D-Serine Regulation of NMDA Receptor Activity

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Life on Earth is intimately related to the selection of L-amino acids as protein building blocks and metabolic intermediates. Until recently, D-amino acids were not thought to exist in substantial quantities in higher organisms, and little attention has been directed toward their study. Despite this common belief, abundant quantities of D-serine occur in the mammalian brain, where its levels are higher than those of most essential amino acids and are equivalent to one-third of L-serine levels (*1*, *2*). Brain D-serine is not incorporated into proteins, but rather works as a physiological coagonist of a key neurotransmitter receptor, the *N*-methyl-D-aspartate–type glutamate receptor (NMDAR) (*3*). Astrocytes, a class of glial cells, release D-serine to activate NMDARs in neurons, indicating that D-serine works as a gliotransmitter that mediates glial-neuronal cross-talk (*4*–*7*). However, new findings suggest that glia are not the sole players responsible for D-serine signaling. Recent data raise major questions about the unique role of astrocytes in D-serine signaling by demonstrating that neurons synthesize and release D-serine as well (*8*) (Fig. 1). A recent study by Puyal and colleagues reports that D-serine immunoreactivity switches from glia to neurons during development, suggesting that glial and neuronal D-serine have distinct roles (*9*). In the following sections, we discuss this new evidence regarding D-serine disposition in the brain, focusing on new ideas concerning the possible roles of glial and neuronal D-serine in NMDAR neurotransmission.

D-Serine: A Physiological Regulator of NMDA Receptors

NMDARs play a key role in excitatory synaptic transmission and have been implicated in many physiological processes, including learning and memory. NMDAR activity is strictly regulated, because its overactivation leads to the massive neuronal death that occurs in some pathological conditions such as stroke and neurodegenerative diseases (*10*). Binding of a coagonist, previously thought to be glycine, is required for opening the NMDAR channel (*11*). In recent years, it has been recognized that D-serine is at least as potent as glycine at the coagonist site and that D-serine may in fact be the physiological coagonist. As a coagonist with glutamate, D-serine mediates NMDAR responses and long-term synaptic changes in the hippocampus, a region linked to learning and memory (*12*, *13*). Likewise, together with glutamate, D-serine mediates NMDAR-dependent cell migration during development of the cerebellum (*14*) and is also the physiological coagonist of NMDAR in the retina (*15*) and hypothalamus (*7*). In models of neurotoxicity, D-serine is the main coagonist required for the cell death caused by NMDAR overactivation, which recapitulates the cellular damage caused by stroke (*16*).

The most compelling evidence that D-serine plays a physiological role in NMDAR activation comes from identification of its biosynthetic enzyme in neurons and glia. Serine racemase, a brain-enriched enzyme, converts L-serine into both D-serine and pyruvate (*17*–*21*). The existence of additional mechanisms regulating extracellular D-serine concentration in the brain, including degradation (*19*, *22*), transport (*23*, *24*), and release (*23*, *25*, *26*), also support a possible transmitter role for D-serine (*5*) (Fig. 1).

Neuronal D-Serine

Unlike classic chemical neurotransmitters, D-serine was originally shown to be specifically produced and released from glia (*18*, *22*, *26*). Therefore, most studies demonstrating a role for D-serine in mediating NMDAR activity attributed its effects solely to glial D-serine without considering a possible neuronal origin. Although glial D-serine is prominent, a number of recent studies have reported the presence of D-serine in neurons as well. Some studies found D-serine in most (*8*) or in a subset of neurons of the cerebral cortex (*27*), whereas others observed D-serine mainly in some neurons of the hindbrain (*9*, *28*).

New findings regarding the localization of serine racemase provide additional evidence that neurons are involved in D-serine synthesis. The use of a new antibody against serine racemase revealed a widespread and prominent neuronal localization in situ (*8*). The presence of serine racemase in neurons is also supported by in situ hybridization studies that show the presence of its mRNA in neuronal populations of the mouse brain, as revealed by the Allen Brain Atlas (*29*) and the Gene Expression Nervous System Atlas (GENSAT) projects (*30*). Moreover, neurons can release D-serine upon ionotropic glutamate receptor stimulation (*8*) and possess a high-affinity transporter for D-serine (*31*), further indicating that neurons probably regulate extracellular D-serine concentrations.

The presence of D-serine in neurons led to an updated model of D-serine signaling (*22*), because the original model did not include a role for neurons in regulating extracellular D-serine concentration. The revised model depicts the release and uptake of D-serine from both neurons and astrocytes (Fig. 1). Neuronal D-serine mediates a fraction of NMDAR-elicited neurotoxicity in neuronal cultures (*8*), indicating that it activates NMDARs in a paracrine or autocrine manner (Fig. 1). On the other hand, glial D-serine plays a role in the neuron-glia cross-talk that modulates NMDAR activity in normal neurotransmission (*32*, *33*).

Role of Glial Versus Neuronal D-Serine

It is conceivable that glial and neuronal D-serine play distinct roles in the many physiological tasks carried out by the NMDAR. Although much remains to be learned, a recent study by Puyal and colleagues sheds light on the relative roles of glial and neuronal D-serine (*9*). The authors carried out a detailed study of the ontogeny of D-serine in vestibular nuclei by determining D-serine content using biochemical and immunohistochemical methods. Puyal and colleagues discovered that D-serine displays an exquisitely choreographed developmental pattern. The authors first noticed that D-serine levels dropped substantially in adult animals. This decrease is associated with an increase in the abundance of D-amino acid oxidase, an enzyme that destroys intracellular D-serine, in astrocytes (Fig. 1). As the

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expression of D-amino acid oxidase increases, a surprising switch in D-serine localization becomes apparent. In the vestibular nuclei of young rats, D-serine is confined to astrocytes, whereas in these brain regions in adult rats, D-serine is found exclusively in the neuronal cells.

The glial-to-neuronal D-serine switch has important implications for D-serine signaling. The authors proposed that glial and neuronal D-serine play distinct roles during development of the vestibular nuclei. The early emergence of glial D-serine in young animals may be primarily associated with the maturation of the vestibular nuclei synaptic connections. On the other hand, the presence of lower—but still meaningful—amounts of D-serine in neurons at later stages of development suggests a different function. One possibility is that neuronal D-serine participates in regulating the basal NMDAR activity of the adult vestibular nuclei. Alternatively, the authors suggest that D-serine may contribute to the neuronal energy metabolism, because the production of D-serine is associated with the production of pyruvate by serine racemase enzyme (*19*, *20*).

It will be important to investigate whether D-serine also shifts from glia to neurons in other brain regions and if this is due to a glial-to-neuronal switch in serine racemase expression. In Puyal's study, a low glutaraldehyde concentration was used to fix D-serine, and a large part of the signal may have been lost due to poor amino acid fixation. Thus, it is likely that glial and neuronal D-serine coexist at many developmental stages. Determination of the ontogeny of serine racemase expression in the vestibular nuclei by immunohistochemistry would nicely complement this work.

Studies on neuronal D-serine pave the way to a better understanding of D-serine signaling and point to new topics for future

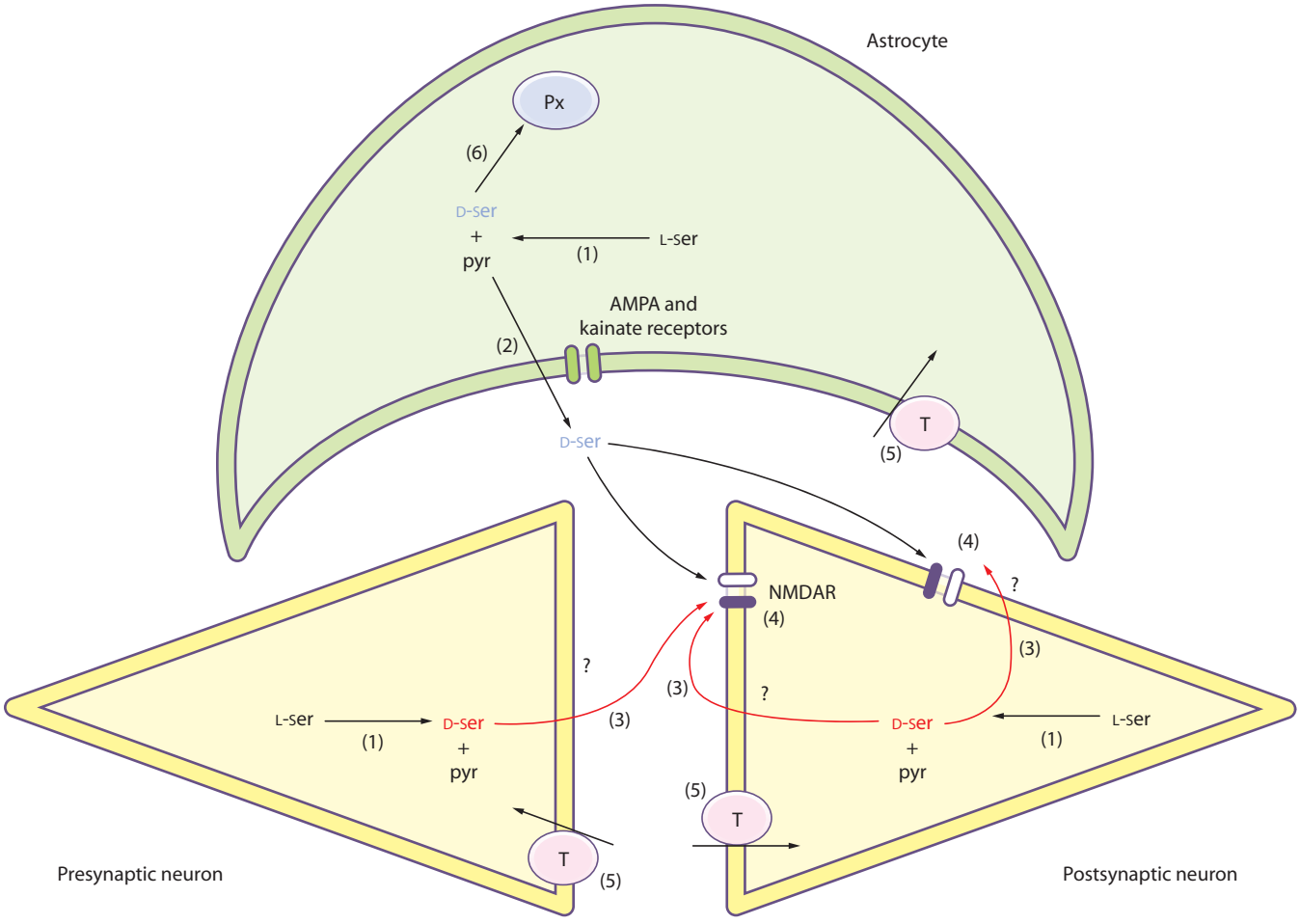

Fig. 1. Updated model for D-serine signaling. The regulation of NMDARs is mediated by the release and uptake of D-serine from both astrocytes and neurons. D-Serine signaling comprises at least six different steps: (1) Production of D-serine (D-Ser) and pyruvate (pyr) from L-serine by the enzyme serine racemase, which is present in both astrocytes and neurons. (2) Release of vesicular D-serine from astrocytes upon AMPA- (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and kainate-type glutamate receptor stimulation or amino acid hetero-exchange catalyzed by a neutral amino acid transporter. (3) Release of neuronal D-serine by means of a nonvesicular pathway upon KCl depolarization, AMPA- and kainate-receptor stimulation, and NMDAR stimulation. It is not clear whether neuronal D-serine synthesis and release occur at pre- or postsynaptic sites. (4) Simulation of synaptic NMDAR and possibly nonsynaptic receptors by D-serine. (5) Reuptake of D-serine by neutral amino acid transporters (T), which presumably helps to terminate the D-serine signal. There is no consensus on whether the neuronal high-affinity L/D-serine transporter occurs at pre- or postsynaptic sites. (6) Destruction of D-serine in glial peroxisomes (Px) by the D-amino acid oxidase enzyme regulates intracellular levels of D-serine.

investigation. Moreover, they raise many questions about the relative roles of glia and neurons in the synthesis, release, and accumulation of D-serine. For instance, the predominant localization of D-serine in glia (*22*) contrasts with the robust neuronal expression of serine racemase observed in a recent study (*8*). One explanation for this apparent discrepancy is that the greater abundance of glial D-serine is not due to greater serine racemase expression, but rather to the accumulation of D-serine taken up from the extracellular medium by astrocytes. Accordingly, intraventricular injection of D-serine strongly labels astrocytes, indicating that these cells preferentially take up extracellular D-serine (*34*). This indicates that glia may be involved in the termination of D-serine signaling by carrying out D-serine reuptake. On the other hand, it is equally possible that the lower abundance of D-serine in neurons is due to lower serine racemase activity. Although L-serine (the substrate for D-serine synthesis) is one of the most abundant amino acids in the brain, neurons have less L-serine than do astrocytes (*35*). Because the threshold of L-serine required for D-serine synthesis in situ is unknown, it is not possible to rule out that the neuronal and the glial serine racemase work at different rates.

The recent advances in defining the disposition and role of D-serine in the nervous system raise many additional questions. A more comprehensive study on the molecular mechanisms of D-serine release and those involved in the termination of D-serine signal will be essential for understanding D-serine's role in the regulation of NMDAR activity. It will also be crucial to define the targets for glial and neuronal D-serine; for instance, does D-serine released from both of these compartments stimulate synaptic NMDARs? Based on the presence of D-serine in neurons, it seems appropriate that we should refine some actions of D-serine previously attributed to glial-derived D-serine by considering the role of neurons in D-serine signaling as well. A final verdict on the relative roles of glia and neurons in D-serine signaling will require the development of techniques to discriminate between glial and neuronal D-serine release.

References

1. A. Hashimoto, T. Nishikawa, T. Hayashi, N. Fujii, K. Harada, T. Oka, K. Takahashi, The presence of free D-serine in rat brain. *FEBS Lett.* **296**, 33–36 (1992).
2. A. Hashimoto, 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 (1993).
3. W. Danysz, A. C. Parsons, Glycine and N-methyl-D-aspartate receptors: Physiological significance and possible therapeutic applications. *Pharmacol. Rev.* **50**, 597–664 (1998).
4. H. Wolosker, R. Panizzutti, J. De Miranda, Neurobiology through the looking-glass: D-serine as a new glial-derived transmitter. *Neurochem. Int.* **41**, 327–332 (2002).
5. S. H. Snyder, C. D. Ferris, Novel neurotransmitters and their neuropsychiatric relevance. *Am. J. Psychiatry* **157**, 1738–1751 (2000).
6. R. F. Miller, D-Serine as a glial modulator of nerve cells. *Glia* **47**, 275–283 (2004).
7. A. Panatier, D. T. Theodosis, J. P. Mothet, B. Touquet, L. Pollegioni, D. A. Poulain, S. H. Oliet, Glia-derived D-serine controls NMDA receptor activity and synaptic memory. *Cell* **125**, 775–784 (2006).
8. E. Kartvelishvily, 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**, 14151–14162 (2006).
9. J. Puyal, M. Martineau, J. P. Mothet, M. T. Nicolas, J. Raymond, Changes in D-serine levels and localization during postnatal development of the rat vestibular nuclei. *J. Comp. Neurol.* **497**, 610–621 (2006).
10. J. A. Kemp, R. M. McKernan, NMDA receptor pathways as drug targets. *Nat. Neurosci.* **5** (suppl.), 1039–1042 (2002).
11. J. W. Johnson, P. Ascher, Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature* **325**, 529–531 (1987).
12. J. P. Mothet, 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**, 4926–4931 (2000).
13. Y. Yang, W. Ge, Y. Chen, Z. Zhang, W. Shen, C. Wu, M. Poo, S. Duan, Contribution of astrocytes to hippocampal long-term potentiation through release of D-serine. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 15194–15199 (2003).
14. P. M. Kim, 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. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 2105–2110 (2005).
15. E. R. Stevens, M. Esguerra, P. M. Kim, E. A. Newman, S. H. Snyder, K. R. Zahs, R. F. Miller, D-serine and serine racemase are present in the vertebrate retina and contribute to the physiological activation of NMDA receptors. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 6789–6794 (2003).
16. M. Shleper, E. Kartvelishvily, H. Wolosker, D-serine is the dominant endogenous coagonist for NMDA receptor neurotoxicity in organotypic hippocampal slices. *J. Neurosci.* **25**, 9413–9417 (2005).
17. H. Wolosker, K. N. Sheth, M. Takahashi, J. P. Mothet, R. O. Brady Jr., C. D. Ferris, S. H. Snyder, Purification of serine racemase: Biosynthesis of the neuromodulator D-serine. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 721–725 (1999).
18. H. Wolosker, 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**, 13409–13414 (1999).
19. V. N. Foltyn, 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. *J. Biol. Chem.* **280**, 1754–1763 (2005).
20. J. De Miranda, 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**, 14542–14547 (2002).
21. J. De Miranda, A. Santoro, S. Engelender, H. Wolosker, Human serine racemase: Molecular cloning, genomic organization and functional analysis. *Gene* **256**, 183–188 (2000).
22. M. J. Schell, 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).
23. C. S. Ribeiro, M. Reis, R. Panizzutti, J. de Miranda, H. Wolosker, Glial transport of the neuromodulator D-serine. *Brain Res.* **929**, 202–209 (2002).
24. K. B. O'Brien, R. F. Miller, M. T. Bowser, D-Serine uptake by isolated retinas is consistent with ASCT-mediated transport. *Neurosci. Lett.* **385**, 58–63 (2005).
25. K. B. O'Brien, M. T. Bowser, Measuring D-serine efflux from mouse cortical brain slices using online microdialysis-capillary electrophoresis. *Electrophoresis* **27**, 1949–1956 (2006).
26. J. P. Mothet, L. Pollegioni, G. Ouanounou, M. Martineau, P. Fossier, G. Baux, Glutamate receptor activation triggers a calcium-dependent and SNARE protein-dependent release of the gliotransmitter D-serine. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 5606–5611 (2005).
27. E. Yasuda, N. Ma, R. Semba, Immunohistochemical evidences for localization and production of D-serine in some neurons in the rat brain. *Neurosci. Lett.* **299**, 162–164 (2001).
28. S. M. Williams, C. M. Diaz, L. T. Macnab, R. K. Sullivan, D. V. Pow, Immunocytochemical analysis of D-serine distribution in the mammalian brain reveals novel anatomical compartmentalizations in glia and neurons. *Glia* **53**, 401–411 (2006).
29. Allen Brain Atlas, as viewed September 2006, www.brain-map.org/.
30. Gene Expression Nervous System Atlas (GENSAT), as viewed September 2006, http://web.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gensat.
31. L. Helboe, J. Egebjerg, M. Moller, C. Thomsen, Distribution and pharmacology of alanine-serine-cysteine transporter 1 (asc-1) in rodent brain. *Eur. J. Neurosci.* **18**, 2227–2238 (2003).
32. P. G. Haydon, G. Carmignoto, Astrocyte control of synaptic transmission and neurovascular coupling. *Physiol. Rev.* **86**, 1009–1031 (2006).
33. R. D. Fields, B. Stevens-Graham, New insights into neuron-glia communication. *Science* **298**, 556–562 (2002).
34. K. Wako, N. Ma, T. Shiroyama, R. Semba, Glial uptake of intracerebroventricularly injected D-serine in the rat brain: An immunocytochemical study. *Neurosci. Lett.* **185**, 171–174 (1995).
35. E. Yasuda, N. Ma, R. Semba, Immunohistochemical demonstration of L-serine distribution in the rat brain. *Neuroreport* **12**, 1027–1030 (2001).

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