Review Article
Neuropathogenesis of HIV-1-associated neurocognitive disorders: a possible involvement of D-serine

Jianxun Xia, Huangui Xiong

Neurophysiology Laboratory, Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198-5880, USA

Abstract: A unique feature of N-methyl-D-aspartate receptors (NMDARs) that distinguishes them from other ionic receptors is that their activation requires more than one agonist to bind simultaneously to distinct binding sites on the receptor. D-serine, a co-agonist binding to the glycine site of NMDARs, has been implicated in several NMDAR-dependent physiological processes, and altered D-serine levels under certain pathophysiological conditions contribute to neural dysfunction via NMDARs in the central nervous system. Entry of HIV-1 in the brain causes neuronal injury leading to cognitive, behavioral and motor impairments known as HIV-associated neurocognitive disorders (HAND). As HIV-1 does not infect neurons, neuronal injury is believed to be primarily mediated by an indirect mechanism, that is, HIV-1-infected and/or immune-activated macrophages and microglial cells release soluble molecules leading to neuronal injury or death. Among the soluble factors is D-serine. In this article we try to address recent progresses on the role D-serine might play in the pathogenesis of neurodegenerative disorders with a particular emphasis of the involvement of D-serine in HIV-1-associated neurotoxicity.

Keywords: D-serine, D-amino acids, NMDA receptors, glycine site, neurodegeneration, central nervous system

An emerging role of D-serine in neurodegenerative disorders

From an evolutionary perspective, amino acids existing in L-form have been assumed to be naturally selected to participate in formation of polypeptides and proteins, and contribute to stabilization of polypeptides by neutral current interactions, whereas D-amino acids are biologically irrelevant [1]. Early in the twentieth century, D-amino acids were considered to be present only in certain bacteria and insects [2, 3]. With the development of chromatographic and *in vivo* microdialysis techniques together with immunohistochemical experiments using stereospecific antibodies, accumulating evidence has unraveled the occurrence of D-amino acids in mammals [4, 5]. Among the D-amino acids isolated (e.g., D-serine, D-alanine, D-glutamate, etc.), D-serine has gained much attention due to its specific physiological functions in CNS development and learning and memory, as well as its pathological roles in excitotoxicity, Alzheimer's disease, schizophrenia and other neurological disorders [3, 6, 7].

Considering D-serine activation of N-methyl-D-aspartate (NMDA) receptor (NMDAR) via glycine-binding site and involvement of excessive activation of NMDAR in a variety of neurodegenerative diseases, such as stroke, amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), Alzheimer's disease (AD), and Huntington's disease (HD) [8], it is plausible that elevated levels of D-serine in diseased brain may contribute to the pathogeneses of many neurodegenerative disorders [9]. It has been shown in animal models of stroke that D-serine levels were elevated together with glycine and L-glutamate [10], and that reperfusion damage following ischemia was attenuated by application of NMDAR glycine site antagonists [11, 12]. Thus, elevated levels of D-serine in stroke might cause neuronal damage via NMDAR-mediated excitotoxicity [13].

In a study for screening ALS therapy using pre-clinical transgenic mouse model, serine racemase (SR) and D-serine concentrations were gradually elevated when disease progressed [14]. Moreover, primary spinal cord neurons

from these mice were more vulnerable to NMDA toxicity than those from control mice in a D-serine-dependent manner as shown by augmented lactate dehydrogenase (LDH) release, and removal of endogenous D-serine by inhibiting SR alleviated NMDAR-mediated cell death in motor neurons [14]. In patients with family and sporadic forms of ALS, the levels of SR and D-serine were found to be greatly increased in spinal cord tissue [14]. Thus, enhanced glutamate toxicity, by increased D-serine concentrations in the spinal cord through alterations in D-serine biosynthesis and metabolism, might represent an underlying mechanism for ALS motor neuronal death [3].

In individuals affected by AD, an increased NMDAR activity has been detected in the brain and memantine, a low-affinity, uncompetitive NMDAR antagonist, was found to be neuroprotective in AD [15]. Along with the finding of increased SR activity in the hippocampus of AD patients, Aβ, a known pathogen of AD that triggers excitotoxic neuronal death, and amyloid precursor protein (APP) have been shown to stimulate release of excitotoxic levels of D-serine from microglia [16, 17] and glutamate from hippocampal neurons *in vitro* [16-18]. Although D-serine levels were not significantly altered in the frontal, prefrontal, parietal and temporal cortex [19-21] in PD and AD patients, it was speculated that modulation of the NMDAR glycine site may influence the severity of neurological disorders.

In addition to its pathogenic role in several aforementioned neurodegenerative disorders, D-serine may also be involved in the pathogenesis of HIV-associated neurocognitive disorders (HAND) via neuroinflammation [22-24], a pathological feature of HAND and other neurodegenerative disorders. The induction of serine racemase expression and D-serine production from glial cells could be triggered by HIV-1 infection, HIV-1 envelope protein gp120 stimulation and/or after proinflammatory activation [22]. So far, few studies have been focused on the role D-serine may play in HAND pathogenesis and studies from our laboratory revealed that D-serine is involved in HIV-1-associated neuropathology. Our laboratory has detected a significant elevation of D-serine levels in HIV-1 envelope protein gp120-treated human astrocyte culture media. Co-culture of rat hippocampal neurons with gp120-treated human astro-

cyte culture media induced neuronal injury that was blocked by either NMDA receptor antagonists or a selective NMDAR antagonist acting on the glycine site on the NMDAR complex (5,7-dichlorokynurenic acid), suggesting neuronal injury induced by gp120-treated human astrocyte culture media was mediated by D-serine via action on the glycine-binding site of NMDA receptors [22] (Xia and Xiong, unpublished data). In this review, we are trying to discuss the role D-serine may play in HAND neuropathogenesis based on current available information.

Neuropathogenesis and neuropathology of HAND

The world has observed the development of HIV-related diseases across countries as a severe global health problem, with the number of people living with HIV infection reaching an estimated 34.0 million (31.4 million–35.9 million) and an estimated 2.5 million (2.2 million–2.8 million) new HIV infections occurring in 2011 (UNAIDS 2012).

It is generally assumed that in HIV-1-infected patients, the virus not only destroy the immune system and leads to acquired immunodeficiency syndrome (AIDS), but also penetrates the central nervous system soon after it infects target peripheral immune cells, presumably via infiltration of HIV-1-infected macrophages and lymphocytes, leading to several neurological disorders, collectively known as HAND. At a late stage of the disease, 15-20% of the patients develop HIV-associated dementia (HAD) [25-27], which involves a variety of neuropathological complications directly triggered by HIV-1, including peripheral neuropathies, vacuolar myelopathy, and a syndrome of cognitive and motor dysfunction [28, 29]. Hence, HAD is also known as AIDS dementia complex (ADC) [30, 31]. The development of HAD is one of the most devastating consequences of HIV-1 infection in CNS. HAD is characterized by neurocognitive impairment (forgetfulness, slowing of thought and poor concentration), emotional disturbance (apathy and social withdrawal), and motor abnormalities (weakness, ataxia, clumsy gait, and tremor) [25, 26, 30, 32-34]. Our current understanding of the clinical features and pathophysiological mechanisms that underlie HAND comes mainly from the study of the clade B subtype of HIV-1, which is most prevalent in

North America and Western Europe. However, there are also other clades that have been identified, and to date, the most common HIV-1 clade worldwide is clade C [35].

The introduction of highly active antiretroviral therapy (HAART) has successfully increased life expectancies by reducing morbidity and mortality of patients infected with HIV-1 and has dramatically decreased incidence of HAD to as low as 10.5%. In addition, improved control of peripheral viral load and the treatment of opportunistic infections continue to prolong survival time. Nonetheless, HAART is insufficient to provide protection from MCMD or HAD, or to reverse the disease in most cases, because it is unable to prevent the entry of HIV-1 into the central nervous system [31, 32, 36]. Consequently, as the incidence of dementia (estimated in the early 1990s as high as 20-30%) has declined to a current 10% in individuals with low cluster of differentiation 4 (CD4) T cell counts and advanced HIV disease [28, 30, 32, 37], as many as 40% of HIV-positive patients still suffer from HAND [27]. Indeed, with the longer lifespan of patients with HIV-1 infection and AIDS in recent years, the prevalence of HAND is on the rise even in patients with well-controlled symptoms [27, 30, 32, 38].

In terms of this complexity, the American Academy of Neurology (AAN) modified the research diagnostic criteria of HAND in 2007 by recognizing three major categories: asymptomatic neurocognitive impairment (ANI), HIV-associated mild neurocognitive disorder (MND), and HIV-associated dementia (HAD) as the most severe form of neurocognitive impairment [39].

It is noteworthy that unlike other encephalopathies, HAND occurs without direct viral infection of neurons. It is generally assumed that HIV enters the CNS within 1-2 weeks after systemic infection via infiltration of infected CD4+ monocytes and perivascular macrophages (Trojan horse hypothesis), because CD4 is a primary receptor for HIV-1 infection and is commonly present on T-cells, monocytes, and macrophages [30]. Productive HIV infection has been observed in microglia, macrophages, and multinucleated giant cells in the CNS but not in neurons, oligodendrocytes and astrocytes. Despite expression of chemokine receptors on neu-rons, expression of CD4+ receptors is absent on neurons [26], which might account for the invalidity of HIV-1 infection of neurons, as HIV-1 infection of aforementioned cells only occurs upon binding of the viral envelope protein gp120 to one of several possible chemokine receptors in conjunction with CD4 [30]. Macrophages and microglia can be infected by HIV-1, but they can also be directly activated by factors released from infected cells. These factors include cytokines and shed viral proteins such as gp120 *etc*. So far, there are several HIV-1 proteins that have been identified to be released from HIV-1-infected cells and/or to be present in the extracellular environment in the HIV-1-infected brain, including the structural proteins gp120 and gp41, and the nonstructural proteins Vpr, Tat, Rev, Vpu and Nef within neurons. Moreover, these proteins have been shown to possess neurotoxic and/or neuro-modulatory features *in vitro* [25, 35]. Accordingly, these findings have led to at least two different hypotheses on how HIV-1 initiates neuronal damage in the brain, which can be described as the "direct injury" hypothesis and the "indirect" or "bystander effect" hypothesis as primary [28, 32].

Neurotoxic factors released from activated (infected/uninfected) cells (macrophages, microglia, astrocytes) include excitatory amino acids (EAAs) such as glutamate and related substances (quinolinate, cysteine) and TNF-α, platlet activating factor (PAF), prostaglandin E_2 (PGE_2), nitric oxide (NO) [30]. EAAs can trigger neuronal apoptosis through excitotoxicity, which involves excessive Ca^{2+} influx and free radical (nitric oxide and superoxide anion) production by overstimulation of glutamate receptors. Certain HIV proteins, such as gp120, Tat, and Vpr, have also been reported to be directly neurotoxic. The toxic viral proteins and factors released from macrophages and microglia may act in concert with astrocyte-secreted glutamate to promote neurodegeneration, even in the absence of extensive viral invasion of the CNS [28, 29, 32]. Furthermore, HAND might share the common features of neuroinflammation and microglial activation with several other neurodegenerative diseases, such as Alzheimer's disease, Multiple Sclerosis, Parkinson's disease and Frontotemporal Lobe Dementia [40].

HIV-associated neuropathology is generally characterized by the infiltration of macrophages into the CNS, widespread reactive astrocytosis, myelin pallor, the formation of microglial nodules, activated resident microglia, and multinucleated giant cells, which possibly result from virus-induced fusion of microglia and/or macrophages in central white and deep gray matter. Selective neuronal loss especially in hippocampus, basal ganglia and caudate nucleus, is also demonstrated by autopsy examination, indicating impaired cognitive and motor functions [30, 41]. In particular, increased numbers of microglia, an elevated TNF-α mRNA in microglia and astrocytes, production of excitotoxins, decreased synaptic and dendritic density, and selective neuronal loss constitute the pathological features most closely associated with the clinical signs of HAND. Furthermore, signs of neuronal apoptosis have also been linked to HAND [29, 32].

Overstimulation of NMDA receptor is involved in neuronal injury of HAND

Activation of ionotropic glutamate receptors, including AMPA receptors (AMPARs) and NMDARs in neurons, induces a transient depolarization of cell membrane potential leading to excitation of neurons under physiological conditions. The AMPARs mediate a fast component of excitatory postsynaptic transmission, while NMDARs are responsible for a component characterized by relatively slower onset and decay time. A typical glutamatergic neurotransmission starts with a fast response generated by activation of AMPARs by presynaptically-released glutamate; and the resultant postsynaptic membrane depolarization relieves the Mg^{2+} blockade to NMDAR under resting conditions and allows limited sodium and mostly calcium ions influx through the NMDAR-coupled ion channel (NMDA channels) to initiate intracellular signaling cascades. This voltage-dependent regulation of NMDAR function results in activity-driven synaptic modulation [42, 43]. Accordingly, NMDARs play critical roles in excitatory neurotransmission and are involved in learning and memory, behavior, and synaptic plasticity [44].

However, extended and/or excessive NMDAR activation can cause excitotoxicity, which is triggered by sustained elevation of the intracellular Ca^{2+} concentration, compromised mito-

chondrial function and cellular energy metabolism, and resultant free radical formation [15, 43, 45]. Excessive stimulation of NMDARs induces several detrimental intracellular signaling pathways that contribute to neuronal cell death by apoptosis or necrosis, depending on the intensity of the initial insult. If the initial excitotoxic insult is severe, the cells die early due to loss of ionic homeostasis, resulting in acute swelling and lysis (necrosis). If the insult is relatively mild, neurons enter a delayed death pathway knows as apoptosis [28, 46].

Excitotoxic substances secreted by infected or immune-activated macrophage/microglia and/or astrocytes as well as viral proteins such as gp120, Vpr, and Tat damage neurons by producing excessive activation of the NMDA subtype glutamate receptors [26, 28, 29, 32, 34, 35, 41]. Indeed, HIV-1-infected or gp120-stimulated mononuclear phagocytes release neurotoxins that stimulate the NMDARs. NMDAR antagonists could block this effect [26, 29, 32, 35]. Besides chemokines and EAAs, HIV-infected or gp120-activated microglia also release inflammatory cytokines, such as TNF-α and IL-1β, which stimulate release of L-cysteine from macrophages. Under either physiological or pathophysiological conditions, L-cysteine can stimulate NMDARs and lead to neuronal apoptosis [47].

Composition and distribution of NMDA receptors within central nervous system

NMDARs are composed of different subunits of the NR1, NR2 (NR2A-D) [48, 49] and NR3 families (NR3A-B) [7, 50-54]. It is generally considered that NMDARs are composed of heterotetrameric complexes of glycine-binding NR1 subunits combined with glutamate-binding NR2 subunits and glycine-binding NR3 subunits [9, 55, 56]. Different combinations of these subunits confer distinct pharmacological features, gating properties and Mg^{2+} sensitivity to the NMDAR complex [7, 57].

Functional NMDARs are widely expressed in neurons and glial cells throughout the brain and spinal cord [54, 58-60]. NR2A and NR2B are the most common NR2 subunits in the adult forebrain. NR2B is most common also in the early postnatal forebrain and is replaced somewhat by NR2A during development. NR2C is abundant in adult cerebellar granule cells.

NR2D is most common in early postnatal development in the diencephalon and brainstem. Within neurons, NMDAR complexes are found primarily at the synaptic sites in addition to the extrasynaptic locations, with the latter composed mainly of NR1/NR2B and NR1/NR2D subtypes [61]. NMDARs that include NR3 subunits are poorly understood, which are most common in the early postnatal brain and are rare in the adult [62, 63].

Different kinds of NMDARs show diverse distribution in the nervous system during development. During early postnatal development, NR2B-, NR2D-, and NR3A-containing NMDARs are abundant and decrease gradually with maturation, whereas NR2A- and NR2C-containing NMDARs become increasingly expressed during maturation process. The typical mature synapse has mainly NR2A-containing NMDARs. NR2B-containing NMDARs reside predominantly in the extrasynaptic membrane [64-66]. The composition of synaptic NMDARs varies throughout the brain. Accordingly, in adults, this separation of NMDARs into synaptic NR2A-containing and extrasynaptic NR2B-containing receptors associates various NMDAR-mediated functions within different brain areas and has significant implications in certain neurological disorders. Synaptic receptors would be activated by glutamate precisely released into the synaptic cleft, whereas extrasynaptic receptors would be activated by extensive release of glutamate followed by spillover into the extrasynaptic spaces. Synaptic NMDAR activation has been suggested to be neuroprotective by initiating the pro-survival signaling pathway, involving induced activity of cAMP response element binding protein (CREB) and gene expression of brain-derived neurotrophic factor (BDNF). Extrasynaptic NMDAR stimulation leads to cell death via the pro-death pathway, by the triggering CREB shut-off pathway and blocking induction of BDNF expression [67, 68].

The ability to specifically modulate synaptic and extrasynaptic NMDARs is of great clinical significance. Application of nonselective NMDA antagonists cannot be efficiently neuroprotective due to their inhibition of both the neurotoxic and neuroprotective pathways of NMDA transmission, thereby disturbing regular neuronal functions. In recent years, search for specific blockers of nonsynaptic NMDARs and selective NR2B antagonists (memantine, ifen-prodil and fluoxetine) has become a primary interest for drug development. Specifically, NR2B-containing NMDARs have been validated as target in the area of research of excitotoxicity prevention [68].

Binding of D-serine as a co-agonist to NR1 subunit is required for regulation of NMDA receptor function

A unique property that distinguishes NMDAR from other transmitter receptors is that its activation requires co-binding of more than one agonist to their respective binding sites. Glutamate, the main NMDAR agonist, does not activate the receptors unless a co-agonist binding site located at the NR1 subunit, generally referred to as the strychnine-insensitive "glycine site", is occupied. In addition to being essential for NMDAR activity, the co-agonist site exerts a neuromodulatory role. Thus, co-agonist binding increases the receptor's affinity for glutamate, decreases it desensitization and promotes NMDAR turnover by internalization [44, 69].

D-serine, an unusual D-amino acid form present in mammalian brain, is recognized as a physiological ligand of the NMDAR co-agonist site. It mediates several NMDAR-dependent physiological and pathological processes, including NMDA receptor transmission, synaptic plasticity, cell migration and neurotoxicity [44, 69]. Like glycine, D-serine has high affinity to the co-agonist site, which is up to threefold more potent than glycine [44]. D-serine is present at very high levels in the mammalian brain and at a much lower concentration in the peripheral tissues. Brain D-serine accounts for one-third of the L-serine and its levels are higher than most essential amino acids [70, 71]. Experiments of brain microdialysis revealed that the extracellular concentration of endogenous D-serine is twice that of glycine in the striatum and comparable to the concentration of glycine in the cerebral cortex [72]. Utilizing chromatographic techniques, a significant amount of D-serine has been identified in rodent and human forebrain, with highest concentration in the cerebral cortex, hippocampus, and striatum, followed by limbic forebrain, diencephalon, and midbrain and low levels in the pons, medulla, cerebellum, and spinal cord [6]. The possibility that D-serine is an endogenous co-agonist of NMDA receptors was demonstrat-

ed by a study showing that D-serine is enriched in areas containing the highest levels of NMDA receptors, including the cerebral cortex and the hippocampus [73].

Main reservoirs of D-serine in brain

Endogenous D-serine is synthesized from L-serine by serine racemase (SR), which requires pyridoxal 5'-phosphate as a cofactor. In addition to racemization, SR deaminates L-serine into pyruvate and ammonia, and the regional localization of SR matches those of endogenous D-serine, suggesting it play a physiological role in D-serine synthesis [44].

Astrocytic origin of D-serine

D-serine is enriched in protoplasmic astrocytes, which are a type of glia that ensheath the synapse and the densities of astrocytes-secreted D-serine are intimately close to neurons containing NMDA receptors [44, 69]. It has also been suggested that D-serine is released from astrocytes by stimulation of the kinate subtype glutamate receptor [74]. All these findings led to the proposal that D-serine is released from glia to activate neuronal NMDA receptors. This proposal suggests a unidirectional flow of D-serine from astrocytes to neurons (termed "unidirectional model") [69].

Neuronal origin of D-serine

Although D-serine was originally thought to be specifically produced and released from astrocytes as a glial-transmitter; subsequent investigations had also shown its presence in neurons, where the D-serine biosynthetic enzyme SR is robustly expressed. D-serine is also synthesized in neurons both *in vitro* and *in vivo*, indicating that D-serine also has a neuronal origin [44]. This suggested another model in which a bidirectional flux of D-serine exists between neurons and astrocytes, and accounts for the activation of NMDA receptors [69].

Microglial origin of D-serine

Apart from the known astrocytic and neuronal reservoirs, D-serine has been concluded more recently to be present in microglial cells; and mRNA for SR has been reported in microglial cells; the observed elevation of SR mRNA levels in microglia in response to APP exposure sug-

gested that D-serine may be involved in the pathogenesis of diseases such as Alzheimer's, acting via an excitotoxic mechanism [75, 76].

All these aforementioned findings raised a question: is D-serine able to be secreted from HIV-infected microglial cells, astrocytes, as well as from non-infected neurons in HAND?

Impaired astrocyte function may contribute to D-serine neurotoxicity in HAND

The role of astrocytes in HAND is thought to contribute to the production or maintenance of excitotoxins like glutamate in several ways. The normal re-uptake of glutamate by astrocytes is impaired in HAND and release of astrocytic glutamate is induced by several factors derived from activated macrophages/microglia, including arachidonic acid and TNF-α [28]. In a pathological context including HIV infection, the inflammatory mediator prostaglandin E_2, which is released from astrocytes, and TNF-α were found to evoke astrocytic glutamate release [77, 78]. Consequently, it is reasonable to propose that the extracellular glutamate is elevated to a level high enough to trigger a Ca^{2+} rise in astrocytes., In turn, astrocytes release further glutamate when their Ca^{2+} is elevated, thereby forming a "vicious cycle", aggravating the excitotoxicity of glutamate as its accumulates as a neurotoxin.

Increase of the intracellular Ca^{2+} concentration in astrocytes caused by activation of glutamatergic receptors has been suggested to trigger exocytotic release of glutamate, ATP and D-serine. These are believed to modulate neuronal excitability and transmitter release and to be involved in diseases as stroke, epilepsy, schizophrenia, Alzheimer's disease and HIV infection [79]. These findings strongly suggested that, in some regions of the brain, glutamate released from nerve terminals might trigger D-serine release from glial cells, which in turn, could modulate NMDARs localized on adjacent neurons [80].

In addition, AMPA and kainite ionotropic glutamate receptor activation promotes the release of D-serine from neurons in a Ca^{2+} and Na^+-dependent manner [81]. Furthermore, activation of microglia by inflammatory stimuli induces overexpression of SR, an effect mediated by the c-Jun terminal kinase [14, 16].
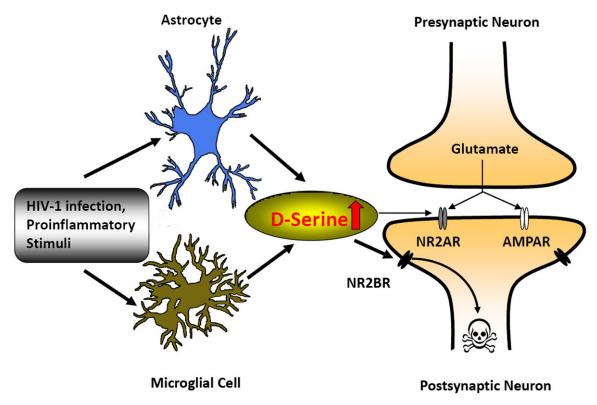
Figure 1. D-serine secreted by proinflammatory activation of astrocytes and/or microglial cells preferentially binds to extrasynaptic NR2B-containing NMDARs leading to neuronal injury. HIV-1 brain infection and/or proinflammatory activation trigger astrocytes and/or microglial cells production of D-serine that preferentially binds to NR2B subtype-containing NMDARs (NR2BRs) located predominantly at the extrasynaptic membrane and induces neuronal injury.

Upon release into the synaptic cleft, D-serine binds to the NMDAR co-agonist site on post-synaptic neuron to promote NMDAR activation, and conversely, NMDAR activation inhibits the production of D-serine [82-85]. This NMDAR-mediated feedback regulation of D-serine synthesis thus provides a pathway to prevent the over-activation of NMDAR and reveals the physiological importance of finely-tuned D-serine concentrations [86]. Nonetheless, as evidenced by Takahashi et al., D-serine is more prone to accumulate in CNS upon excessive production or reduced metabolism compared to glycine [87]. This imbalance provides a novel notion that D-serine diffuses more easily to distant extrasynaptic locations from the release sites and over-activates NMDARs in the proximity, which may play an essential role in the extensive deleterious NMDAR activation resulting in excitotoxicity [3].

Taken together, the discoveries discussed herein lead to a hypothesis that D-serine, an endogenous co-agonist of NMDAR receptor, could be released from astrocytes, neurons, as well as microglial cells during HIV-1 infection and play an important role in the pathogenesis of HAND. It preferentially binds to the extrasynaptic NR2B-containing NMDARs, hence leading to cell death via activation of NR2B-mediated pro-death pathway (**Figure 1**). Using high performance liquid chromatography (HPLC) and cell viability assay, we have more recently found that gp120 treatment induced a significant release of D-serine from cultured human astrocytes, leading to an enhancement of NMDAR-mediated excitotoxicity when co-cultured with rat hippocampal neurons. Whole-cell patch clamp recording revealed that bath application of D-serine potentiated NMDAR-mediated excitatory postsynaptic currents recorded in the CA1 region of rat hippocampal slices, predominantly via activation of extrasynaptic NR2B-containing NMDARs.

Summary

D-serine, as a co-agonist of NMDAR, mediates NMDA receptor transmission, synaptic plasticity, cell migration and neurotoxicity by binding to

the strychnine-insensitive "glycine site" located at the NR1 subunit. Endogenous D-serine has been suggested to be present in astrocytes, neurons, and microglia cells. D-serine could be released from glial cells during HIV-1 brain infection and/or proinflammatory activation of these cells. The released D-serine may play a critical role in the pathogenesis of HAND, but the mechanisms for regulating D-serine release and resultant neurotoxicity remain to be determined. The involvement of D-serine in the pathogenesis of neurodegenerative disorders makes it a potential and novel target for the development of therapeutic strategies in ameliorating NMDAR-mediated neural injuries, especially those caused by activation of NMDAR via glycine-binding site. Such therapeutic potential could be achieved through pharmacological regulation of D-serine levels within the brain, control D-amino acid oxidase (DAAOX) degradation of D-serine or blockade of glycine site of NMDAR.

Acknowledgements

Address correspondence to: Dr. Huangui Xiong, Neurophysiology Laboratory, Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198-5880, USA. Tel: 402-559-5140; Fax: 402-559-3744; E-mail: hxiong@unmc.edu

References

[1] Lamzin VS, Dauter Z, Wilson KS. How nature deals with stereoisomers. Curr Opin Struct Biol 1995; 5: 830-6.

[2] Corrigan JJ. D-amino acids in animals. Science 1969; 164: 142-9.

[3] Fuchs SA, Berger R, de Koning TJ. D-Serine: The right or wrong isoform? Brain Res 2011; 1401: 104-17.

[4] Kreil G. Peptides containing a D-amino acid from frogs and molluscs. J Biol Chem 1994; 269: 10967-70.

[5] Martínez-Rodríguez S, Martínez-Gómez AI, Rodríguez-Vico F, Clemente-Jiménez JM, Las Heras-Vázquez FJ. Natural occurrence and industrial applications of D-amino acids: an overview. Chem Biodivers 2010; 7: 1531-48.

[6] Fuchs SA, Berger R, Klomp LW, de Koning TJ. D-amino acids in the central nervous system in health and disease. Mol Genet Metab 2005; 85: 168-80.

[7] Billard JM. D-Amino acids in brain neurotransmission and synaptic plasticity. Amino Acids 2012; 43: 1851-60.

[8] Danysz W, Parsons CG. Glycine and N-methyl-D-aspartate receptors: physiological significance and possible therapeutic applications. Pharmacol Rev 1998; 50: 597-664.

[9] Pernot P, Maucler C, Tholance Y, Vasylieva N, Debilly G, Pollegioni L, Cespuglio R, Marinesco S. d-Serine diffusion through the blood-brain barrier: effect on d-serine compartmentalization and storage. Neurochem Int 2012; 60: 837-45.

[10] Lo EH, Pierce AR, Matsumoto K, Kano T, Evans CJ, Newcomb R. Alterations in K+ evoked profiles of neurotransmitter and neuromodulator amino acids after focal ischemia-reperfusion. Neuroscience 1998; 83: 449-58.

[11] Hicks CA, Ward MA, Ragumoorthy N, Ambler SJ, Dell CP, Dobson D, O'Neill MJ. Evaluation of glycine site antagonists of the NMDA receptor in global cerebral ischaemia. Brain Res 1999; 819: 65-74.

[12] Petty MA, Neumann-Haefelin C, Kalisch J, Sarhan S, Wettstein JG, Juretschke HP. In vivo neuroprotective effects of ACEA 1021 confirmed by magnetic resonance imaging in ischemic stroke. Eur J Pharmacol 2003; 474: 53-62.

[13] Choi DW, Rothman SM. The role of glutamate neurotoxicity in hypoxic-ischemic neuronal death. Annu Rev Neurosci 1990; 13: 171-82.

[14] Sasabe J, Chiba T, Yamada M, Okamoto K, Nishimoto I, Matsuoka M, Aiso S. D-serine is a key determinant of glutamate toxicity in amyotrophic lateral sclerosis. EMBO J 2007; 26: 4149-59.

[15] Lipton SA, Rosenberg PA. Excitatory amino acids as a final common pathway for neurologic disorders. N Engl J Med 1994; 330: 613-22.

[16] Wu S, Barger SW. Induction of serine racemase by inflammatory stimuli is dependent on AP-1. Ann N Y Acad Sci 2004; 1035: 133-46.

[17] Wu S, Basile AS, Barger SW. Induction of serine racemase expression and D-serine release from microglia by secreted amyloid precursor protein (sAPP). Curr Alzheimer Res 2007; 4: 243-51.

[18] Brito-Moreira J, Paula-Lima AC, Bomfim TR, Oliveira FB, Sepúlveda FJ, De Mello FG, Aguayo LG, Panizzutti R, Ferreira ST. Abeta Oligomers Induce Glutamate Release from Hippocampal Neurons. Curr Alzheimer Res 2011; 8: 552-62.

[19] Nagata Y, Borghi M, Fisher GH, D'Aniello A. Free D-serine concentration in normal and Alzheimer human brain. Brain Res Bull 1995; 38: 181-3.

[20] Kumashiro S, Hashimoto A, Nishikawa T. Free D-serine in post-mortem brains and spinal cords of individuals with and without neuropsychiatric diseases. Brain Res 1995; 681: 117-25.

[21] Chouinard ML, Gaitan D, Wood PL. Presence of the N-methyl-D-aspartate-associated glycine receptor agonist, D-serine, in human temporal cortex: comparison of normal, Parkinson, and Alzheimer tissues. J Neurochem 1993; 61: 1561-4.

[22] Xia JX, et al. D-serine potentiates NMDA receptor-mediated excitatory postsynaptic current (EPSCs) in hippocampal slices: Implications for HIV-1-associated neurotoxicity, in 40th Annual Meeting, Society for Neuroscience. Washington DC: 2011.

[23] Vesce S, Rossi D, Brambilla L, Volterra A. Glutamate release from astrocytes in physiological conditions and in neurodegenerative disorders characterized by neuroinflammation. Int Rev Neurobiol 2007; 82: 57-71.

[24] Maingat F, Vivithanaporn P, Zhu Y, Taylor A, Baker G, Pearson K, Power C. Neurobehavioral performance in feline immunodeficiency virus infection: integrated analysis of viral burden, neuroinflammation, and neuronal injury in cortex. J Neurosci 2009; 29: 8429-37.

[25] van de Bovenkamp M, Nottet HS, Pereira CF. Interactions of human immunodeficiency virus-1 proteins with neurons: possible role in the development of human immunodeficiency virus-1-associated dementia. Eur J Clin Invest 2002; 32: 619-27.

[26] Ozdener H. Molecular mechanisms of HIV-1 associated neurodegeneration. J Biosci 2005; 30: 391-405.

[27] Lindl KA, Marks DR, Kolson DL, Jordan-Sciutto KL. HIV-associated neurocognitive disorder: pathogenesis and therapeutic opportunities. J Neuroimmune Pharmacol 2010; 5: 294-309.

[28] Kaul M, Garden GA, Lipton SA. Pathways to neuronal injury and apoptosis in HIV-associated dementia. Nature 2001; 410: 988-94.

[29] Kaul M, Zheng J, Okamoto S, Gendelman HE, Lipton SA. HIV-1 infection and AIDS: consequences for the central nervous system. Cell Death Differ 2005 Aug; 12 Suppl 1: 878-92.

[30] del Palacio M, Alvarez S, Muñoz-Fernández MÁ. HIV-1 infection and neurocognitive impairment in the current era. Rev Med Virol 2012; 22: 33-45.

[31] Xia C, Luo D, Yu X, Jiang S, Liu S. HIV-associated dementia in the era of highly active antiretroviral therapy (HAART). Microbes Infect 2011; 13: 419-25.

[32] Kaul M, Lipton SA. Mechanisms of neuronal injury and death in HIV-1 associated dementia. Curr HIV Res 2006; 4: 307-18.

[33] Boissé L, Gill MJ, Power C. HIV infection of the central nervous system: clinical features and neuropathogenesis. Neurol Clin 2008; 26: 799-819, x.

[34] Yadav A, Collman RG. CNS inflammation and macrophage/microglial biology associated with HIV-1 infection. J Neuroimmune Pharmacol 2009; 4: 430-47.

[35] Mattson MP, Haughey NJ, Nath A. Cell death in HIV dementia. Cell Death Differ 2005; 12 Suppl 1: 893-904.

[36] Kaul M, Lipton SA. Mechanisms of neuroimmunity and neurodegeneration associated with HIV-1 infection and AIDS. J Neuroimmune Pharmacol 2006; 1: 138-51.

[37] McArthur JC, Hoover DR, Bacellar H, Miller EN, Cohen BA, Becker JT, Graham NM, McArthur JH, Selnes OA, Jacobson LP, et al. Dementia in AIDS patients: incidence and risk factors. Multicenter AIDS Cohort Study. Neurology 1993; 43: 2245-52.

[38] McArthur JC, Haughey N, Gartner S, Conant K, Pardo C, Nath A, Sacktor N. Human immunodeficiency virus-associated dementia: an evolving disease. J Neurovirol 2003; 9: 205-21.

[39] Antinori A, Arendt G, Becker JT, Brew BJ, Byrd DA, Cherner M, Clifford DB, Cinque P, Epstein LG, Goodkin K, Gisslen M, Grant I, Heaton RK, Joseph J, Marder K, Marra CM, McArthur JC, Nunn M, Price RW, Pulliam L, Robertson KR, Sacktor N, Valcour V, Wojna VE. Updated research nosology for HIV-associated neurocognitive disorders. Neurology 2007; 69: 1789-99.

[40] Block ML, Hong JS. Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. Prog Neurobiol 2005; 76: 77-98.

[41] Ghafouri M, Amini S, Khalili K, Sawaya BE. HIV-1 associated dementia: symptoms and causes. Retrovirology 2006; 3: 28.

[42] Bigge CF. Ionotropic glutamate receptors. Curr Opin Chem Biol 1999; 3: 441-7.

[43] Doble A. The role of excitotoxity in neurodegenerative disease: implications for therapy. Pharmacol Ther 1999; 81: 163-221.

[44] Wolosker H, Dumin E, Balan L, Foltyn VN. D-amino acids in the brain: D-serine in neurotransmission and neurodegeneration. FEBS J 2008; 275: 3514-26.

[45] Olney JW. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. Science 1969; 164: 719-21.

[46] Bonfoco E, Krainc D, Ankarcrona M, Nicotera P, Lipton SA. Apoptosis and necrosis: Two distinct events induced, respectively, by mild and intense insult with N-methyl-D-aspartate or nitric

oxide/superoxide in cortical cell cultures. Proc Natl Acad Sci U S A 1995; 92: 7162-7166.

[47] Yeh MW, Kaul M, Zheng J, Nottet HS, Thylin M, Gendelman HE, Lipton SA. Cytokine-stimulated, but not HIV-infected, human monocyte-derived macrophages produce neurotoxic levels of l -cysteine. J Immunol 2000; 164: 4265-70.

[48] Monyer H, Sprengel R, Schoepfer R, Herb A, Higuchi M, Lomeli H, Burnashev N, Sakmann B, Seeburg PH. Heteromeric NMDA receptors: molecular and functional distinction of subtypes. Science 1992; 256: 1217-21.

[49] Meguro H, Mori H, Araki K, Kushiya E, Kutsuwada T, Yamazaki M, Kumanishi T, Arakawa M, Sakimura K, Mishina M. Functional characterization of a heteromeric NMDA receptor channel expressed from cloned cDNAs. Nature 1992; 357: 70-4.

[50] Ciabarra AM, Sullivan JM, Gahn LG, Pecht G, Heinemann S, Sevarino KA. Cloning and characterization of chi-1: a developmentally regulated member of a novel class of the ionotropic glutamate receptor family. J Neurosci 1995; 15: 6498-508.

[51] Sucher NJ, Akbarian S, Chi CL, Leclerc CL, Awobuluyi M, Deitcher DL, Wu MK, Yuan JP, Jones EG, Lipton SA. Developmental and regional expression pattern of a novel NMDA receptor-like subunit (NMDAR-L) in the rodent brain. J Neurosci 1995; 15: 6509-20.

[52] Das S, Sasaki YF, Rothe T, Premkumar LS, Takasu M, Crandall JE, Dikkes P, Conner DA, Rayudu PV, Cheung W, Chen HS, Lipton SA, Nakanishi N. Increased NMDA current and spine density in mice lacking the NMDA receptor subunit NR3A. Nature 1998; 393: 377-81.

[53] Chatterton JE, Awobuluyi M, Premkumar LS, Takahashi H, Talantova M, Shin Y, Cui J, Tu S, Sevarino KA, Nakanishi N, Tong G, Lipton SA, Zhang D. Excitatory glycine receptors containing the NR3 family of NMDA receptor subunits. Nature 2002; 415: 793-8.

[54] Cull-Candy S, Brickley S, Farrant M. NMDA receptor subunits: diversity, development and disease. Curr Opin Neurobiol 2001; 11: 327-35.

[55] Furukawa H, Singh SK, Mancusso R, Gouaux E. Subunit arrangement and function in NMDA receptors. Nature 2005; 438: 185-92.

[56] Yao Y, Mayer ML. Characterization of a soluble ligand binding domain of the NMDA receptor regulatory subunit NR3A. J Neurosci 2006; 26: 4559-66.

[57] Sucher NJ, Awobuluyi M, Choi YB, Lipton SA. NMDA receptors: from genes to channels. Trends Pharmacol Sci 1996; 17: 348-55.

[58] Hollmann M, Heinemann S. Cloned glutamate receptors. Annu Rev Neurosci 1994; 17: 31-108.

[59] Robert JW, Kate P, Steve S, Nathalie S and Ronald SP. Trafficking of NMDA receptors. Annu Rev Pharmacol Toxicol 2003; 43: 335-58.

[60] Salter MG, Fern R. NMDA receptors are expressed in developing oligodendrocyte processes and mediate injury. Nature 2005; 438: 1167-71.

[61] Brickley SG, Misra C, Mok MH, Mishina M, Cull-Candy SG. NR2B and NR2D subunits coassemble in cerebellar Golgi cells to form a distinct NMDA receptor subtype restricted to extrasynaptic sites. J Neurosci 2003; 23: 4958-66.

[62] Wong HK, Liu XB, Matos MF, Chan SF, Pérez-Otaño I, Boysen M, Cui J, Nakanishi N, Trimmer JS, Jones EG, Lipton SA, Sucher NJ. Temporal and regional expression of NMDA receptor subunit NR3A in the mammalian brain. J Comp Neurol 2002; 450: 303-17.

[63] Mueller HT, Meador-Woodruff JH. Distribution of the NMDA receptor NR3A subunit in the adult pig-tail macaque brain. J Chem Neuroanat 2005; 29: 157-72.

[64] Tovar KR, Westbrook GL. The incorporation of NMDA receptors with a distinct subunit composition at nascent hippocampal synapses in vitro. J Neurosci 1999; 19: 4180-8.

[65] Townsend M, Yoshii A, Mishina M, Constantine-Paton M. Developmental loss of miniature N-methyl-D-aspartate receptor currents in NR2A knockout mice. Proc Natl Acad Sci U S A 2003; 100: 1340-5.

[66] Pérez-Otaño I, Ehlers MD. Homeostatic plasticity and NMDA receptor trafficking. Trends Neurosci 2005; 28: 229-38.

[67] Hardingham GE, Bading H. Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. Nat Rev Neurosci 2010; 11: 682-96.

[68] Vizi ES, Kisfali M, Lőrincz T. Role of nonsynaptic GluN2B-containing NMDA receptors in excitotoxicity: Evidence that fluoxetine selectively inhibits these receptors and may have neuroprotective effects. Brain Res Bull 2013 Apr; 93: 32-8.

[69] Wolosker H. NMDA receptor regulation by D-serine: new findings and perspectives. Mol Neurobiol 2007; 36: 152-64.

[70] Hashimoto A, Nishikawa T, Hayashi T, Fujii N, Harada K, Oka T, Takahashi K. The presence of free D-serine in rat brain. FEBS Lett 1992; 296: 33-6.

[71] Hashimoto A, Nishikawa T, Oka T, Takahashi K. Endogenous D-serine in rat brain: N-methyl-D-aspartate receptor-related distribution and aging. J Neurochem 1993; 60: 783-6.

[72] Hashimoto A, Oka T, Nishikawa T. Extracellular concentration of endogenous free D-serine in

the rat brain as revealed by in vivo microdialysis. Neuroscience 1995; 66: 635-43.

[73] Schell MJ, Molliver ME, Snyder SH. D-serine, an endogenous synaptic modulator: localization to astrocytes and glutamate-stimulated release. Proc Natl Acad Sci U S A 1995; 92: 3948-52.

[74] Snyder SH, Kim PM. D-amino acids as putative neurotransmitters: focus on D-serine. Neurochem Res 2000; 25: 553-60.

[75] Shreder KR, Liu Y, Nomanhboy T, Fuller SR, Wong MS, Gai WZ, Wu J, Leventhal PS, Lill JR, Corral S. Design and synthesis of AX7574: a microcystin-derived, fluorescent probe for serine/threonine phosphatases. Bioconjug Chem 2004; 15: 790-8.

[76] Williams SM, Diaz CM, Macnab LT, Sullivan RK, Pow DV. Immunocytochemical analysis of D-serine distribution in the mammalian brain reveals novel anatomical compartmentalizations in glia and neurons. Glia 2006; 53: 401-11.

[77] Bezzi P, Carmignoto G, Pasti L, Vesce S, Rossi D, Rizzini BL, Pozzan T, Volterra A. Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. Nature 1998; 391: 281-5.

[78] Bezzi P, Domercq M, Brambilla L, Galli R, Schols D, De Clercq E, Vescovi A, Bagetta G, Kollias G, Meldolesi J, Volterra A. CXCR4-activated astrocyte glutamate release via TNFalpha: amplification by microglia triggers neurotoxicity. Nat Neurosci 2001; 4: 702-10.

[79] Hamilton NB, Attwell D. Do astrocytes really exocytose neurotransmitters? Nat Rev Neurosci 2010; 11: 227-38.

[80] Oliet SH and Mothet JP. Regulation of N-methyl-D-aspartate receptors by astrocytic D-serine. Neuroscience 2009; 158: 275-83.

[81] Kartvelishvily E, Shleper M, Balan L, Dumin E, Wolosker H. Neuron-derived D-serine release provides a novel means to activate N-methyl-D-aspartate receptors. J Biol Chem 2006; 281: 14151-62.

[82] Mustafa AK, Kumar M, Selvakumar B, Ho GP, Ehmsen JT, Barrow RK, Amzel LM, Snyder SH. Nitric oxide S-nitrosylates serine racemase, mediating feedback inhibition of D-serine formation. Proc Natl Acad Sci U S A 2007; 104: 2950-5.

[83] Shoji K, Mariotto S, Ciampa AR, Suzuki H. Mutual regulation between serine and nitric oxide metabolism in human glioblastoma cells. Neurosci Lett 2006; 394: 163-7.

[84] Shoji K, Mariotto S, Ciampa AR, Suzuki H. Regulation of serine racemase activity by D-serine and nitric oxide in human glioblastoma cells. Neurosci Lett 2006; 392: 75-8.

[85] Balan L, Foltyn VN, Zehl M, Dumin E, Dikopoltsev E, Knoh D, Ohno Y, Kihara A, Jensen ON, Radzishevsky IS, Wolosker H. Feedback inactivation of D-serine synthesis by NMDA receptor-elicited translocation of serine racemase to the membrane. Proc Natl Acad Sci U S A 2009; 106: 7589-94.

[86] Labrie V, Wong AH, Roder JC. Contributions of the d-serine pathway to schizophrenia. Neuropharmacology 2012; 62: 1484-503.

[87] Takahashi K, Hayashi F, Nishikawa T. In vivo evidence for the link between L- and D-serine metabolism in rat cerebral cortex. J Neurochem 1997; 69: 1286-90.