Potential of D-Serine Involves Degeneration of Dopaminergic Neurons in MPTP/p Mouse Model of Parkinson's Disease

Ming Lu, Yi Fan, Mi Tang, Xia Qian, Jianhua Ding & Gang Hu

Jiangsu Key Laboratory of Neurodegeneration, Department of Pharmacology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, China

Correspondence

Gang Hu, M.D., Ph.D., Jiangsu Key Laboratory of Neurodegeneration, Department of Pharmacology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, P. R. China.
Tel.: 86-25-86863169;
Fax: 86-25-86863108;
E-mail: ghu@njmu.edu.cn

doi: 10.1111/j.1755-5949.2011.00275.x

Parkinson's disease (PD) is one of the most common neurodegenerative disorders, characterized by a progressive loss of dopaminergic (DA) neurons in the substantia nigra (SN) and depletion of the dopamine in the striatum [1]. Over the last decades, astrocytes are extensively considered as active partners in the progression of PD, modulating the survival and function of DA neurons.

Astrocytes, the most abundant cell type in the brain, are involved in a variety of important neuronal functions [2]. Besides providing the physical and metabolic supports for neurons, the astrocytes were also regarded as passive contributors to synaptic transmission, with very little electrical activity propagating through the glial syncytium. Additionally, the astrocytes also play a critical role in controlling the extracellular space, regulating local pH and ion homeostasis and reuptake of released neurotransmitters, which are essential for normal neuronal activity [3]. Rather different from their initial image as passive supportive cells in the central nervous system, the astrocytes are now recognized with an ability of releasing a set of gliotransmitter-like substances, such as glutamate, ATP, adenosine, and D-serine (D-Ser) to regulate synaptic communication within neuronal networks. Growing evidence indicates that D-Ser is an important player in the neuron-glial dialogue [4].

D-Ser serves as a physiological co-agonist with glutamate at N-methyl D-aspartate (NMDA) receptors [5]. Currently, multiple lines of evidence suggest that excessive production or release of D-Ser might be associated with chronic neurodegeneration. For example, previous investigation showed that the levels of D-Ser and serine racemase (SR) were greatly increased in the spinal cord of patients with familial and sporadic forms of amyotrophic lateral sclerosis (ALS) as well as in Alzheimer's disease, while they were downregulated in schizophrenia [6, 7]. However, the implication

of D-Ser in PD remains unclear and negligent. The present study was therefore designed to investigate the changes of D-Ser and SR in MPTP/p mouse model to gain an insight into the role of D-Ser, a typical gliotransmitter, in the development of PD.

Twelve-week-old male c57bl/6j mice were used in the experiments. The chronic MPTP intoxication protocol is similar to that described previously: 20 mg/kg MPTP (Sigma Chemicals, St. Louis, MO, USA) in saline was injected subcutaneously, and 250 mg/kg probenecid (Sigma Chemicals, St. Louis, MO, USA) in DMSO was injected intraperitoneally every 3.5 d over a period of 5 weeks. Mice were killed 1 week after the final injection. Vehicle mice were treated with saline and probenecid. MPTP handling and safety measures were in accordance with published guidelines. After mice were perfused with 4% paraformaldehyde (PFA), brains were dissected and maintained in 4% PFA overnight. At the end of gradient dehydration, the brain tissues were then sectioned by Leica freezing microtome at 30 μ m. For immunofluorescence, sections were incubated overnight with pooled primary antibodies: D-Ser (1:1500; AbD Serotec), SR (1:1000; Santa Cruze) together with mouse anti-NeuN (1:200; Chemicon) or mouse anti-TH (1:4000; Sigma) or mouse anti-GFAP antibody (1:1000; Chemicon) followed by goat anti-rat TRITC (red) (1:1000; invitrogen), and goat anti-mouse FITC (green) (1:1000; invitrogen). The level of D-Ser was detected by high-performance liquid chromatography (HPLC).

As shown in Figure 1, it was found that D-Ser and SR were localized in both astrocytes and tyrosine hydroxylase positive (TH⁺) neurons of the SN. Similarly, D-Ser was also expressed in astrocytes and neurons of striatum. These findings, supported by previous report, suggest that the gliotransmitter, D-Ser, resides in PD-related encephalic region at physiological condition. The

Figure 1 The location of D-Ser and SR in both astrocytes (GFAP⁺) and neurons (TH⁺, NeuN⁺). Double immunofluorescence revealed that D-Ser and SR expressed in SN (A), (B) and striatum (C) at physiological condition.

levels of D-Ser and SR may be involved in the development of PD in MPTP/p mouse model.

After administration of MPTP for 5 weeks, TH⁺ neurons were significantly reduced and astrocytes were activated in SN (Figure 2A). This result indicated that the PD mouse model was established successfully. Meanwhile, the expressions of D-Ser and SR were dramatically upregulated in astrocytes of SN. However, the elevated levels of D-Ser and SR could not be observed in TH⁺ neurons of SN, where abundant cell bodies of DA neurons are located (Figure 2A). Interestingly, D-Ser and SR were markedly increased in both astrocytes and nondopaminergic neurons in striatum, where is the location of DA neuron projects (Figure 2B). These results were confirmed by the detection of HPLC. We found that the level of D-Ser significantly rose in the striatum of MPTP-treated mice exclusively and this augment of gliotransmitter could not be observed in mPFC and hippocampus (Figure 2C). All these findings indicate that D-Ser, which functions as a gliotransmitter from astrocytes, is changed specifically in SN and striatum and participates in the neurodegeneration of PD.

Recent literatures suggest that the naturally occurring amino acids glycine and D-Ser act as full agonists at the NMDA-receptor-

associated glycine site. Striatal NMDA receptors are crucial for dopamine–glutamate interactions and their stimulation has been shown to enhance dopamine release and synthesis within the striatum [8]. On the other hand, overactivation or dysfunction of NMDA receptor, the key glutamate receptor, is the main culprit of a large number of neurodegenerative diseases. D-Ser binds with a high affinity to a co-agonist site on the NMDA receptor. Along with glutamate, it mediates several important physiological and pathological processes, including NMDA receptor transmission, synaptic plasticity, and neurotoxicity [9]. Furthermore, NMDA receptor antagonists have shown promise in reversing motor symptoms in animal PD models [10], indicating that these receptors may represent an innovative therapeutic target for PD.

In the current study, we revealed that both astrocytes and neurons express D-Ser and SR in midbrain. Furthermore, chronic MPTP treatment markedly intensified the fluorescence of D-Ser and SR in astrocytes of SN due to astrocytic activation. The results suggest that increased D-Ser in astrocytes and neurons may overactivate NMDA receptors and give rise to MPTP hypersensitivity in mice. These findings demonstrate for the first time

Figure 2 The changes of D-Ser and SR in SN and striatum of MPTP-treated mice. Immunofluorescence of D-Ser and SR was enhanced in astrocytes but not in TH⁺ neurons of SN (A). Expression and release of D-Ser were both elevated in striatum but not in mPFC and hippocampus (B), (C). Data were expressed as mean $\pm$ S.E.M., $n = 8$, * $p < 0.05$ vs. saline group.

that D-Ser is associated with the development of PD. However, more detailed stories of gliotransmitters, including the exact identity of secretory signals, the mechanisms of secretion, and their functional significance, are still in need of further exploration.

This study was supported by the grants from the National Key Basic Research Program of China (No. 2011CB504100 and No. 2009CB521906) and the National Natural Science Foundation of China (No. 81030060 and No. 30973517).

References

- Moore DJ, West AB, Dawson VL, Dawson TM. Molecular pathophysiology of Parkinson's disease. *Annu Rev Neurosci* 2005;**28**:57–87.
- Seifert G, Schilling K, Steinhauser C. Astrocyte dysfunction in neurological disorders: A molecular perspective. *Nat Rev Neurosci* 2006;**7**(3):194–206.
- Allen NJ, Barres BA. Neuroscience: Glia—more than just brain glue. *Nature* 2009;**457**(7230):675–677.
- Oliet SH, Mothet JP. Molecular determinants of D-serine-mediated gliotransmission: From release to function. *Glia* 2006;**54**(7):726–737.
- Kielian T, Esen N. Effects of neuroinflammation on glia–glia gap junctional intercellular communication: A perspective. *Neurochem Int* 2004;**45**(2–3):429–436.
- Wolosker H, Dumin E, Balan L, Foltyn VN. D-amino acids in the brain: D-serine in neurotransmission and neurodegeneration. *FEBS J* 2008;**275**(14):3514–3526.
- Billard JM. D-serine signalling as a prominent determinant of neuronal–glial dialogue in the healthy and diseased brain. *J Cell Mol Med* 2008;**12**(5B):1872–1884.
- Cheramy A, L'Hirondel M, Godeheu G, Artaud F, Glowinski J. Direct and indirect presynaptic control of dopamine release by excitatory amino acids. *Amino Acids* 1998;**14**(1–3):63–68.
- Katsuki H, Nonaka M, Shirakawa H, Kume T, Akaike A. 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* 2004;**311**(2):836–844.
- Johnson KA, Conn PJ, Niswender CM. Glutamate receptors as therapeutic targets for Parkinson's disease. *CNS Neurol Disord Drug Targets* 2009;**8**(6):475–491.