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INVITED REVIEW

Serine racemase: an unconventional enzyme for an unconventional transmitter

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Abstract The discovery of large amounts of D-serine in the brain challenged the dogma that only L-amino acids are relevant for eukaryotes. The levels of D-serine in the brain are higher than many L-amino acids and account for as much as one-third of L-serine levels. Several studies in the last decades have demonstrated a role of D-serine as an endogenous agonist of *N*-methyl-D-aspartate receptors (NMDARs). D-Serine is required for NMDAR activity during normal neurotransmission as well as NMDAR overactivation that takes place in neurodegenerative conditions. Still, there are many unanswered questions about D-serine neurobiology, including regulation of its synthesis, release and metabolism. Here, we review the mechanisms of D-serine synthesis by serine racemase and discuss the lessons we can learn from serine racemase knockout mice, focusing on the roles attributed to D-serine and its cellular origin.

Keywords D-serine · NMDA · Glutamate · Astrocytes · Gliotransmission · Glia

D-Serine in the brain and discovery of serine racemase

D-Serine was identified in the brain by Nishikawa, Hashimoto and coworkers (Hashimoto et al. 1992) when they were trying to develop brain-penetrating drugs to enhance NMDAR function, like *N*-myristoyl-D-serine. Serendipitously, they found large amounts of endogenous D-serine in the brains of control rats when trying to detect D-serine-derived compounds in injected animals (Nishikawa 2005). The surprising discovery of D-serine in the brain led some laboratories to embark on the task of unraveling D-serine origin and function.

Despite the existence of high levels of D-serine in the brain, the lack of knowledge about its origin hampered much progress in the field. One major question was whether D-serine was an endogenous or an exogenous amino acid. Experiments employing intraperitoneal injection of L-serine showed that it might be a precursor for D-serine synthesis (Dunlop and Neidle 1997; Takahashi et al. 1997). Moreover, Esaki and co-workers found that partially purified extracts from silkworm *Bombyx mori* (a eukaryote) could convert L- into D-serine (Uo et al. 1998). On the other hand, another study suggested that D-serine was synthesized by the glycine cleavage system (Iwama et al. 1997). The levels of D-serine were greatly reduced in patients lacking activity of the glycine cleavage system, while glycine levels in postmortem brains were several folds higher than controls (Iwama et al. 1997).

Using conventional biochemical purification, the D-serine biosynthetic enzyme was isolated and cloned from rat brain (Wolosker et al. 1999a, b). The enzyme, dubbed serine racemase (SR), converts L- into D-serine and does not use glycine as a substrate. SR is inhibited by high levels of glycine in a competitive manner (Dunlop and Neidle 2005; Strisovsky et al. 2005), which may explain the reduction of D-serine observed in patients exhibiting non-ketotic hyperglycemia due to mutations in the glycine cleavage system (Iwama et al. 1997).

The discovery of the D-serine biosynthetic enzyme preceded our understanding about its role in brain function.

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SR belongs to the fold type II of pyridoxal 5′-phosphate (PLP)-dependent enzymes (De Miranda et al. 2000). The cofactor binds to a lysine at the catalytic site of SR (Lys56) to form an internal aldimine, like in other members of the PLP-dependent family of enzymes (Wolosker et al. 1999a). Interestingly, SR has striking similarity to the serine/threonine dehydratase enzyme of *E. coli*, rather than to classical amino acid racemases, like alanine racemase (Wolosker 2011).

In addition to PLP, SR binds divalent cations (mainly Mg^{2+}, but also Ca^{2+}), and also has a nucleotide binding site which binds the complex Mg.ATP with high affinity. Mg^{2+} binds at a cation binding site located outside the catalytic site. Chelating Mg^{2+} greatly reduces the enzyme activity (Cook et al. 2002; De Miranda et al. 2002), and it is likely that the Mg^{2+}-binding site is important for proper folding. The crystal structure of the yeast homolog of mammalian SR shows that Mg^{2+} coordinates with the carboxylic groups of Glu-208 and Asp-214, residues that are conserved in mammalian SR as well (Goto et al. 2009). Nucleotides (mainly the complex Mg.ATP) also stimulate enzyme activity (De Miranda et al. 2002; Neidle and Dunlop 2002). ATP is not hydrolyzed by the enzyme and binds to a groove formed at the intersection between the domain interface and the subunit interface (Goto et al. 2009; Smith et al. 2010). Like divalent cations, Mg.ATP seems to stabilize the folding of SR.

The discovery of the physiological cofactors of SR also disclosed the main chemical reaction catalyzed by SR: the α, β-elimination of water from L-serine to form pyruvate and NH_4 (De Miranda et al. 2002). This reaction is reminiscent of the homology of SR to serine dehydratases. For each D-serine generated, about four molecules of pyruvate are produced. Lower, but significant elimination with D-serine and L-threonine are also detectable (Foltyn et al. 2005). α, β-Elimination with L-threonine, however, is not accompanied by any racemization. Several artificial substrates for α, β-elimination have been identified (e.g., L-serine *O*-sulfate, L-threo-3-hydroxyaspartate), but none of them are epimerized, indicating that the racemization reaction is more selective toward serine (Panizzutti et al. 2001; Strisovsky et al. 2005).

The physiological roles of SR α, β-elimination are not clear. Pyruvate (from L-serine) and 2-oxobutyrate (from L-threonine) are important metabolites. However, the rate of pyruvate formation from other sources (e.g., glycolysis) is several orders of magnitude faster than the rate of α, β-elimination of L-serine, making it unlikely that SR-derived pyruvate plays an important metabolic role.

The α, β-elimination by SR provides some indication on how this enzyme evolved. Its structural similarity with eliminases, rather than racemases, likely reflects convergent evolution. SR probably originated from a serine dehydratase gene that partially lost its eliminase activity and acquired serine racemization; both activities are well known to be catalyzed by the cofactor PLP.

Another possible function of the α, β-elimination is to regulate D-serine levels (Foltyn et al. 2005). SR also eliminates with D-serine, and this results in partial consumption of synthesized D-serine. It is possible that this activity limits D-serine production in cells and may be especially relevant in forebrain regions that lack significant levels of D-amino acid oxidase enzyme, a peroxisomal protein that degrades D-amino acids (Hashimoto et al. 1993; Nagata et al. 1999). SR mutants lacking α, β-elimination activity more efficiently produce D-serine in vitro and in intact cells. However, the role of α, β-elimination with D-serine has not yet been demonstrated in vivo.

SR reaction mechanisms

The rate of racemization by SR is about 100-fold lower than the bacterial alanine racemase. The catalytic constant (Kcat) of SR racemization ranges between 3 and 45 min^{-1}. These values indicate that it takes more than 1 s for SR to release one molecule of D-serine. This relatively low efficiency of racemization fits the slow D-serine turnover in the brain (about 17 h half-life) (Dunlop and Neidle 1997).

SR reactions involve a combination of two classical PLP-mediated reactions, through the formation of a common intermediate, the resonance-stabilized carbanion (Fig. 1) (Foltyn et al. 2005). Racemization is attained by deprotonation of the external aldimine (L-serine-PLP complex) leading to the formation of a planar carbanion. The proton abstraction from the α-carbon is made possible by the special orientation of the neutral amino group of Lys56 toward the α-proton of L-serine-PLP and the deprotonated hydroxyl group of Ser84 toward the α-proton of D-serine-PLP, in a so-called two-base mechanism (Fig. 1) (Goto et al. 2009). D-Serine-PLP is generated by the reprotonation of the carbanion at the opposite side of the molecule.

β-Elimination is attained by protonation on the substrate β-hydroxy group which results in removal of water (hence, a dehydratase reaction). This is associated with the formation of aminoacrylate–PLP, an unstable intermediate that spontaneously breaks down into pyruvate and ammonia (Fig. 1).

SR regulation

A major unanswered question in the field is with regard to the regulation of D-serine dynamics. Several regulatory mechanisms and protein interactions have been proposed to

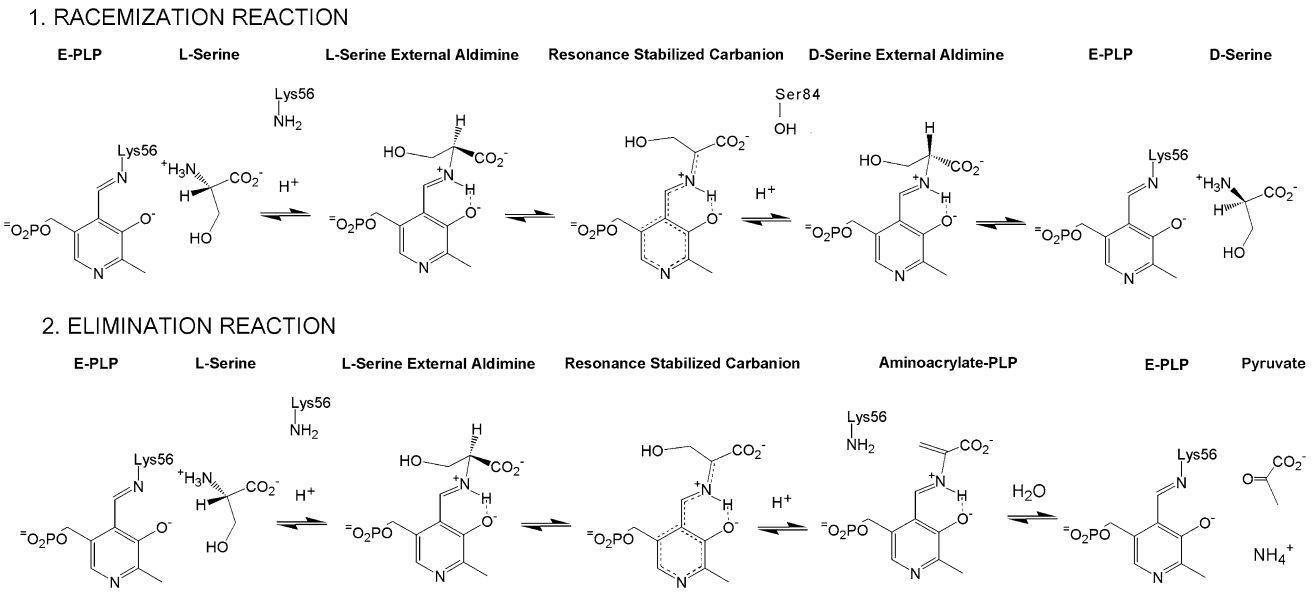

Fig. 1 Reactions catalyzed by SR. For further details, see Foltyn et al. (2005) and Goto et al. (2009)

play a role in modulating SR activity and D-serine release, but the in vivo relevance of the large majority of proposed mechanisms is not known. One complicating factor is that there is no consensus on the cell types that produce D-serine. In this context, some studies focused on astrocytes or microglia, while others investigated D-serine synthesis in neurons. It is not clear which mechanisms are cell specific, but some forms of regulation might be similar in different types of cells.

In astrocytes, mouse SR binds to Grip-1 and Pick-1 (Baumgart et al. 2007; Fujii et al. 2006; Hikida et al. 2008; Kim et al. 2005). The binding occurs through the C-terminal region of mouse SR, which contains a PDZ binding consensus. Binding of SR to Grip-1 increases D-serine production (Kim et al. 2005). Snyder and co-workers proposed that glutamate acting on α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) causes Grip-1 dissociation from the receptors, with consequent binding to SR and enhancement of D-serine production/release (Kim et al. 2005). A direct effect of Pick-1 in SR activity has not been demonstrated, but Pick-1 deficient mice display lower brain D-serine, indicating that it may regulate the mechanisms of D-serine synthesis (Hikida et al. 2008). Of note, the last four amino acids of SR required for binding to the PDZ domains of Grip-1 and Pick-1 are absent in rat and bovine SR, indicating that this interaction may modulate species-specific behaviors (Dumin et al. 2006; Konno 2003).

Another interactor of SR is Golga-3, a protein that binds to the cytosolic face of the Golgi apparatus (Dumin et al. 2006). Interaction with Golga-3 also disclosed a pool of SR that was strongly attached to the membrane fraction. Golga-3 stabilizes SR levels through inhibition of its ubiquitination which leads to slower degradation by the ubiquitin–proteasome system (Dumin et al. 2006). The ubiquitin ligase enzyme (E3) that attaches ubiquitin chains to SR, however, has not been identified yet.

In addition to regulation by protein interactors, SR is regulated by glutamate receptors by different mechanisms. SR binds to and is inhibited by phosphatidylinositol (4,5)-bisphosphate (PIP2) in membranes (Mustafa et al. 2009). Activation of the metabotropic glutamate receptors (mGluR5) increases D-serine synthesis by promoting degradation of PIP2 via activation of phospholipase C. This relieves the inhibition of SR and activates D-serine synthesis (Mustafa et al. 2009). PIP2 affects the nucleotide site of SR, as its inhibition of the enzyme is competitive with Mg.ATP. SR mutants that are unable to bind to PIP2 display higher activity when transfected into cells and are insensitive to mGluR5 activation, confirming that interaction with PIP2 mediates mGluR5 effects on SR.

On the other hand, activation of NMDARs inhibits SR activity. Following NMDAR stimulation, SR translocates from the cytosol (where it resides) to dendritic membranes. This is associated with SR inactivation toward D-serine production (Balan et al. 2009). SR is palmitoylated at serine/threonine residues (*O*-palmitoylation), which is likely to contribute to membrane binding (Balan et al. 2009). Another biochemical mechanism that may control SR interaction with membranes is Thr227 phosphorylation, found only in the membrane-bound SR. Mutation of Thr227Ala decreases the levels of membrane-bound SR under non-stimulated conditions (Balan et al. 2009). SR translocation to the membrane may constitute a feedback

inhibition mechanism to decrease NMDAR activity (through reduction in D-serine synthesis) and prevent neurotoxicity at neighboring synapses. Notably, NMDAR-mediated translocation to the membrane is irreversible, even after blockade with NMDAR antagonists (Balan et al. 2009). The data indicate that NMDAR-mediated translocation is a last resort mechanism used by cells that are already committed to die. NMDAR-dependent inactivation of SR may protect neighboring cells by preventing further D-serine production and limiting the spread of excitotoxicity.

Another mechanism by which NMDAR activation controls SR activity involves NOS activation and SR nitrosylation at Cys113 (Mustafa et al. 2007). This leads to a decrease in SR activity via displacement of ATP binding (Mustafa et al. 2007). Thus, in addition to promoting SR translocation to the membrane (Balan et al. 2009), NMDAR activation inhibits SR activity via reversible *S*-nitrosylation. Diffusion of NO to non-neuronal cells will inactivate glial SR. On the other hand, NMDA-mediated SR translocation is NO independent, indicating that different cell types use different regulatory mechanisms involving different mediators.

Phosphorylation of mouse SR at Thr71 stimulates the rate of D-serine synthesis by increasing the turnover of the enzyme (Foltyn et al. 2010). Metabolic labeling indicates that this site comprises at least 80 % of the total SR phosphoprotein. Phosphorylation of SR at Thr71 was confirmed in vivo by phosphoproteome analysis of mouse brain (Wisniewski et al. 2010; Huttlin et al. 2010). The specific kinase has not been identified, though it likely belongs to the class of proline-directed kinases. This phosphorylation site, however, is not conserved in the human SR, indicating that it may mediate rodent-specific regulation/behavior.

SR inhibitors

In light of the role of SR in neurotoxicity, drugs that curb D-serine synthesis may be useful to treat neurodegenerative diseases. A number of SR inhibitors have been employed to demonstrate a role of SR in synthesizing D-serine and regulating NMDAR-dependent physiological processes. Early papers used a general inhibitor of PLP-dependent enzymes, like aminooxyacetic acid (Wolosker et al. 1999b) or L-serine *O*-sulfate, an artificial substrate for α, β-elimination that blocks D-serine synthesis by replacing the natural substrate L-serine (Panizzutti et al. 2001). Subsequent studies employed phenazine and phenazine derivatives to block SR-mediated granule cell migration in the developing cerebellum (Kim et al. 2005). Furthermore, Konvalika and co-workers found that some dicarboxylic substrate analogs are high-affinity competitive SR inhibitors (Strisovsky et al. 2005). These include malonate and L-erythro-3-hydroxyaspartate that work in the micromolar range. The latter was recently employed to demonstrate the role of astrocytic D-serine in regulating hippocampal LTP (Henneberger et al. 2010). Nevertheless, there is no data on the selectivity of any SR inhibitor, and their use to study complex physiological processes, like neurotransmission, should be avoided, unless SR knockout (KO) mice or shRNA strategy can be used as controls to ensure the inhibitor specificity toward SR. The recent description of SR structure and the availability of in silico screening and docking approaches will facilitate the development of more selective inhibitors.

Serine racemase-knockout mouse strains

The development of SR-KO mice was crucial to clarify the physiological and pathological roles of SR. To date, three SR-KO mouse strains have been developed (Inoue et al. 2008; Basu et al. 2009; Labrie et al. 2009) (Table 1). The first SR-KO mouse strain was generated by homologous recombination in embryonic stem (ES) cells derived from the C57BL/6 strain, and the resulting insertional mutation-type SR-KO mice therefore have a pure C57BL/6 genetic background suitable for the analysis of brain functions (Miya et al. 2008). This strain is now referred to as SR-KO^{HM}. The second, flox-type, SR-KO mouse strain was generated by homologous recombination in 129Sv-derived ES cells and backcrossed with the C57BL/6 strain (Basu et al. 2009). This strain is referred to as SR-KO^{JTC}. The third SR-KO mouse strain was identified from the F1 progeny of ethylnitrosourea (ENU)-mutagenized C57BL/6 male mice and DBA/2 female mice (Labrie et al. 2009). The ENU-induced mutation was identified as a nonsense mutation resulting in the conversion of tyrosine 269 of the SR protein to a stop codon (SrrY269* strain). The F1 founder carrying the SrrY269* mutation was backcrossed with the C57BL/6 strain. The main difference among these mouse strains is their genetic background. Recently, cell-type selective conditional SR-KO mice, namely, astrocyte-selective SR conditional knockout (aSR-KO^{JTC}) and forebrain glutamatergic neuron-selective SR knockout (nSR-KO^{JTC}) mice have been reported (Benneyworth et al. 2012).

Production of D-serine catalyzed by SR in vivo

The three strains of SR-KO mice show no detectable SR protein, and their brain D-serine levels are 10–20 % of the wild-type (WT) controls. The levels of L-serine, glycine

Table 1 Summary of phenotypes of three SR-KO mouse strains

Strains	SR-KO^{HM}	SR-KO^{JTC}	SrrY269*
Genetic background	C57BL/6	129 Sv ES derived × C57BL/6 (N7)	C57BL/6 ENU mutagenesis × DBA derived × C57BL/6 (N8)
Biochemical changes			
SR protein	No	No	No
D-Ser	Reduced (10 % of WT)	Reduced (10 %)	Reduced (10–20 %)
L-Ser	No change	No change	Increased (frontal cortex)
NMDAR activity	Reduced (excitotoxicity assay)	Reduced (electrophysiology)	Not tested
Behavioral tests			
PPI	No change	No change	Impaired
Locomotion	No change	Enhanced (male)	No change
Learning and Memory	Impaired (contextual learning)	Impaired (Morris water maze)	Impaired (Morris water maze)

and glutamate in the cerebral cortex of SR-KO^{HM} and SR-KO^{JTC} mice are equivalent to those of WT controls (Inoue et al. 2008; Basu et al. 2009), though one study reported higher L-serine levels in the frontal cortex of SrrY269* mice (Labrie et al. 2009). These findings indicate that D-serine production in the mouse brain is predominantly catalyzed by SR (Table 1).

The origin of the residual 10–20 % of D-serine is not known. Conceivably, residual D-serine may originate in peripheral tissues. Specific expression of SR was detected in the liver using SR-KO^{HM} mice as negative controls (Horio et al. 2011). SR-KO^{HM} mice display reduced levels of D-serine in some peripheral organs like the kidney, muscle and testis. However, the levels of D-serine in the liver, spleen, pancreas, epididymis, heart, lung and eye of SR-KO^{HM} mice are comparable to those in WT mice (Horio et al. 2011). Possible alternative pathways for D-serine synthesis include the glycine cleavage system (Iwama et al. 1997), the hydrolysis of phosphoserine by phosphoserine phosphatase (Wood et al. 1996) or exogenous D-serine from intestinal bacterial flora.

Localization of SR in vivo

To fully clarify the role of D-serine in the brain, it is important to precisely define the cellular localizations of SR and D-serine. Early data on SR distribution indicated that the enzyme was glial (Wolosker et al. 1999a). However, subsequent immunohistochemical and in situ hybridization studies revealed the localization of the SR protein (Kartvelishvily et al. 2006) and its mRNA (Yoshikawa et al. 2007) primarily in neurons. More definitive data on SR distribution was obtained using SR-KO^{HM} mice as negative controls (Miya et al. 2008). This approach ensured the specificity of the antibodies and revealed that SR was predominantly localized in pyramidal neurons of the cerebral cortex and hippocampal CA1 region. Double-immunofluorescence experiments revealed that SR colocalizes with neuron-specific nuclear protein (NeuN), but not with astrocytic markers, namely, glial fibrillary acidic protein (GFAP) and 3-phosphoglycerate dehydrogenase (Phgdh). In the striatum, SR is expressed by γ-aminobutyric acid (GABA)ergic medium spiny neurons. Furthermore, in the adult cerebellum, weak but significant SR signals are detected in GABAergic Purkinje cells. The data indicate that SR is expressed by the main neuronal populations in most brain regions, irrespective of the excitatory or inhibitory nature of the cells. Therefore, neurons are likely to be the main source of D-serine (Miya et al. 2008). These results, however, cannot exclude the possibility of SR expression in glial cells in vivo at levels below the sensitivity of the available antibodies. In primary cultured cells, SR is expressed in both neurons and astrocytes at similar levels, indicating that the pattern of SR expression in astrocytes in vitro differs from that observed in vivo.

Recently, the notion that SR is predominantly neuronal gathered additional support from the analysis of nSR-KO^{JTC} mice. These mice exhibit lower D-serine and SR expression in the brain, especially in cortical glutamatergic neurons (Benneyworth et al. 2012). Astrocyte-specific SR-KO mice (aSR-KO^{JTC}) exhibit no change in D-serine levels. Notably, the decrease in SR expression observed in nSR-KO^{JTC} mice (about 60 % in the cortex) was more prominent than the decrease in D-serine by HPLC (around 30 % decrease). While these results underscore the importance of neurons in synthesizing D-serine (Benneyworth et al. 2012), they raise additional questions regarding possible alternative sources of D-serine. D-Serine crosses the blood–brain barrier and it is possible that some of brain D-serine comes from the periphery. SR expression in the liver is upregulated in nSR-KO^{JTC} mice, suggesting that

there may be a "cross talk" between the brain and the liver that maintains peripheral and brain D-serine levels (Benneyworth et al. 2012).

Morphological features of brain of SR-KO mice

SR-KO^{JTC} mice display reduced cortical volume (Balu et al. 2012). Neurons in the medial prefrontal cortex (PFC) of SR-KO^{JTC} mice show reduced complexity, total length and spine density of apical dendrites (DeVito et al. 2011). Furthermore, pyramidal neurons in the primary somatosensory cortex (S1) of SR-KO^{JTC} mice also show reduced complexity, total length and spine density of apical and basal dendrites (Balu et al. 2012). However, the gross formation and patterning of barrels in the posteriomedial barrel subfield in S1 are normal. Decreased expression of brain-derived neurotrophic factor (BDNF) may be associated with these morphological changes (Balu et al. 2012).

Gene expression in SR-KO mice

The expression levels of various proteins were analyzed in SR-KO mice, and the observed changes may reflect adaptations to NMDAR hypofunction. The expression of NMDAR subunits, namely, GluN1, GluN2A and GluN2B, which are abundant in the forebrain, are the same in brain homogenates of WT and SR-KO mice (Inoue et al. 2008; Labrie et al. 2009). In contrast, SR-KO^{JTC} mice displayed higher levels of GluN1 and GluN2A subunit expression in postsynaptic density (PSD) fractions obtained from hippocampus, though the levels of these receptors in PFC of SR-KO^{JTC} mice were unaltered (Balu and Coyle 2011). The expression levels of other GluN2B and PSD-95 proteins were unchanged in the PSD fraction from SR-KO^{JTC} mice. Furthermore, another study reported higher expression of GluN1 subunit in the striatum of SR-KO^{JTC} mice (Mustafa et al. 2010). These observed increases in NMDAR subunits may reflect synaptic adaptations to compensate for presumed lower NMDAR activation in SR-KO, but an effect due to differences in genetic backgrounds of the KO mouse strains cannot be ruled out. The differences described above in the expression of glutamate receptors were not observed in all SR-KO strains. For instance, SR-KO^{HM} mice made in pure C57BL/6 background display no changes in striatal GluN1 subunit protein (Inoue, R., Imai-Tabata, A. and H. M, unpublished results).

The expression levels of AMPARs GluA1 and GluA2 subunits, D-amino acid oxidase and glycine transporter 1 (GlyT1) remained unchanged in the whole-brain SrrY269* homogenates (Labrie et al. 2009). In contrast, SrrY269* overexpress the PICK1, which binds to the carboxy terminus of mouse SR (Labrie et al. 2009). A combination of DNA microarray experiments, RT-PCR and Western blot analysis revealed higher expression of transthyretin, ectonucleotide pyrophosphatase/phosphodiesterase 2, klotho, insulin-like growth factor 2, prolactin receptor and claudin 2 proteins in SrrY269* mice (Labrie et al. 2009). The physiological significance of the increases in the expression levels of these proteins remains unknown.

Some genes were expressed at lower levels in SR-KO, and their reduction might contribute to the phenotype. Notably, SR-KO^{JTC} mice display reduced expression of BDNF protein. Lower BDNF expression provides a possible explanation for the morphological changes in dendritic structure of neurons in the somatosensory cortex observed in the SR-KO^{JTC} mice (Balu et al. 2012).

Neurotransmission in SR-KO mice

Analyses of neurotransmission between Schaffer collateral and hippocampal CA1 by whole-cell patch-clamp recording from brain slices of juvenile (postnatal days 21–28) SR-KO mice revealed slower decay kinetics of NMDAR-mediated excitatory postsynaptic potentials (EPSPs), suggesting a contribution of the GluN2B subunit containing NMDAR in SR-KO^{JTC} mice (Basu et al. 2009). This observation is supported by the higher GluN2B protein expression detected in juvenile SR-KO^{JTC} mice brains (Balu and Coyle 2011). Expression of hippocampal synaptic plasticity was also altered in SR-KO^{JTC} mice. The NMDAR-dependent long-term potentiation (LTP) of synaptic transmission induced using a pairing stimulation protocol is impaired in juvenile SR-KO^{JTC} mice (Basu et al. 2009). The impairment of LTP induction can be rescued using bath-applied D-serine. In contrast to these phenotypes of neurotransmission in juvenile SR-KO^{JTC} mice, we have not detected impairment of neurotransmission in hippocampal CA1 synapses of adult mice in a recent study using SR-KO^{HM} (Watanabe, M., H. M. and Manabe, T., unpublished results).

In the nSR-KO^{JTC} mice, NMDAR-mediated EPSPs were reduced and LTP induction by weak single train tetanus protocol was lower than in WT control mice. In contrast, LTP induced with a strong three-train protocol was comparable to that of WT mice (Benneyworth et al. 2012). These data support the notion that neuron-derived D-Serine regulates synaptic plasticity.

D-Serine is present in the retina, though at levels 20-fold lower than those of L-serine (Sullivan et al. 2011). Whole-cell recordings from SR-KO^{JTC} retinal ganglion cells lack light-evoked NMDAR currents. In contrast to the attenuated NMDAR responses detected by electrophysiological analysis, no obvious visual impairment was observed in

behavioral tests (Sullivan et al. 2011). Though SR and D-serine were initially reported to be exclusively localized to glial Muller cells, subsequent studies showed robust SR expression by ganglion cells, supporting a possible role of neuron-derived D-serine in the retina as well (Dun et al. 2008).

Additional biochemical evidence supporting lower NMDAR activation in SR-KO comes from nitrosylation studies. SR-KO^{JTC} display lower levels of nitrosylation of known nitrosylation targets, a process known to be dependent on NOS activity via NMDAR activation (Mustafa et al. 2010).

Behaviors of SR-KO mice

NMDARs are involved in learning and memory, and these processes have been studied using the Morris water maze paradigm which assesses hippocampal NMDAR-dependent spatial memory (Morris et al. 1986). SR-KO^{JTC} and SrrY269* male mice show an impaired spatial reference memory, indicating a role of D-serine in spatial discrimination (Basu et al. 2009; Labrie et al. 2009). Likewise, SR-KO^{HM} also exhibited impaired contextual learning (Inoue,R. and H.M., unpublished observation). SR-KO^{JTC} mice exhibit disrupted representation of the odor associated with events in distinct experiences monitored by object recognition and the odor sequence test (DeVito et al. 2011). On the other hand, SR-KO^{JTC} mice showed intact relational memory in a test of transitive inference, and displayed normal behaviors in the detection of novel objects and in spatial displacement tasks (DeVito et al. 2011). These findings were correlated to altered dendritic morphology and lower spine density in pyramidal neurons of the prefrontal cortex. Altogether, these observations indicate that D-serine is required for cognitive ability.

SR-KO^{JTC} mice show normal motor coordination and no evidence of anxiety or depression-like behaviors (Basu et al. 2009; Labrie et al. 2009). Male SR-KO^{JTC} mice show increased locomotor activity (Basu et al. 2009). The effects of SR-KO on other sexually dimorphic phenotypes in behavioral assays reported by Basu et al. remain to be determined.

Prepulse inhibition (PPI) of the acoustic startle response (ASR) is one indication of the sensory–motor gating process, and is inhibited by the pharmacological suppression of NMDAR. Inhibition of PPI is also observed in patients with schizophrenia (Cadenhead et al. 2000). Despite data indicating reduced NMDAR function, no consistent inhibition of PPI was observed in SR-KO mice, as the effects were strain specific. One study found no difference in PPI between WT and SR-KO^{HM} mice (Mori and Inoue 2010). Basu et al. (2009) also reported the absence of PPI deficit in SR-KO^{JTC} mice, although they observed elevated ASR in female SR-KO mice. In contrast, SrrY269* mice showed diminished PPI; however, under the same conditions, control mice showed no enhancement of PPI with the increase in the prepulse tones (Labrie et al. 2009).

The effects of psychotomimetic drugs, such as phencyclidine (PCP) and amphetamine (AMPH) as well as methamphetamine (METH), on locomotor activity and sensory-motor gating were also examined in SR-KO mice in the context of PPI. Acute PCP-enhanced startle reactivity was increased in SR-KO^{JTC} mice, but AMPH effects were similar to those of WT mice (Benneyworth et al. 2011). Repeated administration of METH results in behavioral sensitization in WT mice, but not in SR-KO^{HM} mice (Horio et al. 2012). This effect was accompanied by lower METH-induced dopamine release in the nucleus accumbens along with decreased METH-induced phosphorylation of ERK1/2 in the striatum of SR-KO^{HM} mice (Horio et al. 2012).

Altogether, the data indicate that SR-KO shows signs of NMDAR hypofunction in many behavioral paradigms, but the effects seem to vary depending on the genetic background of the strain of mice used (Table 1).

Neurodegeneration in SR-KO mice

SR-KO mice display less susceptibility to excitotoxic insults in several models of neurodegeneration, indicating a role of endogenous D-serine in mediating NMDAR-dependent neurotoxicity. Injection of NMDA in the cerebral cortex induces excitotoxic neuronal cell death, and this effect was attenuated in SR-KO^{HM} mice (Inoue et al. 2008). SR-KO^{HM} mice are also resistant to Aβ peptide-mediated neurodegeneration in vivo, an experimental model relevant to Alzheimer disease (Suh and Checler 2002). Injection of Aβ 1–42 peptide into the hippocampus caused significantly less damage in SR-KO^{HM} mice (Inoue et al. 2008). Aβ-induced neurotoxicity was mostly dependent on the function of the NMDAR, because the damage by Aβ was prevented by pretreatment with the NMDAR antagonist MK-801. These results implicate D-serine in neurodegeneration (Inoue et al. 2008). Furthermore, infarct volume following middle cerebral artery occlusion is significantly reduced in SR-KO^{JTC} mice (Mustafa et al. 2010). Together with previous data using brain slices in vitro which showed that D-serine might be the dominant co-agonist for NMDAR-mediated excitotoxicity (Katsuki et al. 2004; Shleper et al. 2005), the results clearly demonstrate that D-serine plays a role in neurodegeneration. This has implications for neurodegenerative diseases in which NMDARs overstimulation leads to neuronal death, such as Huntington and Alzheimer disease. The prominent

role of D-serine in neurotoxicity is consistent with the notion that D-serine availability is a fail-safe mechanism to prevent excessive NMDAR activity during increases in glutamatergic neurotransmission.

Is D-serine a gliotransmitter?

Several studies indicate that D-serine may be released from glia and function as a "gliotransmitter" to regulate NMDAR transmission and synaptic plasticity. This notion is supported by the presence of D-serine in glia (Schell et al. 1995, 1997; Williams et al. 2006), especially in vesicular structures (Bergersen et al. 2011; Mothet et al. 2005; Williams et al. 2006) that undergo exocytosis upon glutamate receptor stimulation (Mothet et al. 2005). Furthermore, Mothet and co-workers demonstrated that the Ca^{2+}-dependent D-serine pool corresponds to vesicles of the secretory pathway in astrocytes (Martineau et al. 2008). Based on these data, previous electrophysiological effects of endogenous D-serine on NMDARs were mostly interpreted as evidence for gliotransmission (Mothet et al. 2000; Henneberger et al. 2010; Panatier et al. 2006; Yang et al. 2003, 2005). The gliotransmitter hypothesis is still somewhat controversial, as there is no specific pharmacological approach that interferes with glial metabolism or transmitter release without having indirect effects on neuronal metabolism or physiology (Agulhon et al. 2008, 2010). Furthermore, non-vesicular release of D-serine and glutamate from glia has been demonstrated (O'Brien and Bowser 2006; Ribeiro et al. 2002; Rosenberg et al. 2010). The recent data showing that neurons can be a source for D-serine release, and that SR is predominantly localized in neurons (Kartvelishvily et al. 2006; Rosenberg et al. 2010; Benneyworth et al. 2011; Miya et al. 2008), raise further questions regarding the relative roles of neurons versus glia in releasing D-serine. Future studies employing more selective pharmacologic or genetic approaches will be required to differentiate between glia- and neuron-derived D-serine. In any case, a substantial number of existing pharmacologic and genetic models indicate that D-serine is required for optimal NMDAR function and opens new possibilities for developing drugs to modulate NMDAR function.

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