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D-serine: a new word in the glutamatergic neuro-glial language

Review Article

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Summary. Gliotransmission is a process in which astrocytes are dynamic elements that influence synaptic transmission and synaptogenesis. The best-known gliotransmitters are glutamate and ATP. However, in the past decade, it has been demonstrated that D-serine, a D-amino acid, acts as a gliotransmitter in glutamatergic synapses. The physiological relevance of D-serine is sustained by the way in which it modulates the action of glutamatergic neurotransmission, neuronal migration and long-term potentiation (LTP). In addition, the synthesis and degradation mechanisms of D-serine have been proposed as potential therapeutic targets for the treatment of Alzheimer's disease, schizophrenia and related disorders. In the present review, detailed information is provided about the physiological and physiopathological relevance of D-serine, including metabolic and regulation aspects.

Keywords: Gliotransmission – D-serine – Glutamate – NMDA – Glicine site

1. Basic aspects about metabolism and actions of D-serine

1.1 Introduction

For a long time, D-amino acids (D-aac) were ignored by researchers, because they belong to the less biologically active conformation of amino acids. Although the existence of D-aac was confirmed in bacteria, worms and other invertebrates (Corrigan, 1969), it was a few years later that they were detected in mammalian tissues in high levels, especially in the brain (Hashimoto et al., 1992; Chouinard et al., 1993; Nagata et al., 1994). Among the first D-aac discovered in high concentrations in the brain was the D-aspartate (Dunlop et al., 1986). Later on, another very important D-aac called D-serine (D-ser) was found in elevated levels in the mammalian brain (Hashimoto et al., 1993b). Since its discovery, data about the functions of D-ser have increased markedly and it has been investigated along many scientific lines. Although this D-aac is found in peripheral tissues, its high content in the brain, especially in astrocytes, makes it an important factor for understanding neuromodulation (Hashimoto et al., 1995). Classically, synapses were considered as polarized elements where neurotransmitter substances are released from the presynaptic cells and bind to their postsynaptic receptors. However, the central nervous system (CNS) is made up of neurons and glial cells, the latter being the most numerous (Nedergaard et al., 2003). Glial cells are tightly located with neurons, allowing a bidirectional communication between neurons and glia (Volterra and Harris, 1999; Haydon, 2001; Volterra and Meldolesi, 2005). The complex produced by synaptic cells and the surrounding glia is the basis for an emerging concept that reflects on the synapses as tripartite elements and proposes glia as dynamic components that control synaptogenesis (Pfrieger, 2002) and synaptic transmission (Oliet et al., 2004). These functions carried out by astrocytes are mediated by neuromodulators and gliotransmitters released by them. Although glutamate (glu) and ATP are the best-known gliotransmitters, it is now clear that D-ser can be added to the list (Wolosker et al., 1992).

1.2 Synthesis and distribution of D-ser

D-ser is synthesized from L-serine (L-ser) via one enzymatic step catalyzed by the enzyme serine racemase (SR) (Schell, 2004). This enzyme is proposed as the main endogenous source, if not the only one, of D-ser. The SR

catalyses the conversion of D-ser into L-ser, albeit with lower affinity (Wolosker et al., 1999; Konno, 2003). The SR distribution is analoguous to D-ser sharing the highest levels in the forebrain (Wolosker et al., 1999; Xia et al., 2004). In the CNS, SR is the almost exclusive enzyme of glial fibrillary acidic protein (GFAP) positive astrocytes (Wolosker et al., 1999; Xia et al., 2004; Williams et al., 2006), but is also found in several cortical neurons and in hindbrain glutamatergic neurons (Williams et al., 2006). In the peripheral nervous system, SR is mainly located in Schwann cells (Wu and Barger, 2004). The level of D-ser varies during brain development. In the adult stage, the highest level of this D-aac is in the forebrain, while in neonates it is found in the cerebellum (Schell et al., 1995, 1997). Notably, the cerebellar concentrations of D-ser in the adult brain are practically undetectable (Schell et al., 1995). In addition, D-ser distribution resembles that observed for N-methyl-D-aspartate (NMDA) glu receptors. Contrary to this, however, the glycine (gly) distribution, which, like D-ser, is a NMDA coagonist, does not resemble the NMDA receptor distribution, except in the hindbrain, the adult cerebellum and the olfactory bulbs, where D-ser is also present (Schell et al., 1995). In the brain stem, the gly levels are maximal and there is a correspondence between gly distribution and NMDA receptor distribution (Schell et al., 1995). Interestingly, in the pons medulla, spinal cord and cerebellum, where D-ser levels are undetectable, gly levels are elevated (Schell et al., 1997).

1.3 Regulation of D-serine synthesis

The levels of D-ser are controlled by the activity of the SR. This enzyme is regulated by multiple factors (Cook et al., 2002; Dunlop and Neidle, 2005; Strisovsky et al., 2005). Pyridoxal 5′-phosphate (PLP) is the main glial cofactor that stimulates the SR activity. In addition to PLP, other compounds, such as Mg^{2+} and ATP, are capable of stimulating the synthesis of D-ser by increasing the rate of activity of SR (de Miranda et al., 2002; Neidle and Dunlop, 2002; Foltyn et al., 2005). The ion Ca^{2+} is another cofactor that positively modulates this enzyme in astrocytes (Cook et al., 2002). SR can also be regulated by protein–protein interactions. SR binds to glutamate receptor interacting protein (GRIP), which acts as scaffolding protein for the α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors. GRIP contains six PDZ domains, a motif associated with protein–protein interactions. SR selectively binds to the PDZ-6 domain through its C-terminal extreme which contains the PDZ-binding consensus sequence VSV (valine-serine-valine). The interaction between SR and GRIP leads to an increase in the rate of D-ser synthesis (Kim et al., 2005). These authors observed two transfected groups of C6 glioma cells, which express GRIP endogenously, with wild type SR and with a mutant variant in the consensus sequence VSV of the C-terminal extreme of SR. They found that the production of D-ser was reduced by 65% in the cells transfected with the mutant variant of SR, suggesting that the activation of this enzyme depends on its interaction with GRIP (Kim et al., 2005). The physiologic relevance of this observation is based on the stimulation of AMPA receptors, which leads to a strong increase in D-ser synthesis (Kim et al., 2005). AMPA activation promotes the phosphorilation of this receptor with the consequent dissociation from it of GRIP. This allows GRIP to bind to SR. On the other hand, gly and certain L-aspartic acid metabolites (L-aspartic acid, L-asparagine and α, β-threo-3-hydroxy aspartic acid) competitively inhibit SR activity (Dunlop and Neidle, 2005; Strisovsky et al., 2005). Notably, it was found that nitric oxide (NO) decreases the enzymatic activity of SR (Shoji et al., 2006). A summarized view of the different modulators of SR is detailed in Table 1.

1.4 D- and L-serine metabolic cycle

SR constitutes the pathway of important metabolic machinery. As mentioned above, SR converts D-ser into L-ser and vice versa. However, this enzyme can also promote the elimination of water from either L- or D-ser, producing pyruvate and ammonia (de Miranda et al., 2002; Neidle and Dunlop, 2002; Strisovsky et al., 2003; Foltyn et al., 2005). Interestingly, the ability of SR to form pyruvate by eliminating water is greater than the ability to racemize L-ser. In fact, only one molecule of four of L-ser attacked by SR is converted into D-ser, while the other three are converted into pyruvate (Strisovsky et al., 2003, 2005; Foltyn et al., 2005). Pyruvate can enter via different pathways, promoting ATP synthesis through the Krebs cycle or being converted into lactate by the enzyme lactate dehydrogenase (LDH). If pyruvate gains access to the Krebs cycle, the ATP obtained closes a positive metabolic

Table 1. Regulation factors of serine racemase

Positive regulators	Negative regulators
PLP, ATP, Mg^{2+}, Ca^{2+}, GRIP, AMPA stimulation	Gly, L-aspartic acid metabolites, NO

cycle, stimulating SR, which originally synthesized the pyruvate. In addition, the course of pyruvate through the Krebs cycle supports the generation of several important amino acids for glia and neurons (GABA, glutamate, glutamine), from α to ketoglutarate. The conversion of pyruvate into lactate provides a metabolic coupling between neurons and glia due to lactate serving as an energetic precursor for neurons, especially during periods of synaptic hyperactivity, oxidative stress or Zn^{2+}-induced neurotoxicity (Foltyn et al., 2005).

In mammals, D-ser is metabolized by the peroxisomal flavoprotein D-amino acid oxidase (DAAO), an enzyme located in astrocytes of the hindbrain and cerebellum (Schell et al., 1995; Moreno et al., 1999; Urai et al., 2002), converting it into pyruvate. DAAO, has shown to be stereoselective, because it has no effect on L-amino acids or dicarboxylic amino acids (Pilone, 2000). In agreement with these findings, studies have been carried out using knock-out mice lacking the DAAO gene. They showed a significant increase in D-ser levels, especially in the brain stem and cerebellum, two regions containing low D-ser levels in wild type animals (Morikawa et al., 2001). This could suggest a constitutive activation of DAAO in the mentioned regions of wild type mice. On the contrary, D-ser levels did not change significantly in the forebrain of knock-out animals, suggesting that in this region, D-ser levels are regulated by another mechanism (Morikawa et al., 2001). The main degradation process in this area would be the water elimination to form pyruvate, catalyzed by the SR, as explained previously (Foltyn et al., 2005).

1.5 D-serine in the glutamatergic neurotransmission

The general aspects for an understanding of how D-ser acts as a gliotransmitter are the same that control classical chemical neurotransmission (cellular depolarization, release of the transmitter, receptor activation and signal termination). It is known that the stimulation of non-NMDA (AMPA, kainite-KA-) receptors is the main stimulus that promotes the efflux of D-ser from astrocytes (Schell et al., 1995). Mothet et al. (2005) demonstrated in in vitro studies that the activation of AMPA/KA and even metabotropic glu receptors triggers the release of D-ser in a Ca^{2+}-dependent manner. Notably, inhibition of the vesicular proton ATPase decreases the levels of released D-ser. This finding suggests the inhibition of vesicular storage of the gliotransmitter by a transporter protein which has yet to be identified. Recent studies showed that astrocytes can release D-ser and other gliotransmitters by Ca^{2+}-dependent exocytosis (Coco et al., 2003; Bezzi et al., 2004; Volterra and Meldolesi et al., 2005). However, D-ser can be released by a Ca^{2+}-independent mechanism because most cytoplasmatic D-ser is not stored (Kim et al., 2005). In agreement, in conditions of low osmolarity or poor extracellular concentration of divalent cations, D-ser is released through hemichannels, anionic channels or P2X7 receptors, impulsed by a chemical gradient (Volterra and Meldolesi et al., 2005). In addition, D-ser can also be released through the alanine-serine-cysteine transporter (ASCT), commonly by countertransport with L-ser in a Na^{+}-dependent manner (Ribeiro et al., 2002). Although glial cells are the primary source of D-ser release, some neurons release it after NMDA stimulation (Kartvelishvily et al., 2006). Interestingly, it is suggested that neuronal release of D-ser would occur through a Ca^{2+}-independent mechanism, opposite to glial release, that would be mainly Ca^{2+}-dependent (Kartvelishvily et al., 2006). Neurophysiological studies of NMDA receptors suggest that with a certain combination of the NR1 and NR2 subunits, D-ser, after being released, binds to the NMDA gly site with threefold potency in comparison to gly binding (Matsui et al., 1995; Priestley et al., 1995). In addition, it has been demonstrated that D-ser binds to the gly site through the same kind of interaction as gly (Furukawa and Gouaux, 2003). The NMDA receptor has unique properties. It consist of a tetramer of two distinct subunits (Kemp and McKernan, 2002; Prybylowski and Wenthold, 2004). Up to date, three different subunits for this receptor have been cloned: NR1, NR2 (mentioned above) and NR3 (Cull-Candy et al., 2001). Most NMDA receptors are formed by combinations of NR1 and NR2 subunits, containing the recognition sites for coagonist and for glu, respectively. The NR3 subunit, less common, can be assembled with either NR1 or NR2 to depress the NMDA activation (Das et al., 1998). Despite all these data, is there enough evidence for the importance of D-ser as a glutamatergic coagonist?

Indeed, the hippocampus provides an excellent model for studying the function of D-ser in the glutamatergic neurotransmission, because it expresses a high density of D-ser and NMDA receptors, especially in the areas CA1 and CA3 (O'Brien et al., 2005). The hippocampus is one of the brain sites where long-term potentiation takes place through NMDA activation (Nicoll, 2003). Considering that D-ser is an endogenous ligand for the NMDA receptor, it is not surprising that the release of D-ser from astrocytes is implicated in the induction of LTP in the pyramidal synapses of the area CA3 (Yang et al., 2003). In fact, administration of DAAO inhibits this LTP, suggesting that D-ser, more than gly, is the endogenous

NMDA coagonist in this brain area (Barnes, 2003). In agreement with this, it was found that the deficit of LTP is not associated with low levels of gly, supporting the role of D-ser as the main modulator of the NMDA gly site (Mothet et al., 2006).

Like other neurotransmitters, the actions of D-ser must be finished by its clearance from the synaptic cleft by transporter proteins expressed in the membranes of neurons and glial cells (Hayashi et al., 1997; Yamamoto et al., 2001; Javitt et al., 2002; O'Brien et al., 2005). Astrocytes express an Na^+-dependent transporter with low affinity for D- and L-ser (Hayashi et al., 1997). The properties of this transporter are correlated with that observed for the ASCT system, which uptakes D-ser in astrocytes and the retina (Ribeiro et al., 2002; O'Brien et al., 2005). Another transporter of neutral amino acids, Asc-1, which is Na^+-independent, shows high affinity for D-ser and is expressed in presynaptic terminals, dendrites and neuronal bodies (Helboe et al., 2003; Matsuo et al., 2004). The Asc-1 system was shown to be important for the CNS development. Knock-out mice lacking the Asc-1 gene had normal appearance at birth, but their brains and other key organs showed a 30% reduction in their weights compared with wild type mice (Xie et al., 2005). In addition, knock-out animals developed spontaneous convulsive seizures and periodic tremors. Both abnormalities are reduced by administrating the NMDA antagonist MK-801 (3 mg/kg) or by high doses of diazepam (10 mg/kg) (Xie et al., 2005). Taken together, these data suggest an increased synaptic excitability in the Asc-1-lacking mice and that NMDA activation could be one of the main causes of it, because D-ser levels in these animals would be higher than in control mice. On the other hand, the effect obtained after administration of diazepam indicates that in knock-out animals, the GABAergic neurotransmission is intact and that an increase of the inhibitory activity could overcome the neuronal hyperactivity (Xie et al., 2005).

2. Physological and physiopathological relevance of D-serine functions in the glutamatergic neurotransmission

2.1 D-serine as a neuronal migration factor

It is well known that glu has an important role as a neuronal migration factor during CNS development (Yacubova and Komuro, 2003; Kim et al., 2005). One of the best characterized models, where glutamatergic participation through NMDA activation is observed, corresponds to the radial migration of the granule cells of the developing cerebellum (Hatten, 1999). Recent studies have demonstrated that glu is crucial for promoting the mobility of granule cells through the molecular layer by stimulating NMDA receptors (Yacubova and Komuro, 2003). However, the mechanism by which glu causes the migration of granule cells by NMDA activation is controversial, due to the fact that neurons do not form mature synapses until the of their migration into the inner granular cell layer. The hypothesis proposes that glu released by Bergmann glia (BG) activates immature NMDA receptors in a non-synaptic paracrine mode (Yacubova and Komuro, 2003). In addition, there is well-documented literature that elicits the importance of BG in the cerebellar development. Indeed, BG play a relevant role in the growth of Purkinje cells, serving them as the main source of L-ser, which acts as a neurotrophic factor (Altman and Bayer, 1996; Yamada et al., 2000). In fact, BG express SR during cerebellar development, and the D-ser levels released by the BG peak at postnatal day 14 (intense cell migration period) and then decrease markedly (Boehning and Snyder, 2003). Apparently, BG-derived D-ser promotes the granule cells' migration by stimulation of NMDA receptors (Kim et al., 2005). In agreement with this, it was found that the inhibition of SR or the administration of DAAO block the cellular migration by reduction of the Ca^{2+} efflux induced by NMDA activation (Kim et al., 2005). Similar results are observed in cerebellar slices, after administration of fenazine ethosulphate, which strongly reduces the intracellular Ca^{2+} level. This effect is reversed by removing the drug from the medium (Kim et al., 2005). This shows the importance of the intracellular Ca^{2+} in the neuronal migration. It is probable that D-ser promotes synaptogenesis of cerebellar neural networks because its ontogeny in BG parallels the expression of the NR2A and NR2B subunit of the NMDA receptor in Purkinje cells (Schell et al., 1997). Considering that NMDA blockade during neocorticogenesis promotes an abnormal cortical development (Gressens, 2000; Reiprich et al., 2005), it is possible that the disruption of D-ser metabolism at early life stages might lead to similar disorders.

2.2 D-serine, a neuronal death promoter

There is no doubt that NMDA receptors trigger neuronal death in some neuropathological conditions where they are overstimulated or are chronically activated (Kemp and McKernan, 2002; Hardingham and Bading, 2003; Lipton, 2004). Increased extracellular levels of glu due to an enhanced release (Takano et al, 2001) or a decrease in the uptake rate of this amino acid are the commonest

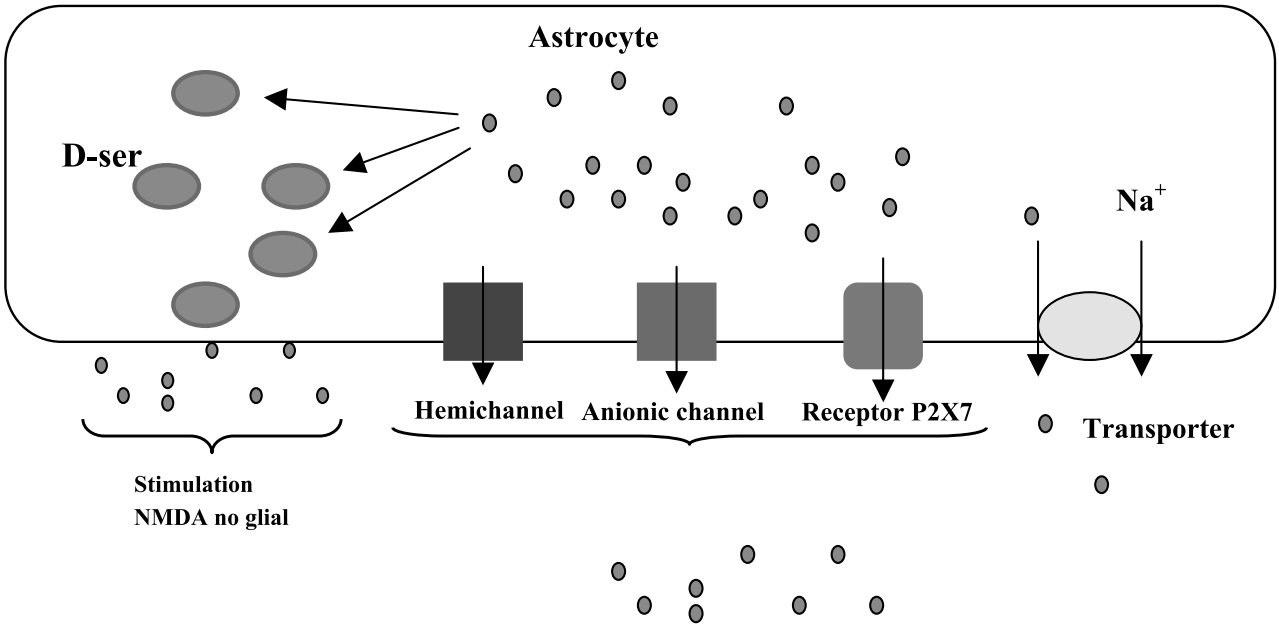

Fig. 1. Mechanism of release of D-ser

causes by which NMDA activation induces neuronal death (Lipton, 2004). In several stress models, excitotoxic cell death mediated by NMDA receptors was observed (Moghaddam, 1993). Olney (1971) defined excitotoxicity as an acute process in which glu or its excitatory structural analogs trigger nerve cell death in the CNS of rodents. The excitotoxic action of glu, via NMDA activation, as a result of its increased release or low uptake, in addition to the excitotoxicity potentiation by glucocorticoids, has been involved in the pathogenesis of stress-induced cerebral damage (Sapolsky et al., 1990; Moghaddam, 1993; Magariños and McEwen, 1995; Kim et al., 1996). In addition, intense NMDA activation produces an increase in the expression of inducible nitric oxide synthase, an enzyme that produces high amounts of NO, which promotes oxidative neuronal damage by forming reactive oxygen species and nitrosilation of diverse proteins (Olivenza et al., 2000). Considering that D-ser acts as the major NMDA coagonist and that NO inhibits the SR activity, it could be thought that NO has double but opposite modulating roles in the excitotoxicity process. On the one hand, it synergies the neuronal damage induced by NMDA stimulation (Olivenza et al., 2000), and on the other, it reduces the coagonist actions of D-ser on this receptor (Shoji et al., 2006). This fact establishes the exciting possibility of the existence of NO-mediated modulating actions on glutamatergic transmission, destined to equilibrate the NMDA activation; this awaits assessment. Oxidative damage is also potentiated by the disruption of glu uptake, because low glial glu levels are correlated with a reduced glutathione production, a well-known endogenous antioxidant (Tan et al., 1998). Glu uptake is mediated by high affinity transporters placed in the plasma membrane of neurons and astrocytes (Danbolt, 2001) developing an essential function in the excitatory neurotransmission and preventing excitotoxicity (Nicholls and Attwell, 1990). These membrane transporters possess redox-sensing properties, due to the existence of sulphidryle groups in its structure. Oxidation of these groups following an oxidative insult might lead to a reduced glu uptake (Trotti et al., 1996). Due to the fact that D-ser modulates the NMDA activity and that it is suspected that glia is involved in excitotoxicity (Aschner et al., 1999; Swanson et al., 2004), it is possible that D-ser threatens neurons' survival, exacerbating the action of glu when there are altered levels of it. D-ser-promoted neurodegeneration is probably caused by an increased AMPA glial activation induced by glu which determines the SR activation by increasing the intracellular Ca^{2+}.

Alzheimer's disease (AD) is a pathology where the excitotoxic effect of D-ser is observed. The amyloid-β-peptide (Aβ) is proposed as the main physiopathological factor of AD (Butterfield and Boyd-Kimball, 2004). Aβ causes an inflammatory reaction in microglia, which triggers excitotoxic neuronal death (Barger and Basile, 2001; Wu and Barger, 2004; Butterfield and Boyd-Kimball, 2004).

In addition, increased NMDA activity has been found in the brains of individuals affected by AD, and meantime, an NMDA antagonist, has been found to have neuroprotective actions (Lipton, 2004). In contrast, the hippocampus of patients with AD shows increased levels in SR activity, and Aβ stimulates, in vitro, the release of excitotoxic levels of D-ser from microglia (Wu et al., 2004). The amyloid-β-peptide increases the levels of D-ser by two possible mechanisms. First, it promotes the stimulation of an activator protein-1 (AP-1) binding sequence located in the first intron of the SR gene, increasing its transcription rate (Wu and Barger, 2004). Second, Aβ regulates SR post-transcriptionally by causing increases in the microglial Ca^{2+} levels (Silei et al., 1999), which up-regulates the enzymatic activity (Cook et al., 2002). These roles of D-ser provide new pharmacological insights for the treatment of neurodegenerative diseases or disorders characterized by significant neuronal damage.

2.3 D-serine and schizophrenia: beyond dopamine and glutamate

Schizophrenia is a complex mental disorder that commonly emerges during adolescence, but its onset is earlier in males that in females (Castle et al., 1998). Although Carlsson postulated the dopaminergic hypothesis in the 1980s (Carlsson, 1988), more recently there has been the suggestion that schizophrenia might be related to glutamatergic hypofunction in the limbic system and forebrain (Coyle et al., 2001). It was found that NMDA receptor blockade by drugs such as phencyclidine and ketamine causes schizophrenic-like symptoms in primates and humans and exacerbates the symptoms of patients with schizophrenia (Lahti et al., 1995). In spite of antipsychotic drugs improving the positive symptoms of the disease (hallucinations, delirium, paranoia and others), they have poor effects on the negative symptoms (cognitive damage, social retreat, etc.). For this reason, researchers began to consider glutamatergic neurotransmission as a possible therapeutic target. In an attempt to counteract the NMDA hypofunction, several pharmacological approaches for the treatment of schizophrenia were tested, administrating modulators of the gly site of the receptor, together with antipsychotic drugs. Among the modulators evaluated were gly (Javitt et al., 1994), D-ser (Tsai et al., 1998) and D-cycloserine (Cascella et al., 1994). Although this approach had moderate success, it was observed that D-ser was nephrotoxic (Carone and Ganote, 1975) and that D-cycloserine, initially used as an antibiotic to treat tuberculosis (Epstein et al., 1955), has a central secondary effect after a year of treatment (Lewis et al., 1957). Despite the observations made by Carone and Ganote (1975) about the nephrotoxic actions of D-ser, Levy and colleagues evaluated the efficacy of gly at high doses (2004) and of D-ser (2005), added to the treatment of schizophrenic patients administrated with olanzapine and risperidone. Even though, in both cases, positive, negative and cognitive symptoms of the disease were improved (Levy et al., 2004, 2005), the doses required of D-ser where much smaller that the doses of gly (30 mg/kg/day and 800 mg/kg/day, respectively), due to the fact that D-ser passes through the blood brain barrier (BBB) more easily than gly (Olendorph, 1971). On the other hand, gly affects inhibitory synapses of the brain stem and spinal cord, by activating its strychnine sensitive receptors (Levy et al., 1999). On the contrary, D-ser did not demonstrate the ability to stimulate the known neurotransmission systems, but it was well tolerated by patients and was efficient in improving the schizophrenic symptoms, which makes it a useful therapeutic tool for the treatment of the disease (Levy et al., 2005). However, in patients treated with clozapine, none of the modulators mentioned, including D-ser (in a dose of 30 mg/kg/day), improves the schizophrenic symptoms, when administrated simultaneously with clozapine (Tsai et al., 1999). Finally, it was observed that a dietary supplement containing L-ser, which enhances the cerebral D-ser levels in rats when administrated systemically, provided an alternative pharmacological strategy (Takahashi et al., 1997; Hashimoto, 2002). As expected, the efficacy of the treatment with antipsychotics and L- or D-ser depends on the ability of these amino acids to pass through the BBB. Contrary to what has been observed for most L-amino acids, Bauer et al. (2005) demonstrated that D-ser has access to the CNS in higher quantity than L-ser. Considering that D- and L-ser have common transport systems, Bauer et al. (2005) proposes a preferred stereoselective transport for D-ser through the BBB. Although this transport system is not elucidated, it is known that subtype 1 of the Na^{+}-independent system L is the predominant uptake mechanism of D- and L-ser in the BBB (Yamamoto et al., 2005) and could be one of the candidates for supplying exogenous D-ser to the CNS. Actually, it is postulated that schizophrenia could have an important genetic component (Lin et al., 1997; Chumakov et al., 2002). Genetic linkage studies have involved DAAO in some forms of schizophrenia, which suggests that changes in the activity of this enzyme could alter the levels of D-ser and consequently the NMDA activation (Chumakov, 2002). A 50 million base pair region on hu-

man chromosome 13, located between q24 and q34, has been associated with schizophrenia in a number of studies (Lin et al., 1997; Blouin et al., 1998; Shaw et al., 1998). Chumakov et al. (2002) focused their studies on this region and identified two putative transcripts, called G72 and G30. The G72 transcript was detected in the amygdala, caudate nucleus and spinal cord. In vitro transcription/translation studies of the G72 transcript demonstrated that this produces a polipeptidic molecule formed by 153 amino acids, while similar analysis for the G30 transcript showed that this does not produce proteic molecules (Chumakov et al., 2002). Surprisingly, it was found that the translation product of G72 transcript was able to interact with DAAO, by protein-protein interactions. In fact, when the protein derived from G72 is added to an excess of DAAO, the activity of this enzyme increases threefold over the basal levels. Relating to this data, Chumakov (2002) proposes a model in which the expression of G72 in schizophrenia produces an enhanced activity of DAAO leading to a decrease in D-ser levels and promoting the NMDA hypofunction. However, the model proposed by Chumakov has disparities with the distribution of DAAO in the mammalian brain. This is due to the fact that this enzyme is almost exclusively found in the cerebellum, the spinal cord and the brain stem (Volpe et al., 1970; Morikawa, 2001), while schizophrenia involves a deficit in the prefrontal cortex and the limbic system (Harrison, 1999). In spite of that, Moreno et al. (1999) reported that DAAO is present in all brain regions. This finding supports the hypothesis that an enhanced DAAO activity could be involved in the glutamatergic hypofunction witnessed in schizophrenia.

2.4 L-ser or D-ser? A conformational contest

Although L-ser and D-ser are only differentiated by their atomic spatial disposition, they carry out very different functions in the CNS (Altman and Bayer, 1996; Yamada et al., 2000; Acosta et al., 2004). L-ser acts as a glia-derived neurotrophic factor (Savoca et al., 1995; Furuya et al., 2000; Acosta et al., 2004), while D-ser is an NMDA coagonist (Hashimoto et al., 1993a; Wolosker et al., 1999), a neuronal migration factor (Kim et al., 2005) and a cell death promoter (Aschner et al., 1999; Swanson et al., 2004).

While the main source of D-ser is through the action of SR on L-ser (Schell, 2004), L-ser is obtained from four different sources: from the diet, from 3-phosphoglycerate, by conversion of glycine through the action of the enzyme serine hydroxymethyltransferase (SHMT) and from the degradation of proteins and phospholipids (de Koning et al., 2003). Two biosynthetic pathways of L-ser from glucose have been identified: the phosphorilated pathway and the non-phosphorilated pathway (Sallach, 1956; Ichihara and Greenberg, 1957), the first being the main source of endogenous L-ser (Neidle and Dunlop, 2002). With reference to this data, it is not difficult to conclude that the body has greater facility to obtain L-ser than to obtain D-ser. In view of the fact that D-ser levels are tightly regulated by multiple factors (de Miranda et al., 2002; Neidle and Dunlop, 2002; Cook et al., 2002; Dunlop and Neidle, 2005; Foltyn et al., 2005; Kim et al., 2005; Strisovsky et al., 2005) because it could be excitotoxic when its extracellular levels are elevated (Aschner et al., 1999; Swanson et al., 2004), it is not surprising that it is much less available than L-ser. Although L-ser and other amino acids, such as L-alanine, can act as an NMDA coagonist (Kleckner and Dingledine, 1988; Thomson, 1990; Hashimoto et al., 1993a, b; Cotman et al., 1995), their potency is 20–30 times weaker than that observed for D-ser, lacking its ability to induce excitotoxicity. Then again, the L- and D-ser distribution, in the adult brain, is similar, being found in the cerebral cortex, the hippocampus and the corpus callosum, the regions where both amino acids are expressed in their highest levels (Schell et al., 1995, 1997; Wolosker et al., 1999; Yasuda et al., 2001). However, D-ser is also found at high concentration in the olfactory bulbs, the hypothalamus and the corpus striatum, but, unlike L-ser, its cerebellar levels are undetectable in the adult animal (Hashimoto et al., 1995; Yasuda et al., 2001). Additionally, it is known that the D-ser levels in the brain areas cited are parallel with the expression of the NMDA receptor (Schell et al., 1997).

Despite the differences mentioned above, the transport system through which glial cells uptake L-ser and D-ser is the same: the ASCT. Although this transporter uptakes both amino acids, it has a higher affinity for L-ser than for D-ser (Hayashi et al., 1997). Regarding the importance of L-ser in the CNS development, we studied, in our laboratory, the uptake of this non-essential amino acid in synaptosomes by the cerebral cortex of rats at different postnatal stages (P5, P7, P13, P21 and adult age) (Cheluja et al., 2006). We found that the uptake profiles of L-ser were similar at each postnatal stage considered, including the adult age, but the kinetic parameters varied with the age. While the maximum velocity of transport was observed at P21, the highest affinity for the substrate was observed at P5 (Cheluja et al., 2006). To date, there is poor information about similar studies carried out for D-ser.

Table 2. Similarities and differences between L-ser and D-ser

	L-ser	D-ser
Biosynthesis and other sources	– Diet – glycine (SHMT) – Glucose – SR	– SR
Degradation	– SR	– SR – DAAO
Distribution	Similar to D-ser	Similar to L-ser
Glial uptake system	ASCT	ASCT
NMDA coagonist	Poor	High
Role in neurodevelopment	Neurotrophic factor	Neuronal migration factor
Implication in neurodegeneration	No	Yes
Related pathologies	– 3-PGDH deficiency – PSP deficiency	– AD – Schizophrenia

Finally, L-ser has been involved in congenital pathologies characterized by a deficit in the expression of enzymes of the phosphorilated pathway of L-ser biosynthesis (de Koning et al., 2003; Acosta et al., 2004). In this context, two disorders were described: 3-phosphoglycerate dehydrogenase (3-PGDH) deficiency and phosphoserine phosphatase deficiency (PSP). Both disorders show severe psychomotor retardation, congenital microcephaly and hypomyelinization (Jaeken et al., 1996; de Koning et al., 2000). Administration of high doses of L-ser, alone or in combination with gly, improved the symptoms of the described disorders (Jaeken et al., 1996; de Koning et al., 1998; Pineda et al., 2000). In an attempt to summarize the main similarities and differences between L- and D-ser, Table 2 was constructed.

Conclusions

Since its discovery, our knowledge about the roles of D-ser in the CNS has markedly increased. It has shown significant relevance in the glutamatergic neurotransmission, but the total mechanism in which it is involved has not yet been found out. However, many important roles with different objectives were described for D-ser, which increase, even more, the potential of the glutamatergic system as a therapeutic target. It is interesting that the CNS needs two different NMDA receptor modulators, D-ser and gly, with the same molecular target, the glycine site, and each one has its own distribution (Schell et al., 1997). However, only one of them, gly, possesses a complete neurotransmitter system, including receptors and specific transporter proteins (Curtis and Jonhston, 1970; De Feudis et al., 1973; Legendre, 2001; Eulenburg et al., 2005). This creates the exquisite complexity of the CNS, giving a modulator role to a well-known neurotransmitter in a different system to which it belongs, depending on the brain region observed (Schell et al., 1997). Although the gly receptors bind the neurotransmitter in a similar way to the NMDA gly site, the last one does it in a strychnine insensitive profile, unlike the first (Perez-León and Salceda, 1995; Rodriguez-Contreras et al., 1998). Despite the fact that a specific receptor for D-ser has not yet been identified, it could be asked whether the NMDA receptor is the only one that binds it and promotes an effect. Is it possible that D-ser elicited any function on the glycinergic receptors? Considering that the administration of exogenous D-ser does not affect such receptors (Levy et al., 2005), the possibility of the existence of a receptor site, different from NMDA, to which D-ser binds and triggers an effect cannot be discarded. It could be thought that, if such a receptor exists, this small molecule might lead to the proposition of the idea of considering ''hybrid'' neurotransmission systems, where two structurally different molecules are responsible, equally, for the transmission of signals in systems considered, up to now, to have only one transmitter molecule. Because D-ser is the main endogenous NMDA coagonist in many brain areas (Schell et al., 1997; Mothet et al., 2006), D-ser was involved in AD (Silei et al., 1999; Wu et al., 2004) and schizophrenia (Takahashi et al., 1997; Hashimoto, 2002), both pathologies affecting the NMDA transmission.

On the other hand, the importance of D-ser in the CNS development, acting as a neuronal migration factor, has been demonstrated (Kim et al., 2005). In addition, if it is considered that L-ser is an essential glial neurotrophic factor for brain development (Savoca et al., 1995; Furuya et al., 2000; de Koning et al., 2003; Acosta et al., 2004), it could be suggested that the expression levels of SR, the molecular link between L- and D-ser, would be a key factor for the function of the developing neuro-glial circuits. The advances of our knowledge about the glutamatergic system, concerning NMDA modulation, could probably provide a new generation of drugs directed to the gly site of this receptor, to the SR or to the DAAO, all important regulators of glutamatergic neurotransmission.

References

Acosta GB, Takarada T, Yoneda Y (2004) L-serine in the brain. In: Yoneda Y (ed) Amino Acids Signaling 04, 2005, Kerala, India, pp 17–31

Altman J, Bayer SA (1996) Migration and distribution of two populations of hippocampal granule cell precursors during the perinatal and postnatal periods. J Comp Neurol 301: 365–381

Aschner M, Allen JW, Kimelberg HK, LoPachin RM, Strit WJ (1999) Glial cells in neurotoxicity development. Ann Rev Pharmacol Toxicol 39: 151–173

Barger SW, Basile AS (2001) Activation of microglia by secreted amyloid precursor protein evokes release of glutamate by cystine exchange and attenuates synaptic function. J Neurochem 76: 846–854

Barnes CA (2003) Long-term potentiation and the aging brain Alzheimer human brain. Brain Res Bull 38: 181–183

Bauer D, Hamacher K, Broer S, Pauleit D, Palm C, Zilles K, Coenen HH, Langen KJ (2005) Preferred stereoselective brain uptake of D-serine a modulator of glutamatergic neurotransmission. Nuclear Med Biol 32: 793–797

Bezzi P, Gundersen V, Galbete JL, Seifert G, Steinhauser C, Pilati E, Volterra A (2004) Astrocytes contain a vesicular compartment that is competent for regulated exocytosis of glutamate. Nat Neurosci 7: 613–620

Blouin JL, Dombroski BA, Nath SK, Lasseter VK, Wolyniec PS, Nestadt G, Thornquist M, Ullrich G, McGrath J, Kasch L, Lamacz M, Thomas MG, Gehrig C, Radhakrishna U, Snyder SE, Balk KG, Neufeld K, Swartz KL, DeMarchi N, Papadimitriou GN, Dikeos DG, Stefanis CN, Chakravarti A, Childs B, Housman DE, Kazazian HH, Antonarakis S, Pulver AE (1998) Schizophrenia susceptibility loci on chromosomes 13q32 and 8p21. Nat Genet 20: 70–73

Boehning D, Snyder SH (2003) Novel neural modulators. Ann Rev Neurosci 26: 105–131

Butterfield DA, Boyd-Kimball D (2004) Amyloid beta-peptide (1-42) contributes to the oxidative stress and neurodegeneration found in Alzheimer disease brain. Brain Pathol 14: 426–432

Carlsson A (1988) The current status of the dopamine hypothesis of schizophrenia. Neuropsychopharm 1: 179–186

Carone FA, Ganote CE (1975) D-serine nephrotoxicity. The nature of proteinuria, glucosuria, and aminoaciduria in acute tubular necrosis. Arch Pathol 99: 658–662

Cascella NG, Macciardi F, Cavallini C, Smeraldi E (1994) D-cycloserine adjuvant therapy to conventional neuroleptic treatment in schizophrenia: an open-label study. J Neural Trans Gen Sect 95: 105–111

Castle D, Sham P, Murray R (1998) Differences in distribution of ages of onset in males and females with schizophrenia. Schizophrenia Res 33: 179–183

Cheluja MG, Scolari MJ, Coelho TM, Blake MG, Boccia MM, Baratti CM, Acosta GB (2006) L-serine and GABA uptake by synaptosomes during postnatal development of rat. Comp Biochem Physiol A Mol Integr Physiol 2006 Feb 13; [Epub ahead of print]

Chumakov I, Blumenfeld M, Guerassimenko O, Cavarec L, Palicio M, Abderrahim H, Bougueleret L, Barry C, Tanaka H, La Rosa P, Puech A, Tahri N, Cohen-Akenine A, Delabrosse S, Lissarrague S, Picard FP, Maurice K, Essioux L, Millasseau P, Grel P, Debailleul V, Simon AM, Caterina D, Dufaure I, Malekzadeh K, Belova M, Luan JJ, Bouillot M, Sambucy JL, Primas G, Saumier M, Boubkiri N, Martin-Saumier S, Nasroune M, Peixoto H, Delaye A, Pinchot V, Bastucci M, Guillou S, Chevillon M, Sainz-Fuertes R, Meguenni S, Aurich-Costa J, Cherif D, Gimalac A, Van Duijn C, Gauvreau D, Ouellette G, Fortier I, Raelson J, Sherbatich T, Riazanskaia N, Rogaev E, Raeymaekers P, Aerssens J, Konings F, Luyten W, Macciardi F, Sham PC, Straub RE, Weinberger DR, Cohen N, Cohen D (2002) Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. Proc Natl Acad Sci USA 99: 13365–13367

Coco S, Calegari F, Pravettoni E, Pozzi D, Taverna E, Rosa P, Matteoli M, Verderio C (2003) Storage and release of ATP from astrocytes in culture. J Biol Chem 278: 1354–1362

Cook SP, Galve-Roperh I, Martinez del Pozo A, Rodriguez-Crespo I (2002) Direct calcium binding results in activation of brain serine racemase. J Biol Chem 277: 27782–27792

Corrigan JJ (1969) D-amino acids in animals. Science 164: 142–149

Cotman CW, Kahle JS, Miller SE, Ulas J, Bridges RJ (1995) The fourth generation of progress. In: Bloom FE, Kupfer DJ (eds) Psychopharmacology. Raven Press, New York, pp 75–85

Cull-Candy S, Brickley S, Farrant M (2001) NMDA receptor subunits: diversity, development and disease. Curr Opin Neurobiol 11: 327–335

Curtis DR, Johnston DA (1970) Strychnine, glycine and vertebrate postsynaptic inhibition. Nature 225: 1258–1259

Danbolt NC (2001) Glutamate uptake. Prog Neurobiol 65: 1–105

Das S, Sasaki YF, Rothe T, Premkumar LS, Takasu M, Crandall JE, Dikkes P, Conner DA, Rayudu PV, Cheung W, Chen HS, Lipton SA, Nakanishi N (1998) Increased NMDA current and spine density in mice lacking the NMDA receptor subunit NR3A. Nature 393: 377–381

De Feudis FV (1979) High-affinity binding processes for GABA, glycine, and beta-alanine in synaptosome-enriched fractions of rats CNS. J Physiol 75: 651–654

de Koning TJ, Duran M, Dorland L, Gooskens R, Van Schaftingen E, Jaeken J, Blau N, Berger R, Poll-The BT (1998) Beneficial effects of L-serine and glycine in the managements of seizures in 3-phosphoglycerate dehydrogenase deficiency. Ann Neurol 44: 261–265

de Koning TJ, Jaeken J, Pineda M, Van Maldergem L, Poll-The BT, van der Knaap MS (2000) Hypomielination and reversible white matter attenuation in 3-phosphoglycerate dehydrogenase deficiency. Neuropediatrics 31: 287–292

de Koning TJ, Snell K, Duran M, Berger R, Poll-The BT, Surtees R (2003) L-Serine in disease and development. Biochem J 371: 653–661

de Miranda J, Panizzutti R, Foltyn VN, Wolosker H (2002) Cofactors of serine racemase that physiologically stimulate the synthesis of the N-methyl-D-aspartate (NMDA) receptor coagonist D-serine. Proc Natl Acad Sci USA 99: 14542–14547

Dunlop DS, Neidle A, McHale D, Dunlop DM, Lajtha A (1986) The presence of free D-aspartic acid in rodents and man. Biochem Biophys Res Commun 141: 27–32

Dunlop DS, Neidle A (2005) Regulation of serine racemase activity by amino acids. Brain Res Mol Brain Res 133: 208–214

Epstein IG, Nair KG, Boyd LI (1955) Cycloserine, a new antibiotic, in the treatment of human pulmonary tuberculosis: a preliminary report. Antibiotic Med Clin Ther 1: 80–93

Eulenburg V, Armsen W, Betz H, Gomeza J (2005) Glycine transporters: essential regulators of neurotransmission. Trends Biochem Sci 30: 325–333

Foltyn VN, Bendikov I, De Miranda J, Panizzutti R, Dumin E, Shleper M, Li P, Toney MD, Kartvelishvily E, Wolosker H (2005) Serine racemase modulates intracellular D-serine levels through an alpha, beta-elimination activity. J Biol Chem 280: 1754–1763

Furukawa H, Gouaux E (2003) Mechanism of activation, inhibition and specificity: crystal structures of the NMDA receptor NR1 ligand-binding core. EMBO J 22: 2873–2885

Furuya S, Tabata T, Mitoma J, Yamada K, Yamasaki M, Makino A, Yamamoto T, Watanabe M, Kano M, Hirabayashi (2000) L-serine and glycine serve as major astroglia-derived trophic factors for cerebellar Purkinje neurons. Proc Natl Acad Sci USA 97: 11528–11533

Goff DC, Coyle JT (2001) The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. Am J Psychiatry 158: 1367–1377

Gressens P (2000) Mechanisms and disturbances of neuronal migration. Pediatr Res 48: 725–730

Hardingham GE, Bading H (2003) The Yin and Yang of NMDA receptor signalling. Trends Neurosci 26: 81–89

Harrison PJ (1999) The neuropathology of schizophrenia. A critical review of the data and their interpretation. Brain 122: 593–624

Hashimoto A (2002) Effect of the intracerebroventricular and systemic administration of L-serine on the concentrations of D- and L-serine in several brain areas and periphery of rat. Brain Res 955: 214–220

Hashimoto A, Nishikawa T, Oka T, Takahashi K (1993a) Endogenous D-serine in rat brain: N-methyl-D-aspartate receptor-related distribution and aging. J Neurochem 60: 783–786

Hashimoto A, Kumashiro S, Nishikawa T, Oka T, Takahashi K, Mito T, Takashima S, Doi N, Mizutani Y, Yamazaki T et al. (1993b) Embryonic development and postnatal changes in free D-aspartate and D-serine in the human prefrontal cortex. J Neurochem 61: 348–351

Hashimoto A, Oka T, Nishikawa T (1995) Anatomical distribution and postnatal changes in endogenous free D-aspartate and D-serine in rat brain and periphery. Eur J Neurosci 7: 1657–1663

Hatten ME (1999) Central nervous system neuronal migration. Ann Rev Neurosci 22: 511–539

Hayashi F, Takahashi K, Nishikawa T (1997) Uptake of D- and L-serine in C6 glioma cells. Neurosci Lett 239: 85–88

Haydon PG (2001) GLIA: listening and talking to the synapse. Nat Rev Neurosci 2: 185–193

Helboe L, Egebjerg J, Moller M, Thomsen C (2003) Distribution and pharmacology of alanine-serine-cysteine transporter 1 (asc-1) in rodent brain. Eur J Neurosci 18: 2227–2238

Ichihara A, Greenberg DM (1957) Further studies on the pathway of serine formation from carbohydrate. J Biol Chem 224: 331–340

Jaeken J, Detheux M, Van Maldergem L, Fouton M, Carchon H, Van Schaftigen E (1996) 3-phosphoglycerate dehydrogenase deficiency: an inborn error of serine biosynthesis. Arch Dis Child 74: 542–545

Javitt DC, Zylberman I, Zukin SR, Heresco-Levy U, Lindenmayer JP (1994) Amelioration of negative symptoms in schizophrenia by glycine. Am J Psychiatry 151: 1234–1236

Javitt DC, Balla A, Sershen H (2002) A novel alanine-insensitive D-serine transporter in rat brain synaptosomal membranes. Brain Res 941: 146–149

Kartvelishvily E, Shleper M, Balan L, Dumin E, Wolosker H (2006) Neuron-derived D-serine release provides a novel means to activate N-methyl-D-aspartate receptors. J Biol Chem 281: 14151–14162

Kemp JA, McKernan RM (2002) NMDA receptor pathways as drug targets. Nat Neurosci [Suppl] ■: 1039–1042

Kim JJ, Foy MR, Thompson RF (1996) Behavioral stress modifies hippocampal plasticity through N-methyl-D-aspartate receptor activation. Proc Natl Acad Sci USA 93: 4750–4753

Kim PM, Aizawa H, Kim PS, Huang AS, Wickramasinghe SR, Kashani AH, Barrow RK, Huganir RL, Ghosh A, Snyder SH (2005) Serine racemase: activation by glutamate neurotransmission via glutamate receptor interacting protein and mediation of neuronal migration. Proc Natl Acad Sci USA 102: 2105–2110

Kleckner NW, Dingledine R (1988) Requirement for glycine in activation of NMDA receptors expressed in Xenopus oocytes. Science 241: 835–837

Konno R (2003) Rat cerebral serine racemase: amino acid deletion and truncation at carboxy terminus. Neurosci Lett 349: 111–114

Lahti AC, Koffel B, LaPorte D, Tamminga CA (1995) Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. Neuropsychopharmacology 13: 9–19

Legendre P (2001) The glycinergic inhibitory synapse. Cell Mol Life Sci 58: 760–793

Levy U, Javitt DC, Ermilov M, Mordel C, Silipo G, Lichtenstein M (1999) Efficacy of high-dose glycine in the treatment of enduring negative symptoms of schizophrenia. Arch Gen Psychiatry 56: 29–36

Levy U, Ermilov M, Lichtenberg P, Bar G, Javitt DC (2004) High dose glycine added to olanzapine and risperidone for the treatment of schizophrenia. Biol Psych 55: 165–171

Levy U, Javitt DC, Ebstein R, Vass A, Lichtenberg P, Bar G, Catinari S, Ermilov M (2005) D-serine efficacy as add-on to risperidone and olanzapine for treatment-refractory schizophrenia. Biol Psych 57: 577–585

Lewis WC, Calden G, Thurston JR, Gilson WE (1957) Psychiatric and neurological reactions to cycloserine in the treatment of tuberculosis. Dis Chest 32: 172–182

Lin MW, Sham P, Hwu HG, Collier D, Murray R, Powell JF (1997) Suggestive evidence for linkage of schizophrenia to markers on chromosome 13 in Caucasian but not Oriental populations. Hum Genet 99: 417–420

Lipton SA (2004) Failures and successes of NMDA receptor antagonist: molecular basis for the use of open-channel blockers like memantine in the treatment of acute and chronic neurologic insults. NeuroRx 1: 101–110

Magariños AM, McEwen BS (1995) Stress – induced atrophy of apical dendrites of hipocampal CA3 neurons neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors. Neurosci 69: 89–98

Matsui T, Sekiguchi M, Hashimoto A, Tomita U, Nishikawa T, Wada K (1995) Functional comparison of D-serine and glycine in rodents: the effect on cloned NMDA receptors and the extracellular concentration. J Neurochem 65: 454–458

Matsuo H, Kanai Y, Tokunaga M, Nakata T, Chairoungdua A, Ishimine H, Tsukada S, Ooigawa H, Nawashiro H, Kobayashi Y, Fukuda J, Endou H (2004) High affinity D- and L-serine transporter Asc-1: cloning and dendritic localization in the rat cerebral and cerebellar cortices. Neurosci Lett 358: 123–126

Moghaddam B (1993) Stress preferentially increases extraneuronal levels of excitatory amino acids in the prefrontal cortex: comparison to hippocampus and basal ganglia. J Neurochem 60: 1650–1657

Moreno S, Nardacci R, Cimini A, Ceru MP (1999) Immunocytochemical localization of D-amino acid oxidase in rat brain. J Neurocytol 28: 169–185

Morikawa A, Hamase K, Inoue T, Konno R, Niwa A, Zaitsu K (2001) Determination of free D-aspartic acid, D-serine and D-alanine in the brain of mutant mice lacking D-amino acid oxidase activity. J Chromatogr B Biomed Sci Appl 757: 119–125

Mothet JP, Pollegioni L, Ouanounou G, Martineau M, Fossier P, Baux G (2005) Glutamate receptor activation triggers a calcium-dependent and SNARE protein-dependent release of the gliotransmitter D-serine. Proc Natl Acad Sci USA 102: 5606–5611

Mothet JP, Rouaud E, Sinet PM, Potier B, Jouvenceau A, Dutar P, Videau C, Epelbaum J, Billard JM (2006) A critical role for the glial-derived neuromodulator D-serine in the age-related deficits of cellular mechanisms of learning and memory. Aging Cell ■: 267–274

Nagata Y, Konno R, Niwa A (1994) Amino acid levels in D-alanine-administered mutant mice lacking D-amino acid oxidase. Metabolism 43: 1153–1157

Nedergaard M, Ransom B, Goldman SA (2003) New roles for astrocytes: redefining the functional architecture of the brain. Trends Neurosci 26: 523–530

Neidle A, Dunlop DS (2002) Allosteric regulation of mouse brain serine racemase. Neurochem Res 27: 1719–1724

Nicoll RA (2003) Expression mechanisms underlying long-term potentiation: a postsynaptic view. Phil Trans R Soc London Ser B Biol Sci 358: 721–726

Nicholls D, Attwell D (1990) The release and uptake of excitatory amino acids. Trends Pharmacol Sci 11: 462–468

O'Brien KB, Miller RF, Bowser MT (2005) D-Serine uptake by isolated retinas is consistent with ASCT-mediated transport. Neurosci Lett 385: 58–63

Oldendorph WM (1971) Brain uptake of radio labeled amino acids, amines and hexoses after arterial injection. Am J Physiol 221: 1629–1639

Oliet SH, Piet R, Poulain DA, Theodosis DT (2004) Glial modulation of synaptic transmission: insights from the supraoptic nucleus of the hypothalamus. Glia 47: 258–267

Olivenza R, Moro MA, Lizasoain I, Lorenzo P, Fernández AP, Rodrigo J, Boscá L, Leza JC (2000) Chronic stress induces the expression of inducible nitric oxide synthase in rat brain cortex. J Neurochem 74: 785–791

Olney JW (1971) Glutamate-induced neuronal necrosis in the infant mouse hypothalamus: an electron microscopy study. J Neuropathol Exp Neurol 30: 75–90

Perez-León JA, Salceda R (1995) Different specific binding sites of [^{3}H]glycine and [^{3}H]strychnine in synaptosomal membranes isolated from frog retina. Neurochem Res 20: 915–922

Pfrieger FW (2002) Role of glia in synapse development. Curr Opin Neurobiol 12: 486–490

Pilone MS (2000) D-Amino acid oxidase: new findings. Cell Mol Life Sci 57: 1732–1747

Pineda M, Vilaseca MA, Artuch R, Santos S, García Gonzalez MM, Aracil A, Van Schaftingen E, Jaeken J (2000) 3-phosphoglycerate dehydrogenase deficiency in a patient with West Syndrome. Dev Med Child Neurol 42: 629–633

Priestley T, Laughton P, Myers J, Le Bourdelles B, Kerby J, Whiting PJ (1995) Pharmacological properties of recombinant human N-methyl-D-aspartate receptors comprising NR1a/NR2A and NR1a/NR2B subunit assemblies expressed in permanently transfected mouse fibroblast cells. Mol Pharmacol 48: 841–848

Prybylowski K, Wenthold RJ (2004) N-Methyl-D-aspartate receptors: subunit assembly and trafficking to the synapse. J Biol Chem 279: 9673–9676

Reiprich P et al. (2005) Neonatal NMDA receptor blockade disturbs neuronal migration in rat somatosensory cortex in vivo. Cereb Cortex 15: 349–358

Ribeiro CS, Reis M, Panizzutti R, de Miranda J, Wolosker H (2002) Glial transport of the neuromodulator D-serine. Brain Res 929: 202–209

Rodríguez-Contreras A, Calderón F, López-Colome AM (1998) Strychnine-insensitive [^{3}H] glycine binding to synaptosomal membranes from the chick retina. Int J Dev Neurosci 16: 413–421

Sallach HJ (1956) Formation of serine from hydroxypyruvate and L-alanine. J Biol Chem 223: 1101–1108

Sapolsky R, Uno H, Robert C, Finch C (1990) Hippocampal damage associated with prolonged glucocorticoid exposure in primates. J Neurosci 10: 2897–2904

Savoca R, Ziegler U, Sonderegger P (1995) Effects of L-serine on neurons in vitro. J Neurosci Methods 61: 159–167

Schell MJ, Molliver ME, Snyder SH (1995) D-serine, an endogenous synaptic modulator: localization to astrocytes and glutamate stimulated release. Proc Natl Acad Sci USA 92: 3948–3952

Schell MJ, Brady RO, Molliver ME, Zinder SH (1997) D-serine as neuromodulator: regional and developmental localizations in rat brain glia resemble NMDA receptors. J Neurosci 17: 1604–1615

Schell MJ (2004) The N-methyl-D-aspartate receptor glycine site and D-serine metabolism: an evolutionary perspective. Phil Trans R Soc Lond 359: 943–964

Shaw SH, Kelly M, Smith AB, Shields G, Hopkins PJ, Loftus J, Laval SH, Vita A, De Hert M, Cardon LR, Crow TJ, Sherrington R, DeLisi LE (1998) A genome-wide search for schizophrenia susceptibility genes. Am J Med Genet 81: 364–376

Shoji K, Mariotto S, Ciampa AR, Suzuki H (2006) Regulation of serine racemase activity by D-serine and nitric oxide in human glioblastoma cells. Neurosci Lett 392: 75–78

Silei V, Fabrizi C, Venturini G, Salmona M, Bugiani O, Tagliavini F, Lauro GM (1999) Activation of microglial cells by PrP and beta-amyloid fragments raises intracellular calcium through L- type voltaje sensitive calcium channels. Brain Res 818: 168–170

Strisovsky K, Jiraskova J, Barinka C, Majer P, Rojas C, Slusher BS, Konvalinka J (2003) Mouse brain serine racemase catalyzes specific elimination of L-serine to pyruvate. FEBS Lett 535: 44–48

Strisovsky K, Jiraskova J, Mikulova A, Rulisek L, Konvalinka J (2005) Dual substrate and reaction specificity in mouse serine racemase: identification of high-affinity dicarboxylate substrate and inhibitors and analysis of the beta-eliminase activity. Biochemistry 44: 13091–13100

Swanson RA, Ying W, Kauppinen TM (2004) Astrocyte influences on ischemic neuronal death. Curr Mol Med 4: 193–205

Takahashi K, Hayashi F, Nishikawa T (1997) In vivo evidence for the link between L- and D-serine metabolism in rat cerebral cortex. J Neurochem 69: 1286–1290

Takano T, Lin JH, Arcuino G, Gao Q, Yang J, Nedergaard M (2001) Glutamate release promotes growth of malignant gliomas. Nat Med 7: 1010–1015

Tan S, Sagara Y, Liu Y, Maher P, Schubert D (1998) The regulation of reactive oxygen species production during programmed cell death. J Cell Biol 141: 1423–1432

Thomson AM (1990) Glycine is a coagonist at the NMDA receptor/channel complex. Prog Neurobiol 35: 53–74

Trotti D, Rossi D, Gjesdal O, Levy LM, Racagni G, Danbolt NC, Volterra A (1996) Peroxynitrite inhibit glutamate transporter subtypes. J Biol Chem 271: 5976–5979

Tsai G, Yang P, Chung LC, Lange N, Coyle JT (1998) D-serine added to antipsychotics for the treatment of schizophrenia. Biol Psychiatry 44: 1081–1089

Tsai GE, Yang P, Chung LC, Tsai I, Tsai C, Coyle JT (1999) D-serine added to clozapine for the treatment of schizophrenia. Am J Psychiatry 156: 1822–1825

Urai Y, Jinnouchi O, Kwak KT, Suzue A, Nagahiro S, Fukui K (2002) Gene expression of D-amino acid oxidase in cultured rat astrocytes: regional and cell type specific expression. Neurosci Lett 324: 101–104

Ventura R, Harris KM (1999) Three-dimensional relationships between hippocampal synapses and astrocytes. J Neurosci 19: 6897–6906

Volpe JJ, Lee G, Laster L, Robinson JC (1970) Regional distribution of isozymes of D-amino-acid oxidase and acetylesterase in developing primate brain. Exp Neurol 28: 76–87

Volterra A, Meldolesi J (2005) Astrocytes, from brain glue to communication elements: the revolution continues. Nat Rev Neurosci 6: 626–640

Williams SM, Diaz CM, Macnab LT, Sullivan RK, Pow DV (2006) Immunocytochemical analysis of D-serine distribution in the mammalian brain reveals novel anatomical compartmentalizations in glia and neurons. Glia 53: 401–411

Wolosker H, Blackshaw S, Snyder SH (1999) Serine racemase: a glial enzyme synthesizing D-serine to regulate glutamate-N-methyl-D-aspartate neurotransmission. Proc Natl Acad Sci USA 96: 13409–13414

Wolosker H, Panuzzutti R, de Miranda J (2002) Neurobiology through the looking-glass: D-serine as a new glial-derived transmitter. Neurochem Int 41: 327–332

Wu S, Barger SW (2004) Induction of serine racemase by inflammatory stimuli is dependent on AP-1. Ann N Y Acad Sci 1035: 133–146

Wu SZ, Bodles AM, Porter MM, Griffin WS, Basile AS, Barger SW (2004) Induction of serine racemase expression and D-serine release from microglia by amyloid beta-peptide. J Neuroinflammation 1: 2

Xia M, Liu Y, Figueroa DJ, Chiu CS, Wei N, Lawlor AM, Lu P, Sur C, Koblan KS, Connolly TM (2004) Characterization and localization of a human serine racemase. Brain Res Mol Brain Res 125: 96–104

Xie X, Dumas T, Tang L, Brennan T, Reeder T, Thomas W, Klein RD, Flores J, O'Hara BF, Heller HC (2005) Lack of the alanine-serine-cysteine transporter 1 causes tremors, seizures, and early postnatal death in mice. Brain Res 1052: 212–221

Yacubova E, Komuro H (2003) Cellular and molecular mechanisms of cerebellar granule cell migration. Cell Biochem Biophys 37: 213–234

Yamada K, Fukaya M, Shibata T, Kurihara H, Tanaka K, Inoue Y, Watanabe M (2000) Dynamic transformation of Bergmann glial fibers proceeds in correlation with dendritic outgrowth and synapse formation of cerebellar Purkinje cells. J Comp Neurol 418: 106–120

Yamamoto N, Tomita U, Umino A, Nishikawa T (2001) Uptake of D-serine by synaptosomal P2 fraction isolated from rat brain. Synapse 42: 84–86

Yang SN, Huang LT, Wang CL, Chen WF, Yang CH, Lin SZ, Lai MC, Chen SJ, Tao PL (2003) Prenatal administration of morphine decreases CREBSerine-133 phosphorylation and synaptic plasticity range mediated by glutamatergic transmission in the hippocampal CA1 area of cognitive-deficient rat offspring. Hippocampus 13: 915–921

Yasuda E, Ma N, Semba R (2001) Immnunohistochemical demonstration of L-serine distribution in the rat brain. Neurochem 12: 1027–1030

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