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Galantamine rescues lead-impaired synaptic plasticity in rat dentate gyrus

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ABSTRACT

Chronic lead exposure causes a variety of impairments in learning and memory and cognitive function. Synaptic plasticity in hippocampus is an extensively studied cellular model of learning and memory, which includes long-term potentiation (LTP) and long-term depression (LTD) in two forms. Depotentiation (DP) is another form of synaptic plasticity. Previous studies show that chronic lead exposure can damage the induction of LTP/LTD in hippocampal CA1 and dentate gyrus (DG) areas. In the present study, we investigated the repair and protection on lead-caused synaptic plasticity impairment by galantamine, using field potential recording on chronic lead exposure rats. The results showed that chronic lead exposure impaired LTP/DP induction in DG area of the hippocampus, and galantamine caused a significant increase on the amplitudes of LTP/DP of lead-exposed rats, but only a small increase in non-exposed group. These results suggest that galantamine could reverse the lead-induced impairments of synaptic plasticity in rats and might be an effective medicine to cure the cognitive deficits induced by lead.

1. Introduction

Hippocampus is the core of learning and memory, long-term potentiation (LTP) is important forms of synaptic plasticity, which is believed to underlie learning and memory (Bliss and Collingridge, 1993). Since found by Bliss in 1970s (Bliss and Lomo, 1973), LTP has been studied widely. The mechanism of induction and maintenance of LTP involves both the augmentation of transmitter releasing in pre-synapse (Lasley and Gilbert, 1996) and the activation of *N*-methyl-D-aspartate (NMDA) receptor in post-synapse (Cohn and Cory-Slechta, 1994; Collingridge, 1992; Collingridge and Bliss, 1995). Also, calcium influx (Cohn and Cory-Slechta, 1994) and retrograde messenger (nitric oxide (NO), etc.) play important roles. Long-term depression (LTD), another important form of synaptic plasticity, was widely studied in recent years. It was induced in cerebellar Purkinje cell initially (Ito et al., 1982), then found to be induced by low frequently stimulate in hippocampus. There are some different types of LTD, one of them is homo-synaptic LTD, which can be induced by low frequency stimulus (LFS) in one pathway, and considered to have relationship with the intracellular calcium concentration and the activation of post-synaptic NMDA receptors (Linden and Connor, 1995). Depotentiation (DP) is another form of synaptic plasticity, which has been known as a reversal of induced LTP caused by application of low-frequency stimulation after LTP. Paired-pulse facilitation (PPF), as an enhancement model of short term synaptic transmission efficiency, is thought to represent the augmentation of pre-synaptic transmitter release (Leung and Fu, 1994). At the same time, the activation of NMDA receptor is also involved in this process (Busselberg et al., 1994).

Many previous studies investigated the mechanism of lead-induced neurotoxicity. At the cellular and molecular level, lead can lead to oxidative damage, alter NO synthase (NOS) activity, disturb the pathway mediated by retrograde messenger NO, and activate the signal transduction pathway such as protein kinase (PKC), nuclear factor-κB (NF-κB), activating protein-1 (AP-1), c-Jun N-terminal kinases (JNK), mitogen-activated protein kinase (MAPK) and caspases. Lead can also repress the currents mediated by NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate receptor in hippocampus, block voltage-dependent calcium channel (VDCC), inhibit NMDA receptor subunits expression, influence the synthesis and release of many kinds of neurotransmitters such as glutamate, acetylcholine (ACh), adrenalin and serotonin (5-hydroxytryptamine, 5-HT), damage

Abbreviations: LTP, long-term potentiation; LTD, long-term depression; DP, depotentiation; DG, dentate gyrus; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; LFS, low frequency stimulus; PPF, paired-pulse facilitation; NOS, NO synthase; PKC, protein kinase; NF-κB, nuclear factor-κB; AP-1, activating protein-1; JNK, c-Jun N-terminal kinases; MAPK, mitogen-activated protein kinase; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; VDCC, voltage-dependent calcium channel; ACh, acetylcholine; 5-HT, 5-hydroxytryptamine; AChRs, ACh receptors; nAChR, nicotinic AChR; GABA, γ-aminobutyric acid; I/O, input/output; IPIs, interpulse intervals; PS, population spike; EPSP, excitatory postsynaptic potential; HFS, high frequency stimulus; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; mGluR, metabotropic glutamate receptor; L-VDCC, L-type voltage dependent calcium channel; 7-ClKN, 7-chlorokynurenic acid.

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LTP/LTD which is induced by NMDA/non-NMDA receptor, decrease the synaptic plasticity, and contribute to the progress of neuronal apoptosis (Busselberg et al., 1994; Cory-Slechta, 1995; Lasley and Gilbert, 2000; Mike et al., 2000; Ramesh et al., 2001).

ACh and ACh receptors (AChRs) are important in the synaptic plasticity of hippocampal neurons and learning and memory. Studies have shown that Pb^{2+} can depress the synthesis and release of ACh, damage the function of α7 nicotinic AChR (nAChR) channel in acute dissociated or cultured hippocampal γ-aminobutyric acidergic (GABAergic) neurons, and reduce the affinity of M2 muscrinic AChR (mAChR) to its ligand (Cory-Slechta, 1995; Mike et al., 2000). But the mechanism of lead-induced deficit of AChR-mediated synaptic plasticity of hippocampal neurons remains unclear.

Galantamine is a drug used for the treatment of mild to moderate Alzheimer's disease. It is an alkaloid that is obtained from the bulbs and flowers of the *Caucasian snowdrop* (Voronov's snowdrop), *Lycoris radiata* (Red Spider Lily), *Galanthus woronowii* (Amaryllidaceae) and related species. Galantamine is a competitive and reversible cholinesterase inhibitor. It is believed it works by enhancing cholinergic function by increasing the concentration of acetylcholine in the brain. Galantamine has also shown activity in modulating the nicotinic cholinergic receptors to increase acetylcholine release. It has been more than 30 years of clinical applications for the treatment of neuromuscular blockade reversal, myasthenia gravis and polio sequelae. Sweeney et al. reported that galantamine improve memory impairment in mice, which suggested that it may be effective on central cholinergic disorder in Alzheimer's disease (Sweeney et al., 1988). Pharmacokinetics study found that galantamine can easily cross the blood–brain barrier, distribute mostly in the frontal lobe, temporal lobe and brain region related to learning and memory with a long effective time (biological half-life is 5–6 h). Galantamine can improve the memory impairment and space positioning capability in rat, enhance the excitement of the central nervous system, and promote the formation of conditioned reflex. Clinical study found galantamine can significantly improve the patient's emotional state and self-care ability and effectively improve memory function (Koontz and Baskys, 2005; Rockwood et al., 2001).

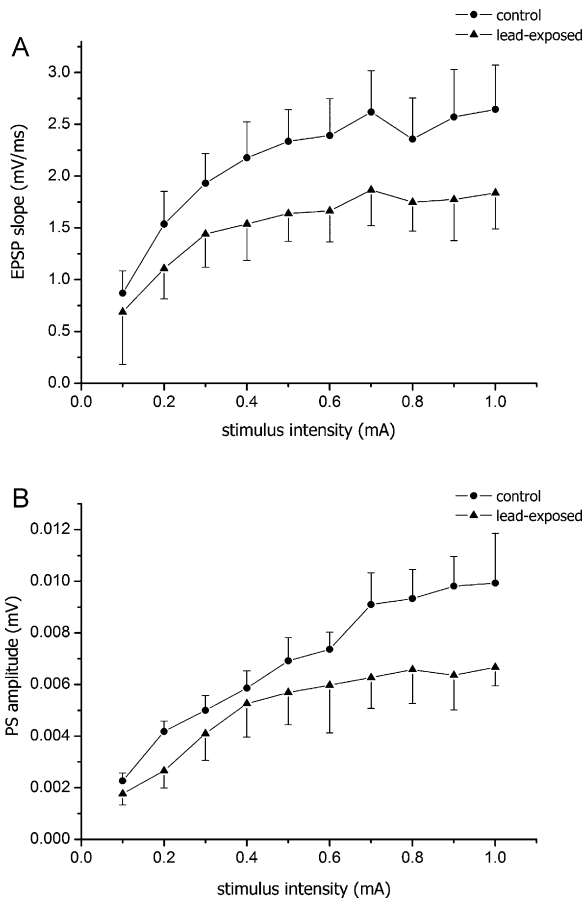

Fig. 1. I/O curves (mean ± S.E.M.) of the EPSP slope (A) and PS amplitude (B) in DG area in control ($n = 8$) and lead-exposed ($n = 8$) rats as a function of stimulus intensity before induction of LTP/DP. (A) The EPSP slope was significantly depressed in lead-exposed group, compared with control group ($F = 40.82$, $P < 0.05$). (B) There is no significant difference in PS amplitude between control and lead-exposed groups ($P > 0.05$).

2. Experimental procedures

2.1. Experimental animals and treatment

The experiments were performed on four groups of adult Wistar rats (postnatal days 60–90, male and female): control, control with a glantamine application, lead-exposed and lead-exposed with a galantamine application. In the present protocol, the offsprings were exposed to lead only via their mother's milk. After pups delivery, the dam had access either to 20 ml tap water (control group) or to water with 0.2% lead acetate depending on the group. After weaning until the experiment, the rats had access to the same solution as their mother. Galantamine was applied by intraperitoneal injection every day (1 mg/day/100 g body weight) two weeks before the electrophysiological recording. Extracellular recording measured in dentate gyrus area of hippocampus were carried out in 60–90 days old animal. No more than one animal per litter was utilized for a given experimental measure.

2.2. Hippocampus lead determination

Lead concentration in the hippocampus was estimated on the animals used for electrophysiology. After decapitation of the animals, the hippocampi were isolated, and digested with an organic tissue solubilizer. The lead concentration in the hippocampus was measured by PlasmaQuad 3 inductive coupled plasma mass spectroscopy (VG Elemental Ltd., UK).

2.3. Stimulation and recording

In each recording session, rats were anaesthetized with urethane 1.8 g/kg, then were fixed in a stereotaxic head-holder. The incisor bar was adjusted 2.4 mm below biauricular line (Bao and Shu, 1991). The skull was exposed and the animal's body temperature, heart rate and electrocardiograph were monitored. A concentric bipolar stimulating electrode was placed in the lateral perforant path (coordinates with the skull surface flat: 8.0 mm posterior to bregma, 4.5 mm lateral to the midline, 2.8–3.0 mm ventral). A 2 M NaCl filled glass pipette recording electrode (3–5 μm tip diameter, 1–3 MΩ resistance) was lowered into the DG (coordinates: 3.8 mm posterior to bregma, 2.5 mm lateral to the midline) until maximal response to prefrontal path stimulus was observed (3.0–3.5 mm ventral).

2.4. Input/output (I/O) function

I/O curves were generated by increasing the stimulus current by steps of 0.1 mA (0.1–1.0 mA) in order to evaluate synaptic potency. Stimulus pulses were delivered at 0.125 Hz and three responses at each current intensity were averaged.

2.5. Paired-pulse ratio (PPR)

PPR was evaluated by increasing the interpulse intervals by steps (IPIs, 10–300 ms). The stimulus current intensity was adjusted at intensity yielding 50% of the maximal amplitude of population spike (PS). Stimulus pairs were delivered at 0.125 Hz and three responses were averaged at each IPI.

2.6. Long-term potentiation and depotentiation

In this study, both LTP and DP were recorded in each animal. First, LTP was evoked. The stimulus intensity selected for baseline measurements was adjusted to yield about 40% of excitatory postsynaptic potential (EPSP) maximal amplitude. After 10 min recording with stimulus applied at 8 s intervals, a high frequency stimulus was applied (HFS: 250 Hz, 1 s). Posttetanic recordings were performed for 1 h with single pulse applied for a frequency of 0.125 Hz. Then after 10 min baseline recording, DP was induced by applying the low frequency stimulation (LFS: 1 Hz, 15 min) after 10 min of baseline recording. 0.125 Hz pulses were then applied for 45 min. At the end of each recording session, small electrolytic lesions (10 mA, 10 s) were made for histological verification of the tip position of the electrodes. Then hippocampus was isolated for measuring lead concentration.