Serine racemase: a key player in neuron activity and in neuropathologies

Barbara Campanini[1], Francesca Spyrakis[2], Alessio Peracchi[3], Andrea Mozzarelli[1,4]

[1]Department of Pharmacy, University of Parma, Parma, Italy, [2]Department of Food Sciences, University of Parma, Parma, Italy, [3]Department of Biosciences, University of Parma, Parma, Italy, [4]National Institute of Biostructures and Biosystems, Rome, Italy

TABLE OF CONTENTS

1. ABSTRACT

Serine racemase is the pyridoxal 5'-phosphate-dependent enzyme that catalyzes L-serine racemisation to D-serine, and L- and D-serine beta-elimination in mammalian brain. D-serine is the essential co-agonist of the N-methyl-D-aspartate receptor, that mediates neurotransmission, synaptic plasticity, cell migration and long term potentiation. High and low D-serine levels have been associated with distinct neuropathologies, aging-related deficits and psychiatric disorders due to either hyper- or hypo-activation of the receptor. Serine racemase dual activity is regulated by ATP, divalent cations, cysteine nitrosylation, post-translational modifications, and interactions with proteins that bind either at the N- or C-terminus. A detailed elucidation of the molecular basis of catalysis, regulation and conformational plasticity, as well as enzyme and D-serine localization and neurons and astrocytes cross-talk, opens the way to the development of enzyme inhibitors and effectors for tailored therapeutic treatments.

2. INTRODUCTION

Racemases are enzymes that reversibly convert L- to D-amino acids and have been identified in all species (1). Racemases described to date belong to two classes, pyridoxal 5'-phosphate (PLP)-independent and PLP-dependent. Serine racemase (SR, EC. 5.1.1.18) belongs to the latter and catalyzes the reversible racemisation of L-serine to D-serine. Not surprisingly, this enzyme is present in bacteria where D-amino acids are used for the formation of cell wall and for the synthesis of defence compounds. However, the discovery of D-serine, as well as other D-amino acids such as D-aspartate, in the brain of higher organisms, including humans (2, 3), was unexpected (4) and triggered a wealth of investigations. Specifically, it was demonstrated that D-serine and the glutamate-, voltage-gated ion channel N-methyl-D-aspartate receptor (NMDAR) share a common pattern of distribution in rat brains (5). Moreover, it was found that in neurons D-serine binds to NMDAR acting as a potent co-agonist of glutamate in the modulation of receptor function (6, 7). The

binding site was identified as the strychnine-independent site/glycine binding site, as originally glycine was considered the main co-agonist of NMDAR.

In the last decade, several studies have addressed issues associated with i) SR structure, ii) SR catalytic reactions and mechanisms, iii) identification of factors regulating SR activity, iv) post-translational modifications and interacting proteins controlling SR localization and activity, v) the distribution of SR and D-serine *in vivo* in different cellular types, vi) NMDAR and SR cross-talks, vii) D-serine exchange processes between neurons and glia; viii) the role of D-amino acid oxidase (DAAO), the only enzyme able to degrade D-serine, beside SR itself, ix) the association of a variety of neuropathologies with either high D-serine levels (e. g. Alzheimer, Parkinson, amyotrophic lateral dystrophy and ischemia) or low D-serine levels (e. g. schizophrenia); x) the development of SR effectors that might be exploited for disease treatment. However, an in-depth understanding of the biological role of D-serine and the relationships between effectors, the catalytic machinery and conformational plasticity of SR are still elusive.

Many excellent reviews have been published on D-serine, SR and NMDAR (8-13). In the present review, we will focus primarily on the biochemical aspects of SR with emphasis on structure-function relationships, catalysis, regulation and on the development of SR ligands that might be suitable for the pharmacological treatment of SR-associated neuropathologies.

3. SERINE RACEMASE STRUCTURE AND DYNAMICS

SR is a PLP-dependent enzyme belonging to the fold-type II structural group, containing enzymes able to catalyze β-elimination and β-replacement reactions, such as tryptophan synthase (14) and *O*-acetylserine sulfhydrylase (15). With respect to other PLP-dependent enzyme the highest sequence identity is found for fission yeast SR and bacterial threonine dehydratase (90% identity). A 27% identity was also found between human SR and human serine dehydratase (SDH) or cystathionine-β-synthase (12, 16). SR and SDH are both able to catalyze the β-elimination of L-serine but SDH is not capable to carry out L-serine racemization (12). The identity among SR from different species ranges between 35% and 91% with the highest values recorded among mammalian SRs and the lowest for yeast and mammalian SRs. Residues involved in the binding of PLP, and effectors, such as cations and ATP (see below) are in general highly conserved (13).

There are currently eight SR structures deposited in the PDB:

1. Human SR (hSR): 3L6B and 3L6R, both with malonate as ligand. 3L6R is selenomethionine hSR (17).

2. Rat SR (rSR): 3HMK and 3L6C, in the holo form and complexed with malonate, respectively (17).

3. *Schizosaccharomyces pombe* SR (SpSR): in the holo form (1V71) with 5'-adenylyl methylenediphosphate (AMP-PCP) (1WTC), modified with PDD (lysino-D-alanyl residue) in the holo form (2ZPU) and modified with PDD and complexed with serine (2ZR8) (18, 19).

All these SRs crystallized as dimers, whereas in solution SR forms dimers with traces of tetramers (12). Dimers are stabilized by the formation of hydrophobic contacts, without the involvement of any disulfide bridge (20-22). Covalently cross-linked dimers were observed in the presence of reactive oxygen and nitrogen species (23). The human and rat dimer in the presence of malonate presents a buried monomer-monomer surface area of about 840 $Å^2$ and 735 $Å^2$, respectively, while the uncomplexed rat dimer surface increases to 827 $Å^2$.

Fold type II enzymes are characterized by the presence of a larger and a smaller domain, both exhibiting the typical open α-β architecture. As represented in Figure 1a showing the structure of hSR complexed with the inhibitor malonate (17), the large domain contains the PLP cofactor covalently bound to Lys56 through a Schiff base, forming the internal aldimine. This domain is made up of residues 1-68 and 157-340 organized in a twisted six-stranded β-sheet, with all but the first strand parallel, surrounded by ten α-helices. Residues 78-155 form the small domain, also arranged in a twisted four-stranded parallel β-sheet surrounded by three α-helices, one located on the solvent side and two at the domains interface. The small and large domains are connected by a flexible hinge region formed by amino acids 69-77, not fully detected in the hSR structure. In the SpSR, the PLP cofactor is bound to Lys56 (human numeration) or to a modified lysinoalanyl residue (18, 19). The interactions with the surrounding residues resemble those typical of fold type II enzymes (24) with the involved residues conserved not only in SR orthologs but also in many other PLP-dependent enzymes (25-27). In particular, the phosphate group interacts with Gly185, Gly186, Gly187, Gly188, known as the Gly loop, Met189 and three water molecules. The 3-hydroxy group forms a hydrogen bond with Asn86 belonging to the asparagine loop (Ser83-Ser84-Gly85-Asn86-His87 (25)), while Ser313 contacts the pyridine nitrogen. Both Asn86 and Ser313 play an essential role in regulating the electronic state of the PLP-Schiff base conjugate (28-30). The pyridine ring is sandwiched by Thr285, Gly239 and Val240 on the *re*-face and Phe55 on the *si*-face, oriented towards the larger domain in the protein core.

All SR structures present a divalent cation octahedrally coordinated in the larger domain, close to the PLP binding site, i.e. Mg^{2+} in SpSR structures and Mn^{2+} in the rat and human enzymes. In hSR, Mn^{2+} is coordinated by the side-chains of Glu210 and Asp216 (conserved in rSR and mutated to Asn in SpSR), the backbone carbonyl of Ala214 (substituted by a glycine in SpSR) and three water molecules (17). Hoffman et al. recently demonstrated that other residues such as Cys217 and Lys221 are fundamental for metal binding as well as for enzymatic activity (31). The absence of Glu210, Asp216 and Ala214 in the *Scapharca* and *Pyrobaculum* SR is consistent with the
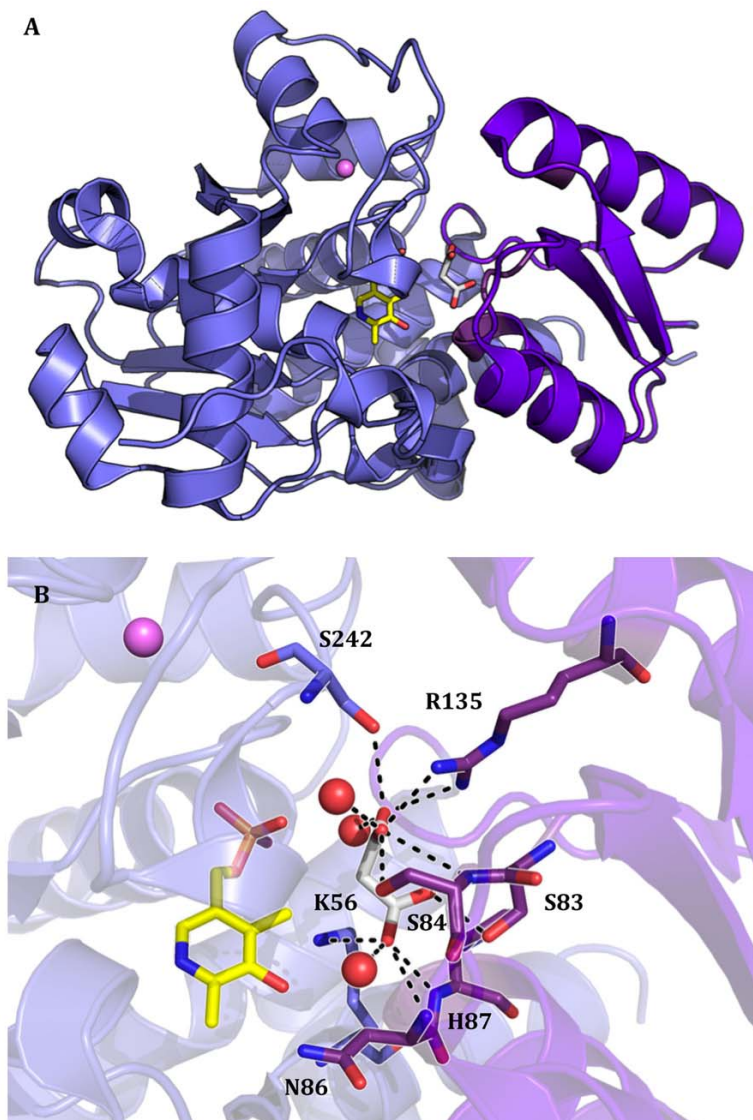
Figure 1. A. Cartoon representation of hSR complexed with malonate (PDB code 3L6B). The large domain is colored in dark violet while the small domain in light violet. The PLP and the malonate are represented by yellow and white sticks. The rose sphere identifies the manganese ion. B. Close up of the binding site. The hydrogen bonds formed between the malonate and the surrounding residues and water molecules (red spheres) are shown by black dashed lines.

absence of increased racemization in the presence of Ca^{2+} or Mg^{2+} (32, 33). Experiments performed with SpSR demonstrated that the enzyme loses its catalytic activity in the presence of EDTA and recovers it upon the addition of Mg^{2+} or Ca^{2+} (18). Also mammalian brain SR is activated by the presence of divalent cations (13, 34). Nevertheless, considering the relative distance of the ion site from the active site, cations are not considered to be directly involved into the catalytic process, rather in the stabilization of protein structure. Neither cations nor ATP appeared to affect SR oligomerization state (20, 35).

Different studies demonstrated that both racemization and α,β-elimination reactions are stimulated upon the addition of Mg•ATP (10, 34). In spite of its relevance for SR function, only an ATP analog, namely AMP-PCP, has been crystallographically detected in SpSR (PDB code 1WTC). The ATP analogue is bound into the groove formed at the domain and subunit interface (18). This binding arrangement was also supported by a hSR model that positions two molecules of AMP-PCP at the dimerization interface (36). Following the yeast numeration, the residues involved in hydrogen bonding the ATP effector are Asn25, Lys52, Met53, Ala114, Tyr119, Asn311 on one monomer and Thr31, Ser33, Thr34, Arg275, Lys277 on the other, and a significant number of water molecules. Many of these residues are conserved in human and rat SRs, while only two are present in *Escherichia coli* threonine dehydratase, in *Salmonella typhimurium O*-acetyl serine sulfhydrylase or in *E. coli*
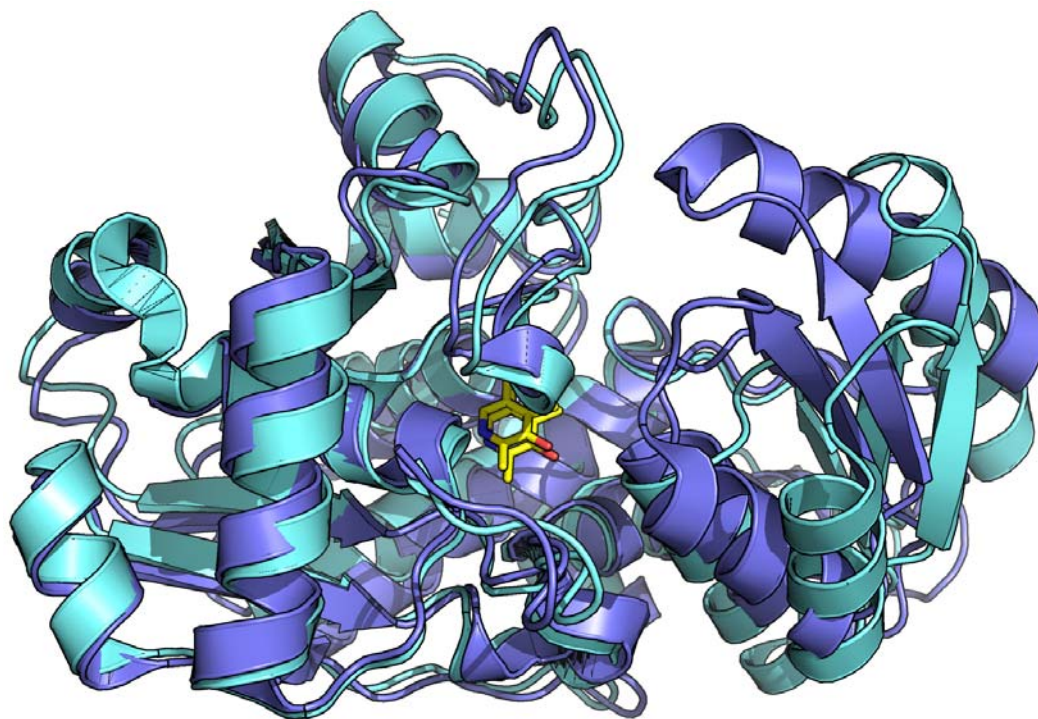
Figure 2. Structural alignment of rSR in the holo (cyan) and malonate-bound (violet) form (PDB code 3HMK and 3L6C, respectively).

tryptophan synthase. A model for the hSR-ATP complex was recently reported by Jiraskova-Vanickova et al. who docked two ATP molecules with a coordinated Mg^{2+} into the hSR dimer (PDB code 3L6B), using the yeast complex as template (12). ATP is localized so that the phosphate groups are buried inside the dimerization interface while the adenosine moiety is directed towards the solution. Overall, ATP is stabilized by ten interactions, four of which are bifurcated and involve the phosphate groups. Gln89 appeared to be particularly relevant by holding in place Thr52 and Tyr121, interacting, respectively, with the phosphate groups and with the adenosine aromatic moiety. All these observations led to the conclusion that the ATP binding site resembles that of other ATP-binding proteins (37), and that only the dimer is able to provide the necessary number of interactions to stabilize the complex. It was also proposed that the last eleven C-terminus residues, not identified in the crystallographic structure, might also contact and stabilize the ATP molecule (12). The comparison with the yeast protein crystallized in the absence of AMP-PCP revealed a different organization of the dimer, thus suggesting that ATP does not affect the conformation of the monomer but the relative orientation of the two subunits. No significant change in the orientation of the cofactor or of the binding pocket side chains is observed. Even if no direct connection exists between PLP and ATP, a hydrogen bond network formed by Met53, Asn84, Gln87, Glu281, Asn311, and water molecules might be responsible for the fine tuning of the active site structure and the stimulation of catalysis. Specifically, ATP might affect the open-closed transition triggered by the binding of substrate and ligands.

The structures of hSR and human selenomethionine SR complexed with malonate (PDB codes 3L6B and 3L6R, respectively), were recently solved (17). In spite of co-crystallization trials with different ligands, i.e. L-serine, glycine, malonate and other small molecules only malonate resulted in highly diffracting co-crystals solved at a resolution of 1.5 Å (17) (Figure 2). The comparison between rSR and hSR in complex with malonate with rSR and SpSR holo structures indicates that binding of the inhibitor has triggered a transition from an open to a closed conformation through a rigid body movement of the small domain (Figure 3). While, in fact, the structural alignment of the human and rat monomer led to a C_{α} RMSD of 0.62 Å, the superimposition of the rat holo structure with that of rSR in complex with malonate resulted in 2.19 Å RMSD. Different PLP-dependent enzymes show a significant movement of the small domain leading to the closure of the active site upon ligand binding. This is the case, for instance, of aspartate aminotransferase belonging to fold-type I (38), threonine synthase and *O*-acetylserine sulfhydrylase belonging to fold-type II (26, 39). In the specific case of SR, the small domain undergoes a decrease of the solvent-exposed surface and a rotation of about 20° towards the large domain, without any change of the large domain dimer interface. The helix H5 and, in particular, the N-terminal loop (Ser83-His87, human numeration) approaches the PLP-lysinoalanine Schiff base forming the binding site for a carboxylate group. The shift of the small domain is supported by a large flexibility degree of the intra-domain interface, which allows it to assume slightly different orientations in the three available crystallographic structures, symptomatic of the fact that the
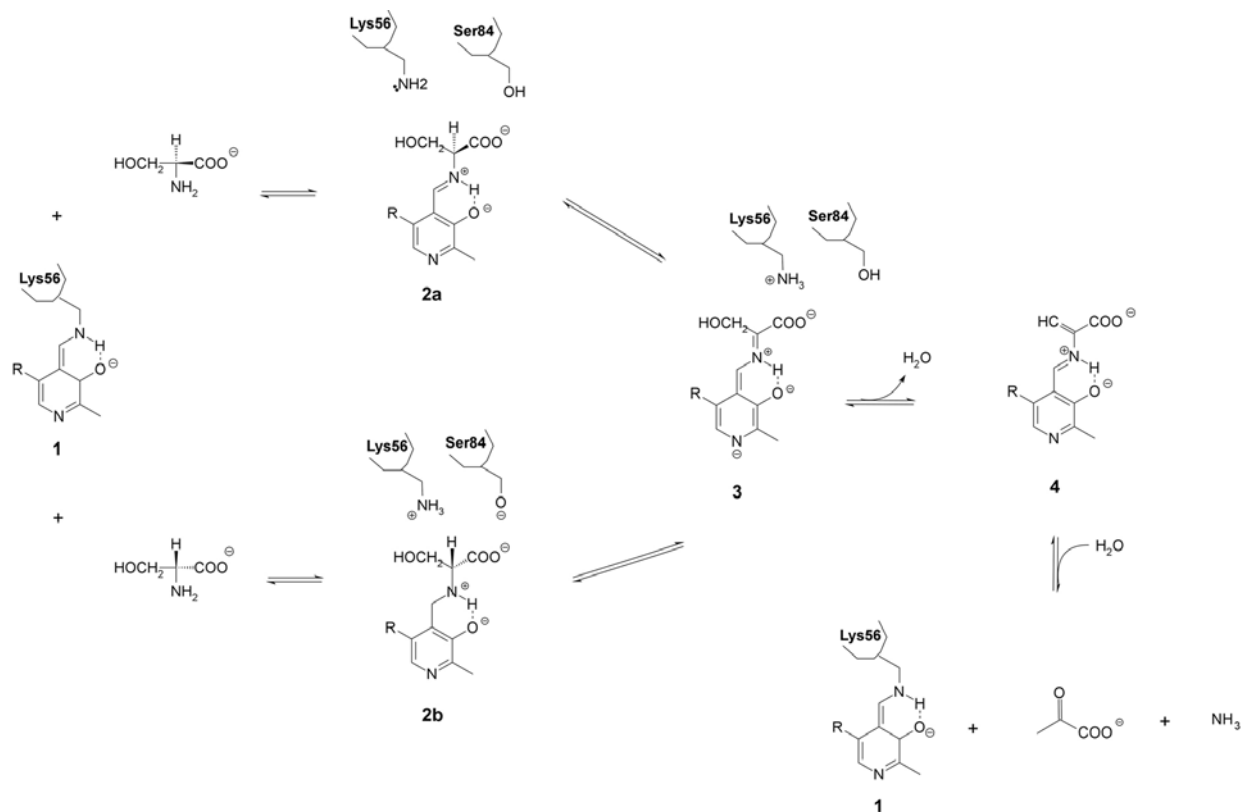
Figure 3. Reaction mechanism of hSR. The deprotonated amino acid substrate reacts with the internal aldimine (1) to give the external aldimine (2a and 2b). External aldimine is deprotonated on the C_α by either Lys56, if the incoming amino acid is L-serine, or by Ser84 if the incoming amino acid is D-serine. A quinonoid intermediate (3) has never been observed for serine racemase, but its existence has been postulated based on studies on bacterial alanine racemase (47). The quinonoid intermediate is reprotonated by either Ser84 or Lys56 and the antipodal amino acid is released with formation of the internal aldimine between Lys56 and PLP. When the hydroxyl group of the quinonoid intermediate is protonated, a β-elimination takes place, with formation of α-aminoacrylate (4), an unstable intermediate that is readily hydrolyzed to ammonia and pyruvate. The protonation can be carried out by either Ser84 or Lys56 depending on the configuration of the incoming amino acid.

motion of the domain should be larger and more continuous in solution, as suggested by an unreleased human holo structure, where the small domain was found completely disordered (17). Moreover, the closure of the binding pocket should occur only after the entrance and recognition of the substrate, which, otherwise, could not access the small cleft between the large and small domains. This arrangement plays a key role in the formation of the active site and, thus, in the catalytic process (18), since the closure observed with malonate binding is the same expected for the natural substrate. As suggested by Smith et al. (17), the rearrangement of the small domain locates Ser84 in such a close proximity of the substrate that is able to donate the proton required to catalyze the isomerization reaction (see below). Again, a similar movement has been detected in the PLP-dependent bacterial aspartate decarboxylase (40), hence suggesting a possible conserved mechanism of reaction.

As already pointed out, the limited number of conformations isolated by X-ray crystallography does not likely represent the actual conformational space explored by the protein. In this perspective, Bruno et al. (41) applied Targeted Molecular Dynamics (TMD) methods with the aim of simulating the transition between the open and the closed form of SR in the presence of either the natural substrate L-serine or the orthosteric inhibitor malonate, and of identifying the key residues and structural features necessary for the protein to stably interact with ligands. The generation of different conformations along the pathway from the open to closed state of SR is also of considerable importance for the identification by *in-silico* screening of new inhibitors that recognize alternative conformations of the enzyme. The simulations were run starting from the "humanized" rSR open conformation, upon the placement of ligands into the binding pocket. TMD analyses showed that, for both complexes, the process of closure of the small domain towards the large domain is a rapid event, occurring in the first 150 ps of simulation. The conformations generated by the dynamics were then clustered. Six representative structures, covering the whole transition from the open to the closed malonate-complexed forms, were selected and used for docking a small library of known SR inhibitors and closely related inactive

analogs. Better results were achieved when docking was applied to the TMD generated conformations rather than to the individual crystallographic structures, indicating the relevance of including protein flexibility in computational simulations (42-44). It was found that the open conformation failed in properly recognizing small active ligands, whereas the closed form highly scored small inactive molecules (41).

4. CATALYSIS

SR catalyses the conversion of L- to D-serine and *vice versa* exploiting the electron withdrawal capacity of PLP. In PLP-dependent enzymes the carbanion formed on the substrate by removal of an α-substituent (for example, the proton or the carboxylate) is stabilized by PLP, that acts as an "electron sink" and delocalizes the negative charge on the pyridine ring (45). PLP-dependent enzymes are widely distributed among organisms and perform most of the reactions of amino acids metabolism (racemization, decarboxylation, transamination) (46). PLP-dependent enzymes are known for their promiscuity, i. e. the same enzyme catalyses more than one reaction on the substrate and/or more than one substrate can be processed by the same enzyme. SR is no exception and in fact can perform β-elimination as well on serine and several amino acid derivatives. In general, racemisation requires the abstraction of the α-proton and reprotonation of C_α on the opposite face, with formation of the antipodal amino acid. The mechanism of PLP-dependent racemization of amino acids has been investigated in detail on bacterial enzymes (1), especially alanine racemase due to its importance as a potential antibiotic target (47-49).

Due to the low expression yields, the reaction mechanism of mammalian SR has never been studied in detail and has mostly been inferred from data on the bacterial enzymes and from structural information (17, 18) (Figure 3). The incoming amino acid, either L- or D-serine with an unprotonated α-amino group, attacks the $C_{4'}$ of the Schiff base of the internal aldimine (Intermediate 1) to form the external aldimine (Intermediate 2) with release of unprotonated Lys56. Racemisation proceeds via a two-base mechanism, with Lys56 and Ser84 acting on the opposite sides of the amino acid. In this mechanism, D- and L-serine are deprotonated by distinct residues (L-Ser by Lys56 and D-Ser by Ser84) and reprotonated on the opposite face of the carbanion by the other one (L-Ser by Ser84 and D-Ser by Lys56). It has been pointed out that this mechanism should reduce the chance for competing reactions to take place, because the acid is pre-positioned to quickly reprotonate the intermediate (47). In fold-type I or IV enzymes the pyridine nitrogen is protonated and the positive charge is stabilized by a conserved negatively charged residue. The functional role of this residue is to increase the electron sink potential of the pyridine ring and thus to further stabilize the carbanionic intermediate. This feature is not present in fold-type II enzymes, where a polar/neutral amino acid is always facing the pyridine nitrogen that is consequently unprotonated (18). For this reason some authors proposed that no quinonoid intermediate (Intermediate 3) forms during catalysis by SR

and the reaction proceeds with formation of a true carbanion with no delocalization of the negative charge to the pyridine ring (18). However, for alanine racemase, it was suggested that formation of a rather unstable quinonoid intermediate helps in controlling reaction specificity by kinetically preventing the conformational changes needed for transamination (47). Further investigations are needed to establish if this is also the case for SR in view of the relative efficiency of the competing β-elimination reaction.

All hSR orthologs characterized to date show some degree of β-elimination, with a relative efficiency with respect to racemisation that decreases going from archea to mammals (12). This observation, together with the finding that serine/threonine dehydratases are the SR closest relatives, suggests that β-elimination could be a residual activity from ancestral enzymes. The first experimental evidence that SR is able to perform β-elimination came from studies on β-substituted amino acids (50). In particular, β-elimination of L-serine-*O*-sulfate is 500 times faster than L-serine racemisation. However, β–elimination on serine was not detected until ATP and Mg^{++} were discovered to be essential cofactors (34, 35). Elimination proceeds with the protonation of the quinonoid intermediate on the β-hydroxy group that is subsequently eliminated as water to form the α-aminoacrylate (17, 18) (Figure 3). Protonation of the β-hydroxy group requires a strong acid. In the case of mammalian serine dehydratase the phosphate group of PLP acts as acid (27). It was proposed that also for SpSR the phosphate group is in the correct orientation to donate a proton to the leaving β-hydroxy group (18). However, hSR seems to work by a different mechanism, where the catalytic Lys56 or Ser84 function as acids to protonate the hydroxyl group of L- and D-serine, respectively (17). Noticeably, functional data show a lower efficiency for D-serine elimination with respect to L-serine. This difference is bigger for the human than the mouse enzyme (22, 51). The α-aminoacrylate is unstable and after release is rapidly hydrolysed to pyruvate and ammonia. The α-aminoacrylate Schiff base is an extremely reactive intermediate that undergoes covalent modifications due to side reactions in PLP-dependent enzymes (52, 53). These syncatalytic modifications often result in the inactivation of the enzyme. Syncatalytic reactions following α-aminoacrylate formation have never been reported for hSR (54), although in the yeast *S. pombe*, formation of α-aminoacrylate is followed by conversion to PLP-lysino-D-alanine with retention of 50-60 % of the initial activity (18, 19).

Whereas SR shows a high substrate specificity for racemisation, that is restricted to serine (55), β-elimination has a broader substrate specificity and is effectively carried out on L-and D-serine, L-threonine, L-Ser-*O*-sulfate, L-threo-3-hydroxyaspartate and β-chloro-L-alanine (7, 22, 34, 50, 55). The elimination is a reaction conserved through evolution (12), suggestive of a biological role. Specifically, under physiological conditions, β-elimination effectively degrades D-serine, thus modulating the concentration of the neurotransmitter, especially in those areas of the brain lacking DAAO. It is

Table 1. Effect of effectors and post-translational modifications on SR activity

Effector/post translational modification	Species/tissue	Reaction	Effect	Reference
Mg-ATP	mSR	β-elimination (L-Ser)	k_{cat}/K_M (↑4x)	(34, 55)
	"	β-elimination (D-Ser)	k_{cat}/K_M (↑13x)	(55)
	"	Racemization (L-Ser)	k_{cat}/K_M (↑2x)	(34, 55)
	"	Racemization (D-Ser)	k_{cat}/K_M (↑7x)	(55)
S-nitrosylation of Cys113	mSR	Racemization (L-Ser)	Specific activity (↓2x)	(61)
			V_{max} (↓)	
Ca^{++}	mSR	Racemization (L-Ser)	Specific activity (↑< 2x)	(20, 21)
PIP2	mSR	Racemization (L-Ser)	Specific activity (↓; IC$_{50}$ = 13 μM)	(62)
			V_{max} (↓)	
Palmitoylation	rSR/brain	Racemization (L-Ser)	Specific activity (↓10x)	(63)
Phosphorylation	mSR (on Thr71)	Racemization (L-Ser)	Specific activity (↑2x)	(64)
GRIP1	mSR/astrocytes		D-serine release from cells (↑)	(66)
		Racemization	↑ (+ 65%)	
PICK1	mSR/astrocytes		D-serine release from cells (↑)	(71)
DISC-1	msr and hSR		D-serine release from cells (↑)	(74)

also conceivable that pyruvate produced by the eliminase reaction could play a role in neuronal energy metabolism, in a similar fashion as the product of serine dehydratase (27). Nevertheless, it has been calculated that the rate of production of pyruvate by SR is less than 0.1 % of that displayed by glycolysis (56).

5. REGULATION

5.1. ATP, and divalent cations

The activity of hSR is modulated at different levels (catalysis, cellular localization, degradation) emphasizing the pivotal role played by this enzyme in the glutamatergic transmission in the central nervous system. The first modulators of hSR to be discovered were ATP and divalent cations (21, 34) (Table 1 and Figure 4). ATP and Mg^{2+} act synergistically and induce a 5-fold increase in the racemisation reaction. In the absence of the cofactors, no formation of pyruvate from L-serine was detectable (7). However, in the presence of ATP and Mg^{2+} the ratio between pyruvate and D-serine produced from L-serine is four. Subsequently, it was demonstrated that SR is activated also by nucleotides different from ATP, like ADP, GTP and, to a minor extent, CTP and UTP, with nucleosides monophosphates being almost ineffective (21). Ca^{2+} and Mn^{2+} also activate SR (20, 21, 34) (Table 1), with Ca^{++} showing an affinity similar to that of Mg^{2+}. However, the physiological significance of a Ca^{2+}-dependent regulation of SR is questionable since SR in resting cells is already fully saturated by Mg^{2+} (34) and an increase in Ca^{2+} concentration induced by a stimulus would probably not result in a further activation of SR. Thus ATP and Mg^{2+} are believed to be the *in vivo* natural effectors of mammalian SR (34) and bind to the enzyme as Mg-ATP complex. Regulation by nucleotides was not detected in SRs from plants (57, 58), whereas it was demonstrated for some bacterial dehydratases where AMP and/or ADP binding affects the oligomerization state and the activity, e. g., threonine dehydratase (59, 60). In the case of mammalian SR, ATP binding does not cause any effect on the aggregation state of the protein (36). In addition, ATP is not hydrolysed during catalysis and, consequently, non-hydrolysable analogs are effective (21, 34, 55). ATP and Mg^{2+} should thus exert their function as allosteric effectors of SR. In SpSR, the binding site of ATP was found to be

connected to the active site by a conserved residue, namely Gln89 (18). Mg-ATP binding might act causing a conformational change that is transmitted to the active site (12, 20). The resulting effect is a decrease of the K_M for L-serine with negligible effects on k_{cat} (see also (12) for further comments on this point).

5.2. Nitrosylation and phosphorylation

ATP binding and the resulting activation of SR seem to be modulated by cysteine nitrosylation (Table 1). In fact, one out of the seven cysteine residues of hSR (Cys2, Cys6, Cys46, Cys113, Cys128, Cys217 and Cys309), namely Cys113, proved to be nitrosylated by S-nitroso glutathione (GSNO) (61). This post-translation modification leads to a decrease of SR activity with respect to controls and the SR mutant Cys113Ser showed no change in activity upon exposure to GSNO. ATP was found to compete with nitrosylation and, concurrently, nitrosylation was found to affect ATP binding (61). Because Cys113 is localized within the ATP binding site, Cys113 nitrosylation likely interferes with ATP binding and enhancement of SR activity, thus explaining the effect of Cys113 nitrosylation on SR activity. It has been speculated that the reaction of NO with Cys113 represents a feedback regulation of NMDAR activity. In fact, it is known that NMDAR activation stimulates neuronal NO-synthase. NO can react with both SR and DAAO causing a decrease in activity and an activation, respectively. Both chemical modifications result in a decrease of D-serine level, that, in turn, leads to a decrease of NMDAR-dependent neurotransmission (61). It might also be that glutathione, and not NO, reacts with Cys113 (12, 13), accounting for the observed effects on activity. In fact, in a work where a different NO donor, DETA NONOate, was used no formation of thionitrosyl derivatives was detected (20). A definite answer might come from experiments using free NO and from a full characterization of cysteine reactivity of hSR. Furthermore, it has been reported that reducing agents, such as dithiothreitol or reduced glutathione (51, 61) are essential for SR activity (20) suggesting the detrimental effect of cystine formation. In the crystallization trials for hSR, in order to improve expression yields, Cys2 and Cys6 were both replaced with aspartate residues (17). A disulfide bond between Cys6 and Cys113 was identified in 3-morpholinosydnonimine
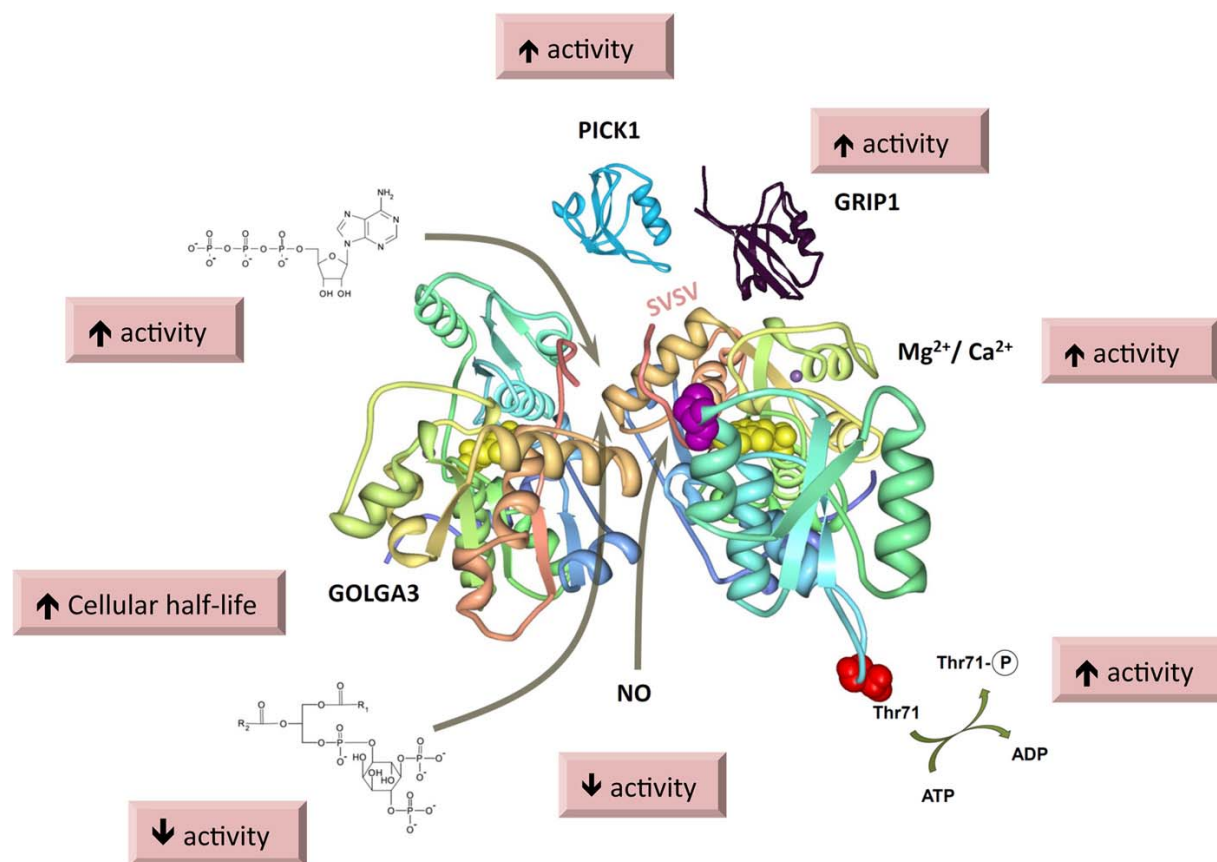
Figure 4. Modulators of SR. The three dimensional structure of the dimer of rSR is represented as a solid ribbon. PLP, Cys113 and Thr71 are shown in space-filling mode, coloured yellow, violet and red, respectively. For each modulator the binding site and the effect exerted on SR activity/half-life is shown.

hydrochloride (SIN-1)-treated SR monomer and dimer (23) whereas Cys217 was found to be involved in metal binding (31).

Activation by ATP participates in a more complex scenario, where subcellular localization of SR also plays an important role. A recent work has demonstrated that in resting glial cells SR is inactive and localized in the cell membrane (62). SR associated with the membrane is inactive because the ATP binding site is occupied by phosphatidylinositol (4, 5) bisphosphate (PIP2) (Table 1). Occupation of the ATP binding site by PIP2 was demonstrated *in vitro* by competition experiments, where the effect of Mg-ATP on SR activity decreases in the presence of PIP2 (62). Stimulation of astrocytes by glutamate released in the synaptic space through the metabotropic glutamate receptors mGluR5, causes the activation of phopholipase C that relieves inhibition on SR by degrading PIP2. Activation of SR increases the intracellular D-serine concentration that is subsequently released into the synaptic space where it will bind to NMDA receptors activating glutamatergic transmission. In a work on rat primary neuronal cultures the association of SR to cell membranes was also demonstrated. However, enzyme activity was proposed to be modulated by a completely different mechanism (63). In the absence of

NMDAR stimulation SR is mainly located in the cytosol, whereas SR is associated to membranes when NMDAR are activated. Association to the membrane is mediated by palmitoylation of the protein and strengthened by phosphorylation of Thr227. Because membrane-bound SR is inactive (Table 1), the biological role of membrane association in neurons should be the prevention of NMDAR overactivation in vicinal cells. By LC-MS analysis another site of phosphorylation, namely Thr71, was discovered (64) (Table 1). The main effect of this post-translational modification on enzyme catalysis seems to be an increase in V_{max}, Further investigations are needed to fully establish the role of Thr71 phosphorylation.

5.3. **Interactome**

In addition to allosteric effectors and post-translational modifications, SR function is regulated *in vivo* by changes in subcellular localization and interaction with specific proteins. Indeed, SR appears to have been molded by evolution to interact with a range of other proteins and subcellular structures, thanks, for example, to the occurrence of a peculiar C-terminus motif (36) that characterizes the human and murine enzyme but is absent in rat and bovine counterparts (9, 65). Although the complete set of interactors of SR has probably yet to be described, several interactions have been uncovered using

yeast two-hybrid screening. Partner proteins were shown to modulate SR function by directly affecting the specific activity of the enzyme but also by altering its subcellular localization and its half-life *via* the control of the rate of protein degradation by the ubiquitin-proteosome system.

In a pioneering study, Kim et al (66) showed that rat or mouse SR interacts with the protein Glutamate Receptor Interacting Protein 1 (GRIP1) and that such an interaction is instrumental to a glutamate-triggered SR activation process. GRIP1 is a large synaptic protein that interacts with AMPA glutamate receptors and contains several PDZ domains (67). One of these, PDZ6, was found to bind to the C-terminal region of SR (36, 66). Activation of AMPA receptors releases GRIP1 from its attachment sites, making it available to bind SR. In turn, this enhances SR activity and D-serine release (36, 66) (Table 1). Such a mechanism contributes to the physiologic regulation of cerebellar granule cell migration by SR (66).

SR was also found to bind to Protein Interacting with C-Kinase (PICK1) (68). This is another PDZ domain-containing scaffold that can bind to a large number of interactors, including AMPA receptors (69). Again, interaction with SR involves the PDZ domain of PICK1 and the C-terminus of SR (68). PICK1 knockout mice showed decreased concentrations of D-serine in the brain, suggesting that PICK1 may be involved in the regulation of SR function in a spatially and temporally specific manner (70) (Table 1). However, the mechanism by which this effect takes place is unclear, since PICK1 does not directly activate SR and the level of SR protein is unchanged in the PICK1-deficient animals (54, 70). A recent study has pointed out the involvement of protein kinase Cα in the PICK1-dependent modulation of mouse SR activity (71).

Another characterized interactor of SR is Golgin subfamily A member 3 (GOLGA3) (72). This is a protein with unknown function that associates to the cytosolic face of the Golgi apparatus. In contrast to the interactions described above, it was found that GOLGA3 binds to SR at the N-terminus of the racemase (72). Interaction with GOLGA3 decreases SR protein degradation through the ubiquitin pathway and indirectly affects D-serine levels (72). The study that characterized the interaction between SR and GOLGA3 also showed that a fraction of SR was strongly bound to the membrane fraction and resisted even washing with high salt concentration (72). The initial study also showed, by colocalization experiments, the presence of SR in the perinuclear region corresponding to the Golgi apparatus of both neurons and astrocytes, suggesting that GOLGA3 could mediate SR binding to the Golgi membrane (72). However, a subsequent study showed that in neurons most membrane-bound SR is located at the plasma membrane and dendrites (63), an interaction mediated by palmitoylation of the protein. As described above, NMDAR activation in primary neuronal cell cultures induces translocation of SR from the cytosol to intracellular and dendritic membranes, where it is inactive toward D-serine synthesis, thus providing a mechanism for feedback regulation of SR and NMDAR activity (63). In glial cells, conversely, the association of SR to membranes

depends on SR interaction with PIP2 (62). Mutations in a potential lipid-binding region of SR abolished SR binding to the membranes and increased the specific activity of the isolated enzyme (62).

A further interaction of SR has been found with Disrupted-In-Schizophrenia-1 (DISC1), one of the few proteins whose mutations have been unquestionably linked to the genetics of schizophrenia (73). A very recent work by Ma et al. (74) has shown that the scaffold protein DISC1 binds to and stabilizes SR. Mutant DISC1 truncated at its C-terminus failed to bind to SR, facilitating ubiquitination and degradation of SR, with a consequent decrease in D-serine production (Table 1). These results suggest a very direct pathway by which DISC1 can modulate the production of D-serine and NMDA neurotransmission, relevant to the pathophysiology of schizophrenia and other neuropsychiatric disorders.

6. SERINE RACEMASE AND D-SERINE DISTRIBUTION AND LOCALIZATION

For several years it was assumed that the predominant, if not exclusive, localization of SR and D-serine was inside glial cells, with only marginal presence in neurons (6, 75-77). However, the exploitation of more specific and sensitive antibodies for *in vivo* recognition of SR (78) and of SR KO mice (79, 80) has significantly changed this view. Present findings indicate that SR is predominantly localized in the cytosol of neurons and only marginally in astrocytes (9, 54, 78, 81). Besides immunocytochemistry works on brain tissues preparations and cell cultures, whose drawbacks are nicely discussed in (82), a very recent work on conditional knockout mice for neuronal and astrocytic SR further substantiates a distribution of the enzyme predominantly in neurons (82). Thus, D-serine might act with either autocrine or paracrine signals to NMDARs localized on neurons. A "shuttle" hypothesis has been recently proposed that involves the transfer of D-serine produced within neurons to astrocytes and then, upon NMDAR stimulation, back in the synaptic space for full receptor activation. Consequently, neurons are suggested to be the primary source of D-serine but astrocytes may act as the primary storage site (54). As a result, the amount of available D-serine for receptor binding is controlled at different stages, including partition of SR neuronal and astrocytes activity between L-serine racemization and L- and D-serine β-elimination, and neuronal and glial export and import systems, including transmembrane proteins called ephrinBs and Eph receptors that are expressed in the synapse and are known to regulate synaptic transmission and plasticity (83).

7. SERINA RACEMASE, NEUROPATHOLOGIES AND PSYCHIATRIC DISORDERS

The relevance of NMDAR in mediating relevant biological events, including NMDAR neurotransmission (84), synaptic plasticity (85), cell migration (66) and long term potentiation (86) is well established. The involvement of NMDAR signals in neurotoxicity and associated apoptotic events as well as psychiatric diseases is also well established (8, 81, 87-89). High and low D-serine levels

cause distinct neuropathologies and aging-related deficits (90), mediated by hyper- and hypo-activation of NMDAR, respectively. To the onset of neurological disorders, studies seems to indicate that the cellular levels of D-serine are not directly related with the levels of SR transcription (70, 90, 94). Thus, only a detailed elucidation of the molecular basis of the complex disease-triggering mechanisms and SR can open the way to tailored therapeutic treatments.

7.1 Schizophrenia

This psychiatric disorder causes many behavioural effects and cognitive deficits and has been linked to low NMDAR activity. There are many causes, ranging from intrinsic receptor abnormalities to inefficient receptor regulation (89). Furthermore, low levels of SR and low concentration of D-serine (76, 91, 92), caused by high levels of DAAO (93, 94), have been linked to schizophrenia (76). However, some studies rule out a genetic association between schizophrenia and serine racemase (95). It was also demonstrated in mice that the conditional deletion of D-3-phosphoglycerate dehydrogenase, the enzyme that catalyzes the first step in L-serine synthesis via the phosphorylated pathway, causes a significant decrease of both L- and D-serine with no effect on the expression level of SR (96). The interaction between SR and DISC1, described above, seems consistent with a role of SR in the pathophysiology of the disease. PICK1, another SR interactor, has also been linked to schizophrenia (68, 69).

7.2. Amyotrophic lateral sclerosis

This disease causes a dramatic loss of motor neurons in the spinal cord and brain leading to paralysis. The consensus hypothesis is that amyotrophic lateral sclerosis (ALS) pathogenesis is due to excitotoxicity mediated by ionotropic glutamate receptors, namely NMDAR, AMPAR and kainate receptor. Specifically, neuronal death is triggered by over-activation of NMDAR caused by high levels of D-serine (97). A recent study has demonstrated that DAAO inactivation is responsible for high levels of D-serine (98). Furthermore, it was observed that D-serine levels are two-fold higher in spinal cords of G93A Cu,Zn-superoxide dismutase (SOD1) mice, the model of ALS (99). ALS mice deprived of SR show earlier symptoms onset of the disease, but the progression phase is slowed and dependent on SR. Surprisingly, administration of D-serine to ALS mice dramatically lowers cord levels of D-serine, leading to changes in ALS onset and survival very similar to SR deletion. It was also found that D-serine treatment causes an increase of cord levels of the alanine–serine–cysteine transporter 1 (Asc-1). The mechanism linking SOD1 mutations to increased D-serine levels is not known. However, these results suggest that SR and D-serine are involved in both the pre-symptomatic and progression phases of ALS (99).

7.3. Alzheimer's disease

This disease causes a global progressive impairment of mental functions resulting from many and not yet fully understood mechanisms. It was reported that NMDARs significantly decrease in selected brain regions of Alzheimer's disease (AD) patients (100). However,

NMDAR activity is higher in AD and memantine, a drug that acts as an antagonist of NMDAR, is able to ameliorate the clinical pattern (101, 102). These findings point to a role of overactivated NMDARs in AD pathophysiology. In AD patients, SR activity is higher in the hippocampus (103) and D-serine levels are increased in the cerebrospinal fluid (104). Furthermore, amyloid β-peptides, the hallmark of AD, stimulate *in vivo* the synthesis and release of D-serine (103).

Other neurodegenerative diseases, such as *Parkinson and Huntington*, have been associated with hyperactivation of NMDAR and with high concentrations of D-serine, resulting from high levels of SR activity and low levels of DAAO activity (81).

7.4. Stroke/ischemia

Excitotoxic signals originate from lack of oxygen, followed by tissue reperfusion. These events are associated with cell death pathways triggered by overactivated NMDAR that are prevented by agents blocking NMDAR activity, such as memantine (81, 102). High levels of D-serine are observed in simulated ischemia models, causing neuronal death (105). Consequently, a protective effect towards ischemic neurotoxicity was observed in SR-depleted cultures of brain mice where a reduced formation of NO and NO-triggered events was observed (106).

8. SERINE RACEMASE INHIBITORS

SR is a rational target for the treatment of diseases described in section 7. However, in spite of its relevance, investigations carried out towards the development of potent inhibitors or effectors are surprisingly rather limited. This fact might be dependent on at least two factors: i) the yield and stability of the enzyme are low, making inhibition studies difficult, and ii) inhibitors should be very specific and cross the blood-brain barrier. In order to generate specific inhibitors, the structural and functional features of SR active site have been exploited as well as the well known reactivity of the coenzyme. SR inhibitors have been developed to reduce the excitotoxic effects of high levels of D-serine. Specifically, these compounds are aimed at reducing SR activity, thus decreasing D-serine concentration, and/or at increasing the β-elimination operating on D-serine, thus speeding up the depletion of the neurotransmitter.

SR effectors that are under development can be grouped based on their mechanism of action:

1. PLP targeting with reversible modifications;
2. PLP targeting with irreversible modifications;
3. Active site targeting;
4. Allosteric effectors;
5. Modulators of SR-protein interactions.

To our knowledge, inhibitors so far identified belong exclusively to the first three groups. In Table 2 the structure and binding affinities of the most potent inhibitors are reported.

Table 2. Structure and activity of the most potent inhibitors of SR identified to date

Inhibitors targeting PLP		Structure	K_i (mM)	Species	Reference
Reversible	Glycine		0.36 1.64	hSR mSR	(51) (22)
	L-erythro-3-hydroxyaspartate		0.01 0.04	hSR mSR	(51) (22)
Irreversible	Succinodihydroxamic acid		0.003	mSR	(109)
	L-aspartic acid β-hydroxamate		0.10	mSR	(109)
Reversible Competitive	Malonate		0.03-0.06 0.07 0.06	hSR mSR rSR	(17, 51) (22) (17)

8.1. PLP targeting with reversible modifications

L- and D-amino acids were assayed as substrate analogs and inhibitors (20, 50, 56). Among them the stronger inhibitor was the NMDAR ligand glycine (56) that exhibits a K_i of 366 and 1640 μM for hSR and mSR, respectively (51). Asparagine, aspartate and oxolacetate also bind to the enzyme indicating that neither an amine group nor a negative charge in the lateral chain are essential for inhibition, and that compounds with bulkier groups can enter in SR active site (56). Sulfhydryl containing amino acids, capable of reacting with PLP to form thiazolidine derivatives, are also SR inhibitors (50, 56). Furthermore, L-serine derivatives, such as L-serine *O*-sulfate, were found to be effective uncompetitive SR inhibitors in the absence of ATP (50). L-serine *O*-sulfate undergoes a β-elimination reaction to sulfate, ammonia and pyruvate. This finding indicates that an α-aminoacrylate species is first formed, and successively hydrolyzed. However, there is not yet an explanation for the observed uncompetitive inhibition.

A comprehensive analysis of compounds derived from serine and L-Ser-*O*-sulfate led to the identification of the so far strongest mSR competitive inhibitor, (2S,3R) L-*erythro* 3-hydroxyaspartate, exhibiting a K_i of 43 μM (22) (Table 2). Interestingly, the K_i for hSR is almost four fold lower than for mSR, 11 μM (51). This compound is a four carbon amino acid analog that reacts with PLP, forming a stable external aldimine. Accordingly to the docked conformation proposed by Jiraskova-Vanickova et al. (12), one carboxylic group interacts with the backbone nitrogen of Ser84 and His87, the other with Arg135 and Asn174, while the hydroxyl moiety contacts the side chain of Ser84. SR is a close relative of bacterial L-*threo* 3-hydroxyaspartate dehydratases and L-*threo*-3-hydroxyaspartate (2*S*,3*S*) is a substrate of the murine (22) and human enzymes (12) that degrade it to oxaloacetate and ammonia. In contrast, D-*threo*-3-hydroxyaspartate (2*R*,3*R*) is only a weak inhibitor (22).

Further studies identified β-haloalanines as SR inhibitors (56), similarly to that observed for other PLP-dependent enzymes belonging to fold type II, as tryptophan synthase (14, 107) and *O*-acetylserine sulfhydrylase (15, 108). This class of compounds reacts with PLP forming an external aldimine that, given the favourable β-leaving group, undergoes a β-elimination reaction with formation of an α-aminoacrylate derivative.

8.2. PLP targeting with irreversible modifications

The first reported SR inhibitor of this class was aminooxoacetic acid, an aspecific PLP-dependent enzyme inhibitor that reacts with the Schiff base linkage between PLP and the enzyme, forming an aldoxime (50). A series of small aliphatic hydroxamic and dihydroxamic acids were assayed as potential mSR inhibitors (109). It was found that the most effective were malonodihydroxamic acid, succinodihydroxamic acid, glutarodihydroxamic acid and L-aspartic acid β-hydroxamate. The K_i value calculated for succinodihydroxamic acid was about 3 μM, the most potent SR inhibitor so far identified (Table 2), whereas L-aspartic acid β-hydroxamate exhibited a K_i of about 100 μM. A clear disadvantage of hydroxamic acid derivatives as SR inhibitors and potential drugs is their lack of specificity, as they react with several PLP-dependent enzymes (109).

Cell migration assays were used for the identification of SR inhibitors. It was found that phenazine, phenazine methosulfate, and phenazine ethosulfate are effective inhibitors of SR with IC_{50} of 3 and 5 μM for the methosulfate and ethosulfate derivatives, respectively (66). However, no information on the assay conditions were reported. Furthermore, much higher IC_{50} values for these compounds were reported by Kovalinka and coworkers (12).

8.3. Active site targeting

This approach is aimed at identifying compounds that do not react with PLP and exploit for binding their

tight functional and geometric complementarity with the active site. A recent example is the interaction between OASS and pentapeptides mimicking the C-terminal of serine acetyltransferase (110, 111).

Several dicarboxylic acids were tested as SR active site inhibitors. It was found that malonate is the best binder with a K_{diss} of 33 µM for hSR (22) (Table 2). A slightly different value, 59 µM, was reported by Smith et al. (17), for hSR, and 111 µM for a mutant hSR in which Cys2 and Cys6 were substituted with Asp residues. A larger discrepancy for the affinity of malonate to mSR was found with a K_i of 71 µM (12, 22, 51), and a Ki of 568 µM to rSR and 1599 µM to the mutant (17). Malonate is a dicarboxylic acid that is orthosteric with L-serine. Its ability to interact with SR is due to the small dimension and the carboxylate groups that make strong ionic bonds with active site residues. As reported in Figure 2, one carboxylate of malonate interacts with the nitrogen backbone of Ser84, Asn86 and His87, with the side chain of Ser83 and with one water molecule, while the other forms a salt bridge type ionic interactions with Arg135, and also contacts Ser84 and Ser242, in addition to a pair of water molecules. As suggested, Arg135 might play a similar role to the arginine finger identified in the active site of G-proteins, involved in the stabilization of the reaction transition state and in the enhancement of the reaction rate (112, 113). On the other side, the N-terminal loop, containing Ser83, Ser84, Gly85, Asn86 and His87, is recognized as the asparagine loop, located in the PLP O3' side, and acting as the recognition site for the ligand in the closed enzyme conformation (25). Interestingly, Arg135, which appears fundamental for the stabilization of the enzyme-inhibitor complex is not conserved in other PLP-dependent enzymes belonging to the fold-type II (18). A series of malonate derivatives were synthesized and tested (12). No significant improvement with respect to the parent molecule was observed. Among a series of dicarboxylic and tricarboxylicacids m*eso*-diaminosuccinic as well as meso-tartaric exhibits a degree of rSR inhibition comparable with malonate.

A different approach towards the identification of SR inhibitors was followed by Dixon et al (114). A 74088 tripeptide library bound to individual beads was screened with a fluorescent-labelled human SR. Sixty peptide-beads were found to be positive and twenty five of them were randomly selected, identified, synthesized and assayed for SR inhibition. It was found that most of the identified peptides contained either histidine or phenylpropionic moieties. The best binders exhibit K_i values between 300 and 600 µM with the inhibition mechanism involving a fast interaction with the enzyme followed by a slow binding.

A still different method is pursued by Spyrakis et al. (quoted in (1)). *In silico* screening using both the open and the closed conformational states of SR, docking, scoring and best hits experimental evaluation was aimed at identifying active site SR binders. Studies are still ongoing (Spyrakis et al., unpublished results).

8.4. Allosteric effectors and modulators of SR-protein interactions

No investigation has been so far carried out aimed at developing compounds that target the multiple sites that allosterically modulate SR activity, i.e. the ATP binding site with the reactive Cys113, the cation binding site and the sites of the interaction between SR and effector proteins, GRIP, PICK1, and GOLGA3. This is a fascinating and challenging approach that might allow to generate effectors that either activate or inhibit SR activity.

9. CONCLUSIONS

Regardless of the intense activities carried out in the past ten years and the importance of SR as a potential drug target for a variety of neuropathologies and psychiatric disorders, some key questions are still unanswered. The relative contribution of neuronal and astrocytic SR to the D-serine pool that modulates synaptic and post-synaptic NMDAR activities is still debated and is strongly connected to our ability of understanding and treating SR-linked neuropathologies. In addition, combinatorial and rational drug design have been so far not very successful in the identification of effective compounds that selective inhibit SR and are potential drugs. This challenging search might benefit from a deeper understanding of the conformational plasticity underlying SR response to effectors.

10. REFERENCES

1. Conti, P., L. Tamborini, A. Pinto, A. Blondel, P. Minoprio, A. Mozzarelli & C. De Micheli: Drug Discovery Targeting Amino Acid Racemases. *Chemical Reviews*, 111, 6919-6946 (2011)

2. Dunlop, D. S., A. Neidle, D. McHale, D. M. Dunlop & A. Lajtha: The presence of free D-aspartate in rodents and man. *Biochem Biophys Res Commun*, 141, 27-32 (1986)

3. Hashimoto, K., T. Nishikawa, T. Hayashi, N. Fujii, K. Harada, T. Oka & K. Takahashi: The presence of free D-serine in rat brain. *FEBS Letters*, 296, 33-36 (1992)

4. Snyder, S. H. & P. M. Kim: D-amino acids as putative neurotransmitters: focus on D-serine. *Neurochem Res*, 25, 553-560 (2000)

5. Hashimoto, K., T. Nishikawa, T. Oka & K. Takahashi: Endogenous D-serine in rat brain: N-methyl-D-aspartate receptor-related distribution and aging. *J. Neurochem.*, 60, 783-786 (1997)

6. Schell, M. J., M. E. Molliver & S. H. Snyder: D-serine, an endogenous synaptic modulator: localization to astrocytes and glutamate-stimulated release. *Proc. Natl. Acad. Sci. U. S. A.*, 92, 3948–3952 (1995)

7. Wolosker, H., K. N. Sheth, M. Takahashi, J. P. Mothet, R. O. Brady, C. D. Ferris & S. H. Snyder: Purification of serine racemase: Biosynthesis of the neuromodulator D-

serine. *Proceedings of the National Academy of Sciences of the United States of America*, 96, 721-725 (1999)

8. Martineau, M., G. Baux & G. P. Mothet: D-Serine signalling in the brain: friend and foe. *Trends in Neurosciences*, 29, 481-491 (2008)

9. Wolosker, H.: NMDA receptor regulation by D-serine: new findings and perspectives. *Mol Neurobiol*, 36, 152-64 (2007)

10. Boehning, D. & S. H. Snyder: Novel neuromodulators. *Annu. Rev. Neurosci.*, 26, 105-131 (2003)

11. Yoshimura, T. & M. Goto: D-Amino acids in the brain: structure and function of pyridoxal phosphate-dependent amino acid racemases. *Febs J.*, 275, 3527–3537 (2008)

12. Jiraskova-Vanickova, J., R. Ettrich, B. Vorlova, H. E. Hoffman, M. Lepsik, P. Jansa & J. Konvalinka: Inhibition of Human Serine Racemase, an Emerging Target for Medicinal Chemistry. *Current Drug Targets*, 12, 1037-1055 (2011)

13. Baumgart, F. & I. Rodriguez-Crespo: D-amino acids in the brain: the biochemistry of brain serine racemase. *FEBS J*, 275, 3538-45 (2008)

14. Raboni, S., F. Spyrakis, B. Campanini, A. Amadasi, S. Bettati, A. Peracchi, A. Mozzarelli & R. Contestabile: Pyridoxal5'-Phosphate-Dependent Enzymes: Catalysis, Conformation, and Genomics. In: Comprehensive Natural Products II. Eds: L. Mander&H.-W. Liu. Elsevier, Oxford (2010)

15. Mozzarelli, A., S. Bettati, B. Campanini, E. Salsi, S. Raboni, R. Singh, F. Spyrakis, V. P. Kumar & P. F. Cook: The multifaceted pyridoxal 5'-phosphate-dependent O-acetylserine sulfhydrylase. *Biochim Biophys Acta*, 1814, 1497-510 (2011)

16. Bruno, S., F. Schiaretti, P. Burkhard, J. P. Kraus, M. Janosik & A. Mozzarelli: Functional properties of the active core of human cystathionine beta-synthase crystals. *J Biol Chem*, 276, 16-9 (2001)

17. Smith, M. A., V. Mack, A. Ebneth, I. Moraes, B. Felicetti, M. Wood, D. Schonfeld, O. Mather, A. Cesura & J. Barker: The structure of mammalian serine racemase: evidence for conformational changes upon inhibitor binding. *J Biol Chem*, 285, 12873-81 (2010)

18. Goto, M., T. Yamauchi, N. Kamiya, I. Miyahara, T. Yoshimura, H. Mihara, T. Kurihara, K. Hirotsu & N. Esaki: Crystal structure of a homolog of mammalian serine racemase from Schizosaccharomyces pombe. *J Biol Chem*, 284, 25944-52 (2009)

19. Yamauchi, T., M. Goto, H. Y. Wu, T. Uo, T. Yoshimura, H. Mihara, T. Kurihara, I. Miyahara, K. Hirotsu & N. Esaki: Serine racemase with catalytically active lysinoalanyl residue. *J Biochem*, 145, 421-4 (2009)

20. Cook, S. P., I. Galve-Roperh, A. Martinez del Pozo & I. Rodriguez-Crespo: Direct calcium binding results in activation of brain serine racemase. *J Biol Chem*, 277, 27782-92 (2002)

21. Neidle, A. & D. S. Dunlop: Allosteric regulation of mouse brain serine racemase. *Neurochem Res*, 27, 1719-24 (2002)

22. Strisovsky, K., J. Jiraskova, A. Mikulova, L. Rulisek & J. Konvalinka: 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, 13091-100 (2005)

23. Wang, W. & S. W. Barger: Cross-linking of serine racemase dimer by reactive oxygen species and reactive nitrogen species. *Journal of Neuroscience Research*, 90, 1218-1229 (2012)

24. Singh, R., F. Spyrakis, P. Cozzini, A. Paiardini, S. Pascarella & A. Mozzarelli: Chemogenomics of pyridoxal 5'-phosphate dependent enzymes. *J Enzyme Inhib Med Chem* (2011)

25. Burkhard, P., C. H. Tai, C. M. Ristroph, P. F. Cook & J. N. Jansonius: Ligand binding induces a large conformational change in O-acetylserine sulfhydrylase from Salmonella typhimurium. *J Mol Biol*, 291, 941-53 (1999)

26. Omi, R., M. Goto, I. Miyahara, H. Mizuguchi, H. Hayashi, H. Kagamiyama & K. Hirotsu: Crystal structures of threonine synthase from Thermus thermophilus HB8: conformational change, substrate recognition, and mechanism. *J Biol Chem*, 278, 46035-45 (2003)

27. Yamada, T., J. Komoto, Y. Takata, H. Ogawa, H. C. Pitot & F. Takusagawa: Crystal structure of serine dehydratase from rat liver. *Biochemistry*, 42, 12854-65 (2003)

28. Goldberg, J. M., R. V. Swanson, H. S. Goodman & J. F. Kirsch: The tyrosine-225 to phenylalanine mutation of Escherichia coli aspartate aminotransferase results in an alkaline transition in the spectrophotometric and kinetic pKa values and reduced values of both kcat and Km. *Biochemistry*, 30, 305-12 (1991)

29. Inoue, K., S. Kuramitsu, A. Okamoto, K. Hirotsu, T. Higuchi, Y. Morino & H. Kagamiyama: Tyr225 in aspartate aminotransferase: contribution of the hydrogen bond between Tyr225 and coenzyme to the catalytic reaction. *J Biochem*, 109, 570-6 (1991)

30. Shaw, J. P., G. A. Petsko & D. Ringe: Determination of the structure of alanine racemase from Bacillus stearothermophilus at 1.9-A resolution. *Biochemistry*, 36, 1329-42 (1997)

31. Hoffman, H., J. Jiraskova-Vanickova, M. Zvelebil & J. Konvalinka: Random mutagenesis of human serine racemase reveals residues important for the enzymatic activity. *Collect. Czech. Chem. Commun.*, 75, 59.79 (2010)

32. Abe, K., S. Takahashi, Y. Muroki, Y. Kera & R. H. Yamada: Cloning and expression of the pyridoxal 5'-phosphate-dependent aspartate racemase gene from the bivalve mollusk Scapharca broughtonii and characterization of the recombinant enzyme. *J Biochem*, 139, 235-44 (2006)

33. Ohnishi, M., M. Saito, S. Wakabayashi, M. Ishizuka, K. Nishimura, Y. Nagata & S. Kasai: Purification and characterization of serine racemase from a hyperthermophilic archaeon, Pyrobaculum islandicum. *J Bacteriol*, 190, 1359-65 (2008)

34. De Miranda, J., R. Panizzutti, V. N. Foltyn & H. Wolosker: Cofactors of serine racemase that physiologically stimulate the synthesis of the N-methyl-D-aspartate (NMDA) receptor coagonist D-serine. *Proc Natl Acad Sci U S A*, 99, 14542-7 (2002)

35. Strisovsky, K., J. Jiraskova, C. Barinka, P. Majer, C. Rojas, B. S. Slusher & J. Konvalinka: Mouse brain serine racemase catalyzes specific elimination of L-serine to pyruvate. *FEBS Lett*, 535, 44-8 (2003)

36. Baumgart, F., J. Mancheno & J. R. Crespo: Insights into the activation of brain serine racemase by the multi-PDZ domain protein GRIP, divalent cations and ATP. *Febs Journal*, 274, 293-293 (2007)

37. Kubala, M., J. Teisinger, R. Ettrich, K. Hofbauerova, V. Kopecky, Jr., V. Baumruk, R. Krumscheid, J. Plasek, W. Schoner & E. Amler: Eight amino acids form the ATP recognition site of Na(+)/K(+)-ATPase. *Biochemistry*, 42, 6446-52 (2003)

38. Jager, J., M. Moser, U. Sauder & J. N. Jansonius: Crystal structures of Escherichia coli aspartate aminotransferase in two conformations. Comparison of an unliganded open and two liganded closed forms. *J Mol Biol*, 239, 285-305 (1994)

39. Burkhard, P., G. S. Rao, E. Hohenester, K. D. Schnackerz, P. F. Cook & J. N. Jansonius: Three-dimensional structure of O-acetylserine sulfhydrylase from Salmonella typhimurium. *J Mol Biol*, 283, 121-33 (1998)

40. Chen, H. J., T. P. Ko, C. Y. Lee, N. C. Wang & A. H. Wang: Structure, assembly, and mechanism of a PLP-dependent dodecameric L-aspartate beta-decarboxylase. *Structure*, 17, 517-29 (2009)

41. Bruno, A., L. Amori & G. Costantino: Addressing the conformational flexibility of serine racemase by combining targeted molecular dynamics, conformational sampling and docking studies. *Molecular Informatics*, 30, 317-328 (2011)

42. Cozzini, P., G. E. Kellogg, F. Spyrakis, D. J. Abraham, G. Costantino, A. Emerson, F. Fanelli, H. Gohlke, L. A. Kuhn, G. M. Morris, M. Orozco, T. A. Pertinhez, M. Rizzi & C. A. Sotriffer: Target flexibility: an emerging consideration in drug discovery and design. *J Med Chem*, 51, 6237-55 (2008)

43. Spyrakis, F., A. BidonChanal, X. Barril & F. J. Luque: Protein flexibility and ligand recognition: challenges for molecular modeling. *Curr Top Med Chem*, 11, 192-210 (2011)

44. Spyrakis, F., P. Cozzini, A. Sarkar & G. E. Kellogg: Applying induced fit in drug discovery: square pegs and round holes? *Curr Top Med Chem*, 11, 131-2 (2011)

45. John, R. A.: Pyridoxal phosphate-dependent enzymes. *Biochim Biophys Acta*, 1248, 81-96 (1995)

46. Percudani, R. & A. Peracchi: A genomic overview of pyridoxal-phosphate-dependent enzymes. *EMBO Rep*, 4, 850-4 (2003)

47. Toney, M. D.: Reaction specificity in pyridoxal phosphate enzymes. *Archives of Biochemistry & Biophysics*, 433, 279-87 (2005)

48. Sun, S. & M. D. Toney: Evidence for a two-base mechanism involving tyrosine-265 from arginine-219 mutants of alanine racemase. *Biochemistry*, 38, 4058-65 (1999)

49. Watanabe, A., T. Yoshimura, B. Mikami & N. Esaki: Tyrosine 265 of alanine racemase serves as a base abstracting alpha-hydrogen from L-alanine: the counterpart residue to lysine 39 specific to D-alanine. *J Biochem*, 126, 781-6 (1999)

50. Panizzutti, R., J. De Miranda, C. S. Ribeiro, S. Engelender & H. Wolosker: A new strategy to decrease N-methyl-D-aspartate (NMDA) receptor coactivation: inhibition of D-serine synthesis by converting serine racemase into an eliminase. *Proc Natl Acad Sci U S A*, 98, 5294-9 (2001)

51. Hoffman, H. E., J. Jiraskova, M. Ingr, M. Zvelebil & J. Konvalinka: Recombinant human serine racemase: Enzymologic characterization and comparison with its mouse ortholog. *Protein Expression & Purification*, 63, 62-67 (2009)

52. Morino, Y., H. Kojima & S. Tanase: Affinity labeling of alanine aminotransferase by 3-chloro-L-alanine. *J Biol Chem*, 254, 279-85 (1979)

53. Passera, E., B. Campanini, F. Rossi, V. Casazza, M. Rizzi, R. Pellicciari & A. Mozzarelli: Human kynurenine aminotransferase II--reactivity with substrates and inhibitors. *Febs J*, 278, 1882-900 (2011)

54. Wolosker, H.: Serine racemase and the serine shuttle between neurons and astrocytes. *Biochimica Et Biophysica Acta-Proteins and Proteomics*, 1814, 1558-1566 (2011)

55. Foltyn, V. N., I. Bendikov, J. De Miranda, R. Panizzutti, E. Dumin, M. Shleper, P. Li, M. D. Toney, E.

Kartvelishvily & H. Wolosker: Serine racemase modulates intracellular D-serine levels through an alpha,beta-elimination activity. *Journal of Biological Chemistry*, 280, 1754-1763 (2005)

56. Dunlop, D. S. & A. Neidle: Regulation of serine racemase activity by amino acids. *Brain Res Mol Brain Res*, 133, 208-14 (2005)

57. Fujitani, Y., T. Horiuchi, K. Ito & M. Sugimoto: Serine racemases from barley, Hordeum vulgare L., and other plant species represent a distinct eukaryotic group: Gene cloning and recombinant protein characterization. *Phytochemistry*, 68, 1530-1536 (2007)

58. Fujitani, Y., N. Nakajima, K. Ishihara, T. Oikawa, K. Ito & M. Sugimoto: Molecular and biochemical characterization of a serine racemase from Arabidopsis thaliana. *Phytochemistry*, 67, 668-674 (2006)

59. Whanger, P. D., A. T. Phillips, K. W. Rabinowitz, J. R. Piperno, J. D. Shada & W. A. Wood: The mechanism of action of 5'-adenylic acid-activated threonine dehydrase. II. Protomer-oligomer interconversions and related properties. *J Biol Chem*, 243, 167-73 (1968)

60. Simanshu, D. K., H. S. Savithri & M. R. Murthy: Crystal structures of Salmonella typhimurium biodegradative threonine deaminase and its complex with CMP provide structural insights into ligand-induced oligomerization and enzyme activation. *J Biol Chem*, 281, 39630-41 (2006)

61. Mustafa, A. K., A. Kumar, B. Selvakumar, G. P. H. Ho, J. T. Ehmsen, R. K. Barrow, L. M. Amzel & S. H. Snyder: Nitric oxide S-nitrosylates serine racemase, mediating feedback inhibition of D-serine formation. *Proc Natl Acad Sci U S A*, 104, 2950–2955 (2007)

62. Mustafa, A. K., D. B. van Rossum, R. L. Patterson, D. Maag, J. T. Ehmsen, S. K. Gazi, A. Chakraborty, R. K. Barrow, L. M. Amzel & S. H. Snyder: Glutamatergic regulation of serine racemase via reversal of PIP2 inhibition. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 2921-2926 (2009)

63. Balan, L., V. N. Foltyn, M. Zehl, E. Dumin, E. Dikopoltsev, D. Knoh, Y. Ohno, A. Kihara, O. N. Jensen, I. S. Radzishevsky & H. Wolosker: Feedback inactivation of D-serine synthesis by NMDA receptor-elicited translocation of serine racemase to the membrane. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 7589-7594 (2009)

64. Foltyn, V. N., M. Zehl, E. Dikopoltsev, O. N. Jensen & H. Wolosker: Phosphorylation of mouse serine racemase regulates D-serine synthesis. *Febs Letters*, 584, 2937-2941 (2010)

65. Konno, R.: Rat cerebral serine racemase: amino acid deletion and truncation at carboxy terminus. *Neurosci Lett*, 349, 111-4 (2003)

66. Kim, P. M., H. Aizawa, P. S. Kim, A. S. Huang, S. R. Wickramasinghe, A. H. Kashani, R. K. Barrow, R. L. Huganir, A. Ghosh & S. H. Snyder: Serine racemase: Activation by glutamate neurotransmission via glutamate receptor interacting protein and mediation of neuronal migration. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 2105-2110 (2005)

67. Dong, H., R. O'Brien, E. Fung, A. Lanahan, P. Worley & H. RL.: GRIP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors. *Nature*, 386, 279-84 (1997)

68. Fujii, K., K. Maeda, T. Hikida, A. K. Mustafa, R. Balkissoon, J. Xia, T. Yamada, Y. Ozeki, R. Kawahara, M. Okawa, R. L. Huganir, H. Ujike, S. H. Snyder & A. Sawa: Serine racemase binds to PICK1: potential relevance to schizophrenia. *Mol Psychiatry*, 11, 150-7 (2006)

69. Dev, K. K. & J. M. Henley: The schizophrenic faces of PICK1. *Trends Pharmacol Sci*, 27, 574-9 (2006)

70. Hikida, T., A. K. Mustafa, K. Maeda, K. Fujii, R. K. Barrow, M. Saleh, R. L. Huganir, S. H. Snyder, K. Hashimoto & A. Sawa: Modulation of D-serine levels in brains of mice lacking PICK1. *Biol Psychiatry*, 63, 997-1000 (2008)

71. Zhuang, Z., B. Yang, M. H. Theus, J. T. Sick, J. R. Bethea, T. J. Sick & D. J. Liebl: EphrinBs regulate D-serine synthesis and release in astrocytes. *J Neurosci*, 30, 16015-24 (2010)

72. Dumin, E., I. Bendikov, V. N. Foltyn, Y. Misumi, Y. Ikehara, E. Kartvelishvily & H. Wolosker: Modulation of D-serine levels via ubiquitin-dependent proteasomal degradation of serine racemase. *J Biol Chem*, 281, 20291-302 (2006)

73. Sanchez-Pulido, L. & C. P. Ponting: Structure and evolutionary history of DISC1. *Hum Mol Genet*, 20, R175-81

74. Ma, T. M., S. Abazyan, B. Abazyan, J. Nomura, C. Yang, S. Seshadri, A. Sawa, S. H. Snyder & M. V. Pletnikov: Pathogenic disruption of DISC1-serine racemase binding elicits schizophrenia-like behavior via D-serine depletion. *Mol Psychiatry* (2012)

75. Wolosker, H., S. Blackshaw & S. H. Snyder: Serine racemase: a glial enzyme synthesizing D-serine to regulate glutamate-N-methyl-D-aspartate neurotransmission. *Proc Natl Acad Sci U S A*, 96, 13409-14 (1999)

76. Verall, L., M. Walker, N. Rawlings, I. Benzel., J. N. C. Kew, P. J. Harrison & P. W. J. Burnet: D-Amino acid oxidase and serine racemase in human brain: normal distribution and altered expression in schizophrenia. *European Journal of Neuroscience*, 26, 1657-1669 (2007)

77. Yasuda, E., N. Ma & R. Semba: Immunohistochemical evidences for localization and production of D-serine in some neurons in the rat brain. *Neuroscience Letters*, 289, 162-164 (2001)

78. Rosenberg, D., E. Kartvelishvily, M. Shleper, C. M. Klinker, M. T. Bowser & H. Wolosker: Neuronal release of D-serine: a physiological pathway controlling extracellular D-serine concentration. *Faseb J*, 24, 2951–2961 (2010)

79. Horio, M., M. Kohno, Y. Fujita, T. Ishima, R. Inoue, I. Mori & K. Hashimoto: Levels of D-serine in the brain and peripheral organs of serine racemase (Srr) knock-out mice *Neurochem Int*, 59, 853–859 (2011)

80. Mori, H. & R. Inoue: Serine Racemase Knockout Mice. *Chemistry and Biodiversity*, 7, 1573-1578 (2010)

81. Hardingham, G. E. & H. Bading: Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. *Nature Rev. Neurosci.*, 11, 682-696 (2011)

82. Benneyworth, M. A., Y. Li, A. C. Basu, V. Y. Bolshakov & J. T. Coyle: Cell selective conditional null mutations of serine racemase demonstrate a predominate localization in cortical glutamatergic neurons. *Cell Mol Neurobiol*, 32, 613-24

83. Zhuang, Z., B. Yang, M. H. Theus, J. T. Sick, J. R. Bethea, T. J. Sick & D. J. Liebl1: EphrinBs Regulate D-Serine Synthesis and Release in Astrocytes. *Journal of Neuroscience*, 30, 16015–16024 (2010)

84. Mothet, J. P., A. T. Parent, H. Wolosker, R. O. Brady, Jr., D. J. Linden, C. D. Ferris, M. A. Rogawski & S. H. Snyder: D-serine is an endogenous ligand for the glycine site of the N-methyl-D-aspartate receptor. *Proc Natl Acad Sci U S A*, 97, 4926-31 (2000)

85. Panatier, A., D. T. Theodosis, J. P. Mothet, B. Touquet, L. Pollegioni, D. A. Poulain & S. H. R. Oliet: Glia-derived D-serine controls NMDA receptor activity and synaptic memory. *Cell*, 125, 775-784 (2006)

86. Henneberger, C., T. Papouin, S. H. Oliet & D. A. Rusakov: Long-term potentiation depends on release of D-serine from astrocytes. *Nature*, 463, 232-6 (2010)

87. Kartvelishvily, E., M. Shleper, L. Balan, E. Dumin & H. Wolosker: Neuron-derived D-serine release provides a novel means to activate N-methyl-D-aspartate receptors. *J Biol Chem*, 281, 14151-62 (2006)

88. Shleper, M., E. Kartvelishvily & H. Wolosker: D-serine is the dominant endogenous coagonist for NMDA receptor neurotoxicity in organotypic hippocampal slices. *J Neurosci*, 25, 9413-7 (2005)

89. Kantrowitz, J. T. & D. C. Javitt: N-methyl-d-aspartate (NMDA) receptor dysfunction or dysregulation: The final common pathway on the road to schizophrenia? *Brain Research Bulletin*, 83, 108-121 (2010)

90. Turpin, F. R., B. Potier, J. R. Dulong, P.-M. Sinet, J. Alliot, S. H. R. Oliet, P. Dutar, J. Epelbaum, J. P. Mothet & J. M. Billard: Reduced serine racemase expression contributes to age-related deficits in hippocampal cognitive function. *Neurobiology of Aging*, 32, 1495–1504 (2011)

91. Hashimoto, K., T. Fukushima, E. Shimizu, N. Komatsu, H. Watanabe, N. Shinoda, M. Nakazato, C. Kumakiri, S. Okada, H. Hasegawa, K. Imai & M. Iyo: Decreased Serum Levels of D-Serine in Patients With Schizophrenia. *Arch. gen. Psychiatry*, 60, 572-576 (2003)

92. Labrie, V., A. H. C. Wong & H. C. Roder: Contributions of the D-serine pathway to schizophrenia. *Neuropharmacology*, 62, 1484-1503 (2012)

93. Pollegioni, L. & S. Sacchi: Metabolism of the neuromodulator D-serine. *Cell. Mol. Life Sci.*, 67, 2387-2404 (2010)

94. Yamada, K., T. Ohnishi, K. Hashimoto, H. Ohba, Y. Iwayama-Shigeno, M. Toyoshima, A. Okuno, H. Takao, T. Toyota, Y. Minabe, K. Nakamura, E. Shimizu, M. Itokawa, N. Mori, M. Iyo & T. Yoshikawa: Identification of Multiple Serine Racemase (SRR) mRNA Isoforms and Genetic Analyses of SRR and DAO in Schizophrenia and D-Serine Levels. *Biol. Psychiatry*, 57, 1493–1503 (2005)

95. Strohmaier, J., A. Georgi, F. Schirmbeck, C. Schmael, R. A. Jamra, J. Schumaker, T. Becker, S. Hofels, N. Klopp, T. Illig, P. Propping, S. Cichon, M. M. Nothen, M. Rietschel & T. G. Schulze: No association between the serine racemase gene (*SRR*) and schizophrenia ina german case-control sample. *Psychiatric Genetics*, 17, 125 (2007)

96. Yang, J. H., A. Wada, K. Yoshida, Y. Miyoshi, T. Sayano, K. Esaki, K. M.O, S. Tomonaga, N. Azuma, N. Watanabe, H. K., K. Zaitsu, T. Machida, A. Messing, S. Itohara, Y. Hirabayashi & S. Furuya: Brain-specific Phgdh Deletion Reveals a Pivotal Role for L-Serine Biosynthesis in Controlling the Level of D-Serine, an N-methyl-D-aspartate Receptor Co-agonist, in Adult Brain. *J. Biol. Chem.*, 285, 41380–41390 (2010)

97. Sasabe, J., T. Chiba, M. Yamada, K. Okamoto, K. Nishimoto, M. Matsuoka & S. Aiso: D-Serine is a key determinant of glutamate toxicity in amyotrophic lateral sclerosis. *EMBO Journal*, 26, 4149–4159 (2007)

98. Sasabe, J., J. Miyoshi, M. Suzuki, M. Mita, R. Konno, M. Matsuoka, K. Hamase & S. Aiso: D-Amino acid oxidase controls motoneuron degeneration through D-serine. *Proc. Natl. Acad. Sci. U. S. A.*, 109, 627–632 (2012)

99. Thompson, M., Y. C. Marecki, S. Marinesco, V. Labrie, H. C. Roder, S. W. Barger & J. P. Crow: Paradoxical roles of serine racemase and D-serine in

G93A mSOD1 mouse model of amyotrophic lateral sclerosis. *J Neurochem*, 120, 598-610 (2012)

100. Maragos, W. F., D. C. Chu, A. B. Young, C. J. D'Amato & J. B. Penney Jr.: Loss of [^{3}H]TCP bindinh in Alzheimer's disease. *Neurosci. Lett.*, 74, 371-376 (1987)

101. Reisberg, B., R. Doody, A. Stoffler, S. F., S. Ferris & H. J. Mobius: Memantine in moderate-to-severe Alzheimer's disease. *N. Engl. J. Med.*, 348, 1333-1341 (2003)

102. Lipton, S. A.: Failures and successes of NMDA receptor antagonists: molecular basis for the use of open-channel blockers like memantine in the treatment of acute and chronic neurologic insults. *NeuroRx*, 1, 101-110 (2004)

103. Wu, S. Z., A. M. Bodles, M. M. Porter, W. S. Griffin, A. S. Basile & S. W. Barger: Induction of serine racemase expression and D-serine release from microglia by amyloid b-peptide. *J. Neuroinflammation*, 1, 2 (2004)

104. Fisher, G., N. Lorenzo, H. Abe, E. Fujita, W. H. Frey, C. Emory, M. M. Di Fiore & D. A. A.: Free D- and L-amino acids in ventricular cerbrospinal fluid from Alzheimer and normal subjects. *Amino Acids*, 15, 263-269 (1998)

105. Katsuki, H., M. Nonaka, H. Shirakawa, T. Kume & A. Akaike: Endogenous D-serine is involved in induction of neuronal death by N-methyl-D-aspartate and simulated ischemia in rat cerebrocortical slices *J. Pharmacol. Exp. Ther.*, 311, 836-844 (2004)

106. Mustafa, A. K., A. S. Ahmad, E. Zeynalov, S. K. Gazi, G. Sikka, J. T. Ehmsen, R. K. Barrow, J. T. Coyle, S. H. Snyder & S. Doré: Serine racemase deletion protects against cerebral ischemia and excitotoxicity. *Journal of Neuroscience*, 27, 1413–1416 (2010)

107. Raboni, S., S. Bettati & A. Mozzarelli: Tryptophan synthase: a mine for enzymologists. *Cell Mol Life Sci*, 66, 2391-403 (2009)

108. Tian, H., R. Guan, E. Salsi, B. Campanini, S. Bettati, V. P. Kumar, W. E. Karsten, A. Mozzarelli & P. F. Cook: Identification of the structural determinants for the stability of substrate and aminoacrylate external Schiff bases in O-acetylserine sulfhydrylase-A. *Biochemistry*, 49, 6093-103 (2010)

109. Hoffman, H. E., J. Jiraskova, P. Cigler, M. Sanda, J. Schraml & J. Konvalinka: Hydroxamic acids as a novel family of serine racemase inhibitors: mechanistic analysis reveals different modes of interaction with the pyridoxal-5'-phosphate cofactor. *J Med Chem*, 52, 6032-41 (2009)

110. Salsi, E., A. S. Bayden, F. Spyrakis, A. Amadasi, B. Campanini, S. Bettati, T. Dodatko, P. Cozzini, G. E. Kellogg, P. F. Cook, S. L. Roderick & A. Mozzarelli: Design of O-acetylserine sulfhydrylase inhibitors by mimicking nature. *J Med Chem*, 53, 345-56 (2010)

111. Spyrakis, F., P. Felici, A. S. Bayden, E. Salsi, R. Miggiano, G. E. Kellogg, P. Cozzini, P. F. Cook, A. Mozzarelli & B. Campanini: Fine tuning of the active site modulates specificity in the interaction of O-acetylserine sulfhydrylase isozymes with serine acetyltransferase. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, In press, doi: 10.1016/j.bbapap.2012.09.009 (2012)

112. Rittinger, K., P. A. Walker, J. F. Eccleston, K. Nurmahomed, D. Owen, E. Laue, S. J. Gamblin & S. J. Smerdon: Crystal structure of a small G protein in complex with the GTPase-activating protein rhoGAP. *Nature*, 388, 693-7 (1997)

113. Scheffzek, K., M. R. Ahmadian, W. Kabsch, L. Wiesmuller, A. Lautwein, F. Schmitz & A. Wittinghofer: The Ras-RasGAP complex: structural basis for GTPase activation and its loss in oncogenic Ras mutants. *Science*, 277, 333-8 (1997)

114. Dixon, S. M., P. Li, R. Liu, H. Wolosker, K. S. Lam, M. J. Kurth & M. D. Toney: Slow-binding human serine racemase inhibitors from high-throughput screening of combinatorial libraries. *J Med Chem*, 49, 2388-97 (2006)

Send correspondence to: Andrea Mozzarelli, Department of Pharmacy, University of Parma, Parma, Italy, Tel:30-0521905138; Fax 39-0521905151, E-mail: andrea.mozzarelli@unipr.it