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D-serine relieves chronic lead exposure-impaired long-term potentiation in the CA1 region of the rat hippocampus *in vitro*

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Abstract

Chronic lead-exposure produces long-lasting astroglial morphological and functional changes, which disturb the neuronal functions in the hippocampus. It has been shown that glia-derived D-serine is an essential signal for *N*-methyl-D-aspartate receptor (NMDAR)-dependent synaptic plasticity in the hippocampal CA1 region. However, the relationship between D-serine and the chronic lead exposure-induced deficit of synaptic plasticity is not clear. In the present study, the properties of D-serine on the chronic lead exposure-impaired synaptic plasticity in the rat hippocampal CA1 region were investigated with electrophysiological recording techniques *in vitro*. We found that 50 μM D-serine rescued the chronic lead exposure-induced deficit of long-term potentiation (LTP). However, this effect could be abolished by 7-chlorokynurenic acid (7-ClKY), which is a specific antagonist of the glycine-binding site of NMDARs. In contrast, D-serine had no effect on the NMDAR-independent LTP, which was induced in the mossy-CA3 synapses. In addition, we found that D-serine rescued the acute Pb^{2+}-impaired NMDAR-mediated excitatory postsynaptic currents (EPSCs) partially. These findings demonstrate that D-serine relieves the chronic lead exposure-induced deficit of synaptic plasticity via NMDAR activation suggesting that administration of D-serine may be a potential therapeutic intervention to treat chronic lead exposure-impaired cognitive functions or affective disorders.

Keywords: Chronic lead exposure; Glia-derived D-serine; NMDARs; Hippocampus; Long-term potentiation

Lead is one of the most important neurotoxic metals in environment. Chronic lead exposure (CLE) during the early developmental period is known to be associated with cognitive and neurobehavioral dysfunction in children and animals [15,20,26]. Generally, activity-dependent synaptic plasticity, such as long-term potentiation (LTP), is believed to be the mechanism underlying certain types of learning and memory [4]. In addition, both *in vivo* and *in vitro*, CLE impairs the induction and maintenance of hippocampal LTP [12]. Thus, the investigation of possible relieving strategies for CLE-impaired synaptic plasticity is significant to cognitive dysfunction.

Neurons and glia talk to each other at synapses. Glia senses the synaptic activity and consequently regulate synaptic efficacy via the release of gliotransmitters. One such gliotransmitter is D-serine, which is considered as an endogenous ligand for the glycine-binding site of NMDARs [10,3]. Furthermore, glia-derived D-serine is an essential signalling molecule in LTP induction via the activation of NMDARs [19,30]. Interestingly, lead exposure produces long-lasting astroglial morphological and functional changes which contribute to excitatory neurotransmission [9,22,24]. On the other hand, lead alters the release of neurotransmitters, such as dopamine, norepinephrine and acetylcholine, by interfering with calcium metabolism or synaptic function [7,21]. In addition, in the rat hippocampal CA1 region, LTP is NMDAR-dependent [2]. Given the potential role of glia-derived D-serine in neurotransmission, we investigated whether D-serine can relieve the CLE-induced deficit of synaptic plasticity.

The present study reveals that D-serine rescues the CLE-induced deficit of NMDAR-dependent LTP and relieves the acute Pb^{2+}-impaired NMDAR-mediated excitatory neurotransmission. These findings provide a promising strategy for studying CLE-dependent alterations in cognitive functions or affective disorders.

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The protocol for chronic exposure to lead has been characterized previously [28]. In brief, Wistar rats were fed on distilled water ad libitum and kept at a carefully controlled ambient temperature (24 ± 1 °C) and relative humidity (50 ± 10%). On parturition day, the dams were randomly divided into two groups from 12 litters:control and lead-exposure. The lead-exposed pups acquired lead via milk of dams whose drinking water contained 0.2% (1090 ppm) lead acetate from parturition, while the control dams remained on distilled water throughout the lactation period and litters were culled to eight pups. At the age of 21 days, offspring were weaned and housed in a colony room with a 12:12 h light, dark schedule and permitted free access to food and distilled water with 0.2% lead acetate. Extracellular recordings were carried out at the age of 23–28 days.

We prepared hippocampal slices as described previously [25]. In brief, the rats were decapitated under deep halothane anesthesia. Hippocampal formation was dissected rapidly and placed in ice-cold artificial cerebral spinal fluid (ACSF). Horizontally sectioned 400 μm thick hippocampal slices were cut and then transferred into a submersion-type chamber with ACSF for at least 1 h to allow their energetic and functional recovery. The ACSF which was saturated and continuously bubbled with the gas mixture (95% O_2 and 5% CO_2) contained (in mM): NaCl, 120; KCl, 2.5; $NaHCO_3$, 26; NaH_2PO_4, 1.25; $CaCl_2$, 1.19; $MgSO_4$, 2.0 and D-glucose, 10. For whole-cell recordings, $MgSO_4$ concentration was 0.2 mM. Then, the slice was gently transferred into a recording chamber and maintained at stable temperature (32 °C). The experimental protocols were approved by the Care and Use of Animals Committee of University of Science and Technology of China and the Department of Biology of the Chinese Academy of Sciences, PR China.

For extracellular recordings, the pipette (2–3 MΩ resistance) filled with 3 M NaCl was placed at the stratum radiatum of the hippocampal CA1 area to record the field excitatory postsynaptic potentials (fEPSPs), in response to the stimulation of Commissural/Schaffer fibers. Input/output curves were measured and baseline recordings were adjusted to evoke approximately 50% of the maximum fEPSP amplitude. In another group, fEPSPs were recorded from the stratum lucidum in the CA3 region and the stimulating electrode was placed in the cell layer of the dentate gyrus to stimulate the mossy fibres. After baseline recordings (20 min, one sweep per 30 s), high frequency stimulation (HFS, 100 Hz, 100 pulses) was applied to induce LTP that was recorded for 40 min. The average magnitudes of LTP were the last 5 min (35–40 min after HFS) fEPSPs slopes and expressed as mean ± S.E.M.% of the baseline fEPSPs slopes. Each point in the figures represents the average slope of six successive fEPSPs responses with Igor software.

For whole-cell recordings, pyramidal neurons in the CA1 region were visualized and identified based on morphology under an upright microscope (E-600-FN, Nikon) with differential interference contrast optics. Patch pipettes for recordings were filled with (in mM): potassium gluconate, 130; KCl, 10; $CaCl_2$, 1; NaCl, 6; HEPES, 20; EGTA, 10; Mg-ATP, 3; Na-GTP, 0.5 and QX-314, 5, pH 7.2 (321 mOsm). Cells were clamped at +40 mV for NMDAR-mediated currents recording. The stimulating electrode was placed in the stratum radiatum of CA1 region and electrical stimuli (0.1 ms in duration) were delivered at a frequency of 0.05 Hz to obtain baseline responses. NMDAR-mediated EPSCs were identified in the presence of D-AP5 (50 μM) besides CNQX (10 μM), strychnine (0.4 μM) and picrotoxin (50 μM). Student's *t*-test was used to analyze the significance between two groups and one-way ANOVA was used to analyze the difference among groups. Statistical significance was determined as $P < 0.05$.

All recordings were performed in the presence of 50 μM picrotoxin to block GABA receptor-mediated events. D-serine, D-amino acid oxidase (DAAO, 0.1 U/ml) and lead acetate (Pb^{2+}) were dissolved in aqueous solution and applied through bath perfusion during experiments or applied directly into ACSF to incubate slices for at least 1 h. All reagents in the present experiments were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

D-serine (50 μM) enhanced LTP magnitudes significantly (D-serine, 242.9 ± 10.3% of baseline, $n = 8$, $P < 0.05$; Control, 197.9 ± 8.3% of baseline, $n = 9$, $P < 0.05$; $P < 0.05$, D-serine versus control; Fig. 1A). Consistent with the results of previous studies, CLE decreased LTP magnitudes (solid circles, 127.8 ± 9.4% of baseline, $n = 5$, $P < 0.05$; $P < 0.05$, control versus CLE; Fig. 1B) compared with the control. However, 50 μM D-serine enhanced the CLE-reduced LTP magnitudes significantly (open circles, 203.3 ± 13.5% of baseline, $n = 7$, $P < 0.05$; $P < 0.05$, D-serine + CLE versus CLE; Fig. 1B). Moreover, in the CLE rat slices incubated in D-serine, a further potentiation could be induced by a subsequent HFS (D-serine + CLE, 118.2 ± 12.0% of the responses 30 min after the first HFS, $P < 0.05$; CLE, 102.2 ± 11.4% of the responses 30 min after the first HFS; Fig. 1B). In addition, HFS failed to induce LTP in the slices treated with DAAO (0.1 U/ml, the enzyme which degrades D-serine, open circles, 114.6 ± 8.1% of baseline, $n = 4$; Fig. 1C), confirming that endogenous D-serine is essential for LTP induction or expression. On the other hand, we found that 100 μM 7-chlorokynurenic acid (7-ClKY, a specific antagonist to the glycine-binding site of NDMARs) blocked the LTP expression in the CLE rat slices treated with D-serine (solid circles, 121.9 ± 9.5% of baseline, $n = 4$; Fig. 1C). These data suggest that D-serine relieves the CLE-induced deficit of LTP via NMDAR activation.

To confirm that D-serine relieves the CLE-induced deficit of LTP via NMDAR activation, HFS was applied on the mossy-CA3 synapses. As shown in Fig. 2A, D-serine had no effect on the mossy-CA3 LTP magnitudes (open circles, 144.1 ± 7.5% of baseline, $n = 7$, $P < 0.05$) compared with the control (solid circles, 151.9 ± 8.0% of baseline, $n = 5$, $P < 0.05$) in normal rat slices. Interestingly, in 10 slices of 4 CLE rats, LTP magnitudes were not influenced by D-serine as well (CLE, open circles, 161.7 ± 11.2% of baseline, $n = 5$, $P < 0.05$; CLE + D-serine, solid circles, 162.9 ± 12.4% of baseline, $n = 5$, $P < 0.05$; $P > 0.05$, D-serine + CLE versus CLE; Fig. 2B). Consistent with the results of previous studies, D-AP5 did not affect the HFS-induced mossy-CA3 LTP (161.7 ± 11.2% of baseline, $n = 5$, $P < 0.05$; Fig. 2C) suggesting that the mossy-CA3 LTP is NMDAR-independent [5].