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CLIOQUINOL AND VITAMIN B12 (COBALAMIN) SYNERGISTICALLY RESCUE THE LEAD-INDUCED IMPAIRMENTS OF SYNAPTIC PLASTICITY IN HIPPOCAMPAL DENTATE GYRUS AREA OF THE ANESTHETIZED RATS *IN VIVO*

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Abstract—Lead (Pb^{2+}) exposure in development induces impairments of synaptic plasticity in the hippocampal dentate gyrus (DG) area of the anesthetized rats *in vivo*. The common chelating agents have many adverse effects and are incapable of alleviating lead-induced neurotoxicity. Recently, CQ, clioquinol (5-chloro-7-iodo-8-hydroxy-quinoline), which is a transition metal ion chelator and/or ionophore with low affinity for metal ions, has yielded some promising results in animal models and clinical trials related to dysfunctions of metal ions. In addition, CQ-associated side effects are believed to be overcome with vitamin B12 (VB12) supplementation. To determine whether CQ treatment could rescue impairments of synaptic plasticity induced by chronic Pb^{2+} exposure, we investigated the input/output functions (I/Os), paired-pulse reactions (PPRs) and long-term potentiation (LTP) of different treatment groups in hippocampal DG area of the anesthetized rat *in vivo* by recording field potentials and measured hippocampal Pb^{2+} concentrations of different treatment groups by PlasmaQuad 3 inductive coupled plasma mass spectroscopy. The results show: CQ alone does not rescue the lead-induced impairments of synaptic plasticity in hippocampal DG area of the anesthetized rats *in vivo*; VB12 alone partly rescues the lead-induced impairments of LTP; however the co-administration of CQ and VB12 totally rescues these impairments of synaptic plasticity and moreover, the effects of CQ and VB12 co-administration are specific to the lead-exposed animals.

Key words: lead, clioquinol, dentate gyrus, hippocampus, rat.

Lead (Pb^{2+}) is one of the most important neurotoxic metals in the environment. It is now well established that the chronic Pb^{2+} exposure in development produces cognitive deficits and neurobehavioral dysfunction in children and in a lot of animal species (Cory-Slechta, 1990; Bellinger et al., 1991; Banks et al., 1997). Significant cognitive impairment of developing brain is reported at blood Pb^{2+} levels as low as 10 μg/dl in children (Needleman and Gatsonis, 1990; Bellinger et al., 1991) and animal studies have supported these findings, indicating that developmental exposure to Pb^{2+} resulting in environmentally relevant blood concentrations also produces cognitive dysfunction (Cory-Slechta et al., 1985, Rice, 1993). In the Pb-exposed animals a blood lead concentration of 30 μg/dl compares well with the about 10 μg/dl blood concentration measured in Pb-exposed children. The hippocampus, which is one of the most important loci of lead-mediated toxicity, plays a key role in high cognitive functions, such as learning and memory (Squire, 1992). Long-term potentiation (LTP) in the hippocampus is a form of activity-dependent synaptic plasticity that may be the electrophysiological substrate for learning and memory (Bliss and Lomo, 1973; Bliss and Collingridge, 1993). Pb-induced impairments of synaptic plasticity both in slices of hippocampus from Pb-exposed animals (Altmann et al., 1993) and in CA1 and dentate gyrus (DG) area of hippocampus in Pb-exposed rats *in vivo* (Gilbert et al., 1996, 1999; Zaiser and Miletic, 1997) have been reported. Our previous studies also showed that chronic Pb^{2+} exposure impaired LTP in DG area of hippocampus in rats *in vivo*, which possibly is associated with chronic lead-induced deficits of learning and memory in rats (Ruan et al., 1998).

Although common chelating agents, such as triethylene tetramine (TETA), penicillamine and desferrioxamine, are currently available for the treatment of Pb^{2+} neurotoxicity, they are shown to have many adverse effects and to be incapable of alleviating some neurotoxic effects of Pb^{2+}. So far, no efficient drugs are available for treating chronic lead-induced deficits, especially, related to learning and memory (Mortensen and Walson, 1993; Porru and Alessio, 1996). CQ, clioquinol (5-chloro-7-iodo-8-hydroxy-quinoline) was first prepared in the early part of the last century and was widely used as an antibiotic. In the 1970s, it was banned in many countries due to being linked to outbreak of subacute myelo-optic neuropathy (SMON) in Japan (Arbiser et al., 1998; Tateishi, 2000; Tabira, 2001). However, a causal relation between CQ and SMON was never proven (Bush and Masters, 2001). In fact, CQ is a transition metal ion chelator and/or ionophore with low affinity for divalent cations, such as: CQ for Zn^{2+} (K_1=7.0)

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Abbreviations: AD, Alzheimer's disease; Aβ, β-amyloid protein; control+CQ+VB12, control with clioquinol and vitamin B12 group; CQ, clioquinol (5-chloro-7-iodo-8-hydroxy-quinoline); DG, dentate gyrus; fEPSP, field excitatory postsynaptic potential; HD, Huntington's disease; HFS, high frequency stimulus; I/O, input/output function; IPI, interpulse interval; lead+CQ, lead-exposed with clioquinol group; lead+CQ+VB12, lead-exposed with clioquinol and vitamin B12 group; lead+VB12, lead-exposed with vitamin B12 group; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate; PD, Parkinson's disease; PPR, paired-pulse reaction; PS, population spike; SMON, subacute myelo-optic neuropathy; VB12, vitamin B12.

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and CQ for Cu^{2+} (K_1=8.9) (Cherny et al., 2001) and it is hydrophobic and freely permeable across the blood–brain barrier (Padmanabhan et al., 1989). Recently, as a novel and potential therapeutic strategy, CQ treatment has yielded some promising results in animal models and clinical trials related to dysfunctions of metal ions. For example, it enhanced Aβ (β-amyloid protein) aggregate dissolution and decreased Aβ toxicity in an Alzheimer's disease (AD) model, where Cu and Zn are elevated in the neocortex and particularly concentrated in amyloid plaques (Cherny et al., 2001); the phase II clinical trial showed that it displayed early positive effects on AD and had no significant CQ neurotoxicity (Ritchie et al., 2003); it prevented MPTP (1-methyl-4-phenyl-1,2,3,6-tetra-pyridine)-induced neurotoxicity *in vivo*, a Parkinson's disease (PD) model, where Fe levels in substantia nigra (SN) have been reported to be elevated (Kaur et al., 2003); it decreased polyQ-expanded protein accumulation and promoted cell survival in an *in vitro* model of Huntington's disease (HD) and decreased symptoms and increased lifespan in an animal model of HD, which is associated with dysfunctions of Cu, Fe or Zn (Nguyen et al., 2005). At the same time, CQ-associated neurological side effects, which may be due to a CQ chelating effect on the vitamin B12 (VB12)-bound Co^{2+}, are now believed to be preventable with VB12 supplementation (Yassin et al., 2000). VB12 plays a key role in CNS function. It participates in the methionine-synthase-mediated conversion of homocysteine to methionine, which is essential for nucleotide synthesis and genomic and non-genomic methylation (Reynolds, 2006).

To determine whether CQ treatment could rescue the lead-induced impairments of synaptic plasticity related to learning and memory, in the present study, we investigated the LTP of different treatment groups in hippocampal DG area of the anesthetized rats *in vivo*. In addition, we also investigated the input/output functions (I/Os) and paired-pulse reactions (PPRs), two auxiliary electrophysiological parameters, and measured Pb^{2+} concentrations in hippocampus of different treatment groups. Our results indicate: CQ alone does not rescue the lead-induced impairments of synaptic plasticity in hippocampal DG area of the anesthetized rats *in vivo*; VB12 alone partly rescues the lead-induced impairments of LTP; however CQ and VB12 synergistically, totally, rescue these impairments in hippocampal DG area of the anesthetized rats *in vivo* and moreover, the effects of CQ and VB12 co-administration are specific to the lead-exposed animals.

EXPERIMENTAL PROCEDURES

Experimental animals and treatment

In the present protocol, the rats were divided into five groups which included control group (control) (n=12), lead-exposed group (lead) (n=12), lead-exposed with clioquinol group (lead+CQ) (n=10), lead-exposed with vitamin B12 group (lead+VB12) (n=9), lead-exposed with clioquinol and vitamin B12 group (lead+CQ+VB12) (n=10) and control with clioquinol and vitamin B12 group (control+CQ+VB12) (n=8). Before pups' delivery, all the mother rats freely had access to tap water. From pups' delivery to weaning, the offspring had access either to tap water (the controls and control+CQ+VB12 group) or to water with 0.2% lead acetate (the lead-exposed group and groups related to the lead-exposed) via their mothers' milk. After weaning, the offspring were weaned to the same solution as that given their mothers, so that chronic exposure to Pb^{2+} was throughout the lifetime that began in the early postnatal period. Before the experiment, the rats of drug-treatment group were intraperitoneally injected with the different agents for the distinct groups for 7 days. The doses of agents were 30 mg/kg/day for CQ and 45 μg/kg/day for VB12 (Cherny et al., 2001). The experiments were carried out on adult Wistar rats weighing 200–280 g. At the age of 60–80 postnatal days, the animals were utilized for extracellular recording in area DG area of hippocampus of anesthetized Wistar rats *in vivo*. No more than two animals per litter were utilized for experimental measurement in the same group. The agents used in this experiment were all bought from Sigma (St. Louis, MO, USA). The care and use of animals in these experiments followed the guidelines and protocol approved by the Care and Use of Animals Committee of the University of Science and Technology of China. All efforts were made to minimize the number of animals used and their suffering. All experiments conformed to the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 80-23, revised 1996).

Stimulation and recording

In each recording session, rats were anesthetized with urethane (1.8 g/kg, i.p. injection) and fixed in a stereotaxic head-holder. The skull was exposed and the animal's body temperature, heart rate and electrocardiogram 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, 4.2 mm lateral to the midline, and 2.8–3.0 mm below the surface of the skull). A micropipette glass recording electrode (3–5 μm tip diameter and 1–3 MΩ resistance) was placed in the DG (coordinates with the skull surface flat: 3.8 mm posterior to bregma, 2.1 mm lateral to the midline, and 3.0–3.5 mm below the surface of the skull). The glass micropipette filled with 2 M NaCl was used for extracellular recordings.

I/O

I/O curves were generated by systematic variation of 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 0.05 Hz and three responses at each current level were averaged.

PPR

PPR was evaluated by increasing the interpulse intervals (IPIs, 10–400 ms). The stimulus current intensity was adjusted at intensity yielding 40–60% of the maximal amplitude of population spike (PS). Stimulus pairs were delivered at 0.05 Hz and three responses were averaged at each IPI.

LTP

In present study, LTP was recorded in each animal. After 20 min baseline recordings, a high frequency stimulus (HFS) was applied (250 Hz, 1 s). Posttetanic recordings were performed for 1 h with single pulse applied at a frequency of 0.05 Hz. At the end of each recording session, small electrolytic lesions (10 μA, 10 s) were made to permit histological verification of the tip position of the electrodes. Hippocampus was isolated for measuring Pb^{2+} concentrations.

Hippocampal Pb^{2+} concentrations

Hippocampal Pb^{2+} concentrations were estimated on the animals used for electrophysiology. After decapitation of the animals, the