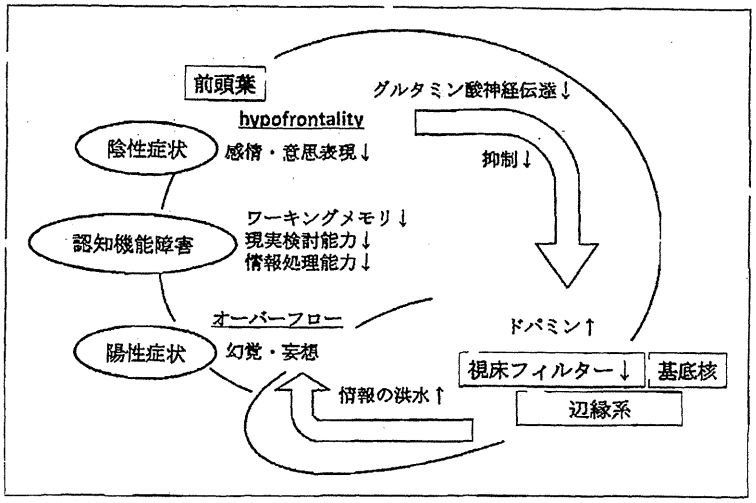

図3 精神症状発現のしくみ

ド・ソーティング課題などで測定されたワーキングメモリが障害されていることがよく知られている．またNMDA型グルタミン酸受容体遮断薬はワーキングメモリを障害し，前頭葉におけるグルタミン酸伝達と認知機能障害の関連が示唆されている．

前頭葉(前頭前野)の機能の低下は下位脳に波及する．[13] 例えば，前述のように前頭前野におけるドパミン神経の障害は，線条体におけるドパミン神経の過活動を引き起こす．また前頭前野から投射するグルタミン酸神経伝達の障害が，線条体や辺縁系のGABA抑制神経の活動を低下させ，ドパミン神経に脱抑制・過活動をもたらす．辺縁系でのドパミン過剰は扁桃核や海馬などの感情回路を興奮させ，大量の感情や記憶を呼び起こし，いわゆる「感情を伴う情報の洪水」が生じる．また視床は入力される情報の大きさを決めるフィルターとして機能する部分であるが，通常は基底核(線条体)により抑制されている．しかし基底核のドパミン過剰となると，視床が脱抑制されて興奮し，フィルターの機能を果たすことができなくなる．こうして視床により十分にフィルターされない「感情を伴う情報の洪水」が，前頭葉に送られることとなる．

下位脳より過剰な情報を受け取った前頭葉は，機能が低下しているために正確な情報処理が行えずにオーバーフローしてしまう．内部からの情報と外部からの情報を照合し，合理的に正しく判断する認知機能を現実検討能力というが，統合失調症ではこの機能が低下し，内部情報である自らの「心の声」を正しく処理できずに外部情報として捉え，幻聴を発現するかもしれない．また，辺縁系から送られたフィルターされていない不安感や恐怖感などを伴った情報は，電話中にたまたま聞こえた雑音と結び付き，正しい判断がされずに「秘密警察に盗聴されている」と確信して妄想を発展させるかもしれない．

7 今後の展望

以上述べてきたように，統合失調症の病態メカニズムを考える場合に，陽性症状と関連するドパミン仮説は，陽性症状・陰性症状・認知機能障害すべてと関連するグルタミン酸仮説の「下流」にあると考えられる．このような観点から，現在の統合失調症治療薬であるドパミンD_2受容体遮断薬に続く新規治療薬として，NMDA型グルタミン酸受容体機能促進薬の開発が模索されている．

図1で示したように，NMDA受容体上にはグルタミン酸結合部位の他にグリシン結合部位がある．グリシン結合部位の刺激は単独では神経伝達を生じないが，グルタミン酸結合部位の作動薬がNMDA受容体を十分に活性化するためにはグリシン結合部位の刺激が必須である．このことから，グリシン結合部位の作動薬はNMDA受容体のコ・アゴニストと呼ばれる．グルタミン酸結合部位の直接刺激は細胞死やけいれんを誘発する危険があるが，グリシン

結合部位作動薬ではこうした現象が生じにくいため，統合失調症患者に対してNMDA受容体コ・アゴニストの治療効果が検証されている．

統合失調症患者にグリシンが投与され，抗精神病薬との併用により陰性症状に対して効果があることが1988年に初めて報告された.[14] しかし，グリシンは脳内移行性が低いため60 g/日という高用量を用いなければならず，経口摂取の負担や腎臓におけるアンモニアの発生が問題となった．

D-セリンは，哺乳類の生体内分子としてはL-体アミノ酸のみが存在するという従来の定説を覆す新たな脳内在性分子として筆者らが1992年に見いだしたものであるが,[15,16] このアミノ酸はNMDA受容体に対してコ・アゴニストとして作用する．統合失調症患者に2 g/日を経口投与し，陽性症状・陰性症状・認知機能障害の改善を認めたという報告が1998年になされた.[17] しかし，D-セリンもグリシンと同様に脳内移行性が低いために高用量を必要とし，また腎毒性を持つ可能性がある点が問題となった．ただしD-セリンは脳内在性アミノ酸であるため，外部からの投与により治療効果を期待するより，D-セリンの調節をもたらす薬剤の開発の方が長期的な治療戦略としてははるかに合理性を持つと考えられる．このようなことから我々は現在，シナプス間隙におけるD-セリン濃度の制御およびD-セリンの生合成・代謝に関わる分子機構の解明に関する研究を進めている．

D-サイクロセリンは，細胞壁ペプチドグリカン生合成阻害作用を持ち，抗結核薬として40年以上使用されてきており，安全性に関する臨床データが蓄積されている.[18] 抗結核薬の効果とは全く別に，中枢神経系においてはNMDA受容体の部分アゴニストとして作用する．脳への移行性も高く，統合失調症への治療効果が期待されている．1994年以降行われてきた抗精神病薬と併用して，統合失調症患者に投与する臨床試験では250 mg/日という高用量では症状を悪化させるが，50 mg/日という適度な用量によって陰性症状に改善が見られることが複数報告されている.[19] これは，高用量では逆にD-サイクロセリンがグリシン結合部位においてアンタゴニストとして作用するため，NMDA受容体機能が低下することによるものと推測される．我々は現在，D-サイクロセリン50 mg/日と抗精神病薬を統合失調症患者に投与するプラセボ対照二重盲検試験を行っており，精神症状に対する効果およびD-サイクロセリン，D-セリンの血中動態を検証している．

8 おわりに

以上，本稿では統合失調症の分子生物学的な病態メカニズムとして，ドパミン仮説およびグルタミン酸仮説という2つの大きな理論を紹介した．さらに，これらが統合失調症の精神症状に関連するメカニズムを説明した．ドパミンD_2受容体遮断薬を主流とする現在の薬物療法では，統合失調症患者の社会的機能を妨げている陰性症状や認知機能障害を改善しているとはいえず，グルタミン酸仮説を裏付ける分子機構の解明と，それを基礎とする新規治療薬の開発が望まれる．

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ARTICLE

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Mouse strain differences in phencyclidine-induced behavioural changes

Akihiro Mouri[1,2,3], Takenao Koseki[1,3], Shiho Narusawa[1,3], Minae Niwa[1,3,4,5], Takayoshi Mamiya[1,3], Shin-ichi Kano[5], Akira Sawa[5,6,7,8] and Toshitaka Nabeshima[1,3]

[1] *Department of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Meijo University, Nagoya, Japan*
[2] *Division of Scientific Affairs, Japanese Society of Pharmacopoeia, Tokyo, Japan*
[3] *The Academic Frontier Project for Private Universities, Comparative Cognitive Science Institutes, Meijo University, Nagoya, Japan*
[4] *Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[5] *Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD, USA*
[6] *Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD, USA*
[7] *Graduate Program in Cellular and Molecular Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA*
[8] *Graduate Program in Human Genetics, Johns Hopkins University School of Medicine, Baltimore, MD, USA*

Abstract

Administration of phencyclidine (PCP) is acknowledged to generate a model of psychosis in animals. With the identification of genetic susceptibility factors for schizophrenia and bipolar disorder, great efforts have been made to generate genetic animal models for major mental illnesses. As these disorders are multifactorial, comparisons among drug-induced (non-genetic) and genetic models are becoming an important issue in biological psychiatry. A major barrier is that the standard mouse strain used in the generation of genetic models is C57BL/6, whereas almost all studies with PCP-induced models have utilized other strains. To fill this technical gap, we systematically compared the behavioural changes upon PCP administration in different mouse strains, including C57BL/6N, C57BL/6J, ddY, and ICR. We observed strain differences in PCP-induced hyperlocomotion and enhanced immobility in the forced swim test (ddY>>C57BL/6N and 6J>ICR). In contrast, there was no strain difference in the impairment of recognition memory in the novel object recognition memory test after withdrawal of chronic PCP administration. This study provides practical guidance for comparing genetic with PCP-induced models of psychosis in C57BL/6. Furthermore, such strain differences may provide a clue to the biological mechanisms underlying PCP-induced endophenotypes possibly relevant to major mental illnesses.

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Key words: Behavioural sensitization, forced swim test, mouse strain difference, phencyclidine, schizophrenia.

Introduction

It is challenging to model major psychiatric disorders, such as schizophrenia, in mice (Flint & Shifman, 2008). At present, two approaches are utilized. First, on the basis of similarities between drug-induced psychosis in adults and schizophrenia (Javitt & Zukin, 1991; Luby *et al.* 1959), drug-treated, especially phencyclidine (PCP)-treated animals are utilized (Jentsch & Roth, 1999; Mouri *et al.* 2007*a*). PCP is an antagonist of the NMDA-type glutamate receptor, and PCP-induced models have been used for screening many compounds in neuropsychopharmacology (Hashimoto *et al.* 2005; Kunitachi *et al.* 2009; Mouri *et al.* 2007*a*; Noda *et al.* 1995). A major drawback of these models is that, even if they mimic the pathophysiology of schizophrenia, they do not encompass the neurodevelopmental abnormalities that underlie the pathogenesis of the disease (Fatemi & Folsom, 2009). Second, on the basis of disease-associated susceptibility genes for schizophrenia, many groups have generated genetically engineered mice as possible

Address for correspondence: T. Nabeshima, Ph.D., Department of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Meijo University, 150 Yagotoyama, Tempaku-ku, Nagoya 468-8503, Japan.
Tel.: 81-52-839-2735 *Fax*: 81-52-839-2738
Email: tnabeshi@meijo-u.ac.jp

models for the disease (Desbonnet *et al.* 2009; Hikida *et al.* 2007; Mohn *et al.* 1999). An advantage of this approach is that mice with genetic changes in disease susceptibility factors involved in neurodevelopment can offer the potential to address the aetiology-associated biology during development (Fatemi & Folsom, 2009). Furthermore, with rapid advances in controlling spatial- and temporal-specific genetic alternations, genetic models offer the potential to dissect neuronal circuitry-dependent phenotypic changes in detail (Pletnikov, 2009). Nonetheless, caution should be exercised in the interpretation of data because there is currently no specific causal gene for schizophrenia (Glessner & Hakonarson, 2009). Therefore a strategy to compare both genetic and non-genetic (in most cases, drug-induced) animal models is clearly warranted.

A crucial criterion in characterizing the behaviour of these genetically engineered models is that the mice are backcrossed with C57BL/6 and standardized to be in the C57BL/6 genetic background (Hikida *et al.* 2007; Mohn *et al.* 1999). Despite some reports on the effect of repeated PCP treatment on cognition in C57BL/6J strain (Beraki *et al.* 2008, 2009), most studies with PCP-induced endophenotypes have used strains other than C57BL/6, such as ddY (Mouri *et al.* 2007*b*; Murai *et al.* 2007; Noda *et al.* 1995). The present study was designed to fill this gap and enable systematic experiments in both genetic and non-genetic (PCP-induced) models in parallel with those in the C57BL/6 strain. Therefore, we compared behavioural changes and sensitivity to chronic PCP administration in different strains, including C57BL/6N, C57BL/6J, ddY, and ICR.

Methods

Mice

Male mice of the ddY, ICR, C57BL/6N, and C57BL/6J strains (6-wk-old) were obtained from Japan SLC (Shizuoka, Japan). The animals were housed in plastic cages and kept in a regulated environment (24±1 °C, 50±5% humidity), with a 12-h light/dark cycle (lights on at 08:00 hours). Food and tap water were available *ad libitum*. All experiments were performed in accordance with the Guidelines for Animal Experiments of Meijo University Faculty of Pharmaceutical Sciences. The procedures involving animals and their care were conducted in conformity with the international guidelines, Principles of Laboratory Animal Care (National Institutes of Health publication 85–23, revised 1985).

Drugs

Phencyclidine hydrochloride [1-(1-phenylcyclohexyl) piperidine hydrochloride (PCP)] was synthesized by the authors according to the method of Maddox *et al.* (1965) and checked for purity. PCP was dissolved in a saline (0.9% NaCl) solution and administered in a volume of 0.1 ml/10 g body weight. The mice (6-wk-old) were treated with PCP (10 and 15 mg/kg s.c.) once a day for 14 d.

Locomotor activity test

To measure spontaneous locomotor activity in a novel environment, a mouse was placed in a transparent acrylic cage with a black frosted Plexiglas floor (45 × 26 × 40 cm), and locomotion was measured for 120 min using digital counters with infrared sensors (Scanet SV-10; Melquest Co. Ltd, Japan). One day after measurement of spontaneous locomotor activity, mice were given saline or PCP at a dose of 10 mg/kg s.c. for 14 d. Locomotor activity was immediately measured for 2 h after PCP treatment on days 1 and 14. PCP challenge-induced hyperactivity was measured separately from locomotor activity during the 14-d treatment. To exclude any effect of PCP remaining in the brain on the challenge-induced hyperactivity, the test was performed 5 d after withdrawal from chronic PCP treatment. This is because the half-life of PCP in the brain was 30.5 min in rats treated repeatedly (Nabeshima *et al.* 1987) and PCP-treated rats did not show withdrawal syndrome behaviour 4 d after the final treatment (Nabeshima *et al.* 1986). One day after habituation to the apparatus, locomotor activity was measured for 120 min immediately after PCP (1 and 3 mg/kg) challenge.

Forced swim test

The forced swim test was conducted according to previous reports (Murai *et al.* 2007; Noda *et al.* 1995) with a minor modification. The test was performed 1 d and 3 d after withdrawal from 14-d PCP treatment as reported previously (Murai *et al.* 2007; Noda *et al.* 1995). A mouse was placed in a transparent glass cylinder (20 cm high, 15 cm diameter), which contained water at 22–23 °C to a depth of 15 cm, and was forced to swim for 360 s. The duration of swimming was measured by a SCANET MV-10 AQ apparatus (Melquest Co. Ltd). Immobility was calculated as follows (in seconds): total time − swimming time = immobility time.