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The effect of sodium selenite on lead induced cognitive dysfunction

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ABSTRACT

The effect of lead (Pb) on spatial memory and hippocampal long-term potentiation (LTP) as a key risk factor has been widely recognized and the oxidative damage has been proposed as a possible mechanism of lead neurotoxicity. Selenium (Se) is a nutritionally essential trace element with known antioxidant potential. In this study we investigated the effect and the underlying mechanisms of Se supplementary on Pb induced cognition and synaptic plasticity impairment. Lactating Sprague-Dawley rats (SD rats) were randomly divided to four groups: 0 ppm lead acetate (Pb); 0 ppm Pb and 0.2 ppm sodium selenite (Se); 100 ppm Pb; 100 ppm Pb and 0.2 ppm Se. Lactating rats were treated with or without Pb and/or Se throughout lactation until weaning. The levels of hippocampal LTP, the spatial memory, the apoptosis of hippocampal neurons, the levels of lactate dehydrogenase (LDH) release, and the serum level of superoxide dismutase (SOD) and malondialdehyde (MDA) were assayed. It had been observed that in Pb group the spatial memory, the induce level of LTP, the serum SOD level decreased, the LDH release level, the neurons apoptosis level, the serum MDA level increased, while in the Se supplements groups, the spatial memory, the induce level of LTP increased significantly. Compared with the Pb group, Se supplements shown down regulated the level of LDH, the neurons apoptosis and the serum MDA, and up regulated the level of serum SOD. We could draw the conclusion that Se supplements could alleviate toxic effect of lead on hippocampal LTP and spatial memory. The treated with selenium around 0.2 ppm may protect against spatial memory dysfunction induced by lead exposure.

1. Introduction

Lead (Pb) is a ubiquitous environmental and industrial pollutant that mainly conveyed to humans through water, food, and occupational sources. Lead can also be transmitted through maternal milk (Isaac et al., 2012; Ettinger et al., 2004), and even low-level lead (0.3%) exposure can cause long-lasting cognitive deficits (Yang et al., 2003). Furthermore, removal of lead exposure at weaning still produced learning deficits in adult rats (Kuhlmann et al., 1997).

Extensive studies trying to explain the mechanism of lead induced cognitive deficits, such as region-specific effects of lead on constitutive nitric oxide synthase (cNOS) activity (Garcia-Arenas et al., 2004), the exchange of synaptic receptors and synaptic plasticity. LTP of the hippocampal excitatory synaptic transmission is thought to be a pattern of manifestation for synaptic plasticity (Bliss and Collingridge, 1993) which is believed to be the mechanism underlying certain types of learning and memory (Bliss and Collingridge, 1993). Studies have demonstrated chronic low level lead exposure during development impaired LTP in hippocampus in rats (Liu et al., 2012; Yang et al., 2006).

Although chelation therapy is currently an available treatment of Pb neurotoxicity, it shows to have many adverse effects such as divalent metal ion imbalance and to be powerless of improving previous nerve injury induced by Pb. Until now, no efficient drugs are available for treating chronic lead induced cognitive deficits (Mortensen and Walson, 1993; Porru and Alessio, 1996). Selenium (Se) is well known as its wide range of pleiotropic effects, ranging from anti-oxidant and anti-inflammatory effects to the production of active thyroid hormone. Low selenium status has been associated with increased risk of mortality, poor immune function. Studies believe that selenium supplementation has antiviral effects, is essential for male and female reproduction, and reduces the risk of autoimmune thyroid disease (Rayman, 2012). Prospective studies have generally

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shown some benefit of higher selenium status on the risk of prostate (Hurst et al., 2012; Richman and Chan, 2012), lung (Jablonska et al., 2008), colorectal (Hu et al., 2012; Takata et al., 2012), and bladder cancers (Lotan et al., 2012; Rayman, 2012). Studies also show it's beneficial on cognitive decline (Akbaraly et al., 2007; Ishrat et al., 2009).

In this study, we asked the question if co-exposed to Se could alleviate the impaired cognition and synaptic plasticity induced by lead and its possible mechanisms of action.

2. Materials and methods

2.1. Materials

Pregnant Sprague-Dawley rats (SD rats) were obtained from the Animal Experiment Center of the Fourth Military Medical University. Analytical pure lead acetate and sodium selenite were purchased from SIGMA (USA). Lactate dehydrogenase (LDH)-cytotoxicity assay kits were purchased from Biovision (Biovision, K726-500, USA). The terminal transferase-mediated dUTP nick end-labeling (TUNEL) assay kit was purchased from Roche (Roche, Germany). All other reagents were purchased from SIGMA (USA). The inverted microscope, the fluorescence microscope and the Laser confocal microscope were bought from OLYMPUS (Tokyo, Japan). The enzyme linked immunosorbent spectrophotometer was obtained from SHIMADZU (Nakagyo-ku, Kyoto, Japan).

2.2. Experimental animals and treatment

Se was used as the sodium selenite (Na_2SeO_3) (Sigma Aldrich, St. Louis, MO, USA). Female SD rats (250–300 g) were divided to four groups: 0 ppm lead acetate; 0 ppm lead acetate and 0.2 ppm sodium selenite; 100 ppm lead acetate; 100 ppm lead acetate and 0.2 ppm sodium selenite. The dosages of lead acetate were choosing according to literatures (Liu et al., 2012; Reza et al., 2008) and our pilot study (Fig. S1A). The dosages of sodium selenite were choosing according to literatures (Smith and Picciano, 1986). Dams were maintained on their respective drink throughout lactation until weaning. Pups were weaned at 21 days of age. At this time, Pb exposed rat pups were removed from the Pb exposure and drank distilled water until the end of the study. Therefore, the Pb exposure and Se supplement only occurred during lactation. All experimental offspring were reared in the same room. The animal care and experimental protocol were carried out in strict accordance with the international standards of animal care guidelines (guide for the care and use of laboratory animals) and were approved by the institutional animal care and use committee of Fourth Military Medical University (Permit Number: 12001).

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2.3. Morris water maze test

Morris water maze (MWM) was evaluated at 42 days of age for the assessing spatial learning ability. The Morris water maze consisted of a circular water tank (120 cm diameter, 50 cm height) that was partially filled with water (25 °C, dyed white with edible pigment). The pool was divided virtually into four equal quadrants labeled 1-2-3-4 in the middle position of each pool wall. A white escape platform (10 cm in diameter) was hidden 2 cm below the surface of the water in a fixed location in one of four quadrants (defined as the target quadrant) of the pool. The platform remained in the same place during the entire experiment. The maze was surround with a curtain and located in a quiet test room, surrounded by many fixed visual cues (*e.g.* the label on each quadrant's pool wall, rack, *etc.*), which were visible from within the pool and could be used by the rats for spatial orientation. The movement of the animals was recorded by a TV camera located over the center of the pool and was connected to a personal computer. Before the training started, rats were allowed to swim freely in the pool for 60 s without the platform and then put on the platform for 30 s in order to let rats be familiar with the experiment condition. Rats were given four trials (once from each starting position) per session for 7 days, with each trial having a ceiling time of 120 s and a trial interval of approximately 30 s. After climbing onto the platform, the animal remained there for 30 s before the commencement of the next trial. Recording was automatically terminated as escape latency when the animal found the target. The time required to reach the platform is known as the escape latency. If rats could not escape to the platform within 120 s by themselves, they were placed on the platform and allowed to remain there for the same amount of time and their escape latency was accepted as 120 s. The mean latency of finding the invisible platform was measured for individual animals on each day. The day after the acquisition phase, a probe test was conducted by removing the platform. Rats were allowed to swim freely in the pool for 60 s. The time spent in the target quadrant, which had previously contained the hidden platform, was recorded. The result was presented as the percent of time spent in the target quadrant.

2.4. Long-term potentiation (LTP) recording in hippocampal slices

2.4.1. Slice preparation

Coronal brain slices (300 μm) from a 6 week-old SD rat, containing the hippocampus, were prepared using standard methods (Wei et al., 2001). Slices were transferred to a submerged recovery chamber containing oxygenated (95% O_2 and 5% CO_2) artificial cerebrospinal fluid (ACSF) (124 mM NaCl, 4.4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 25 mM $NaHCO_3$, 1 mM NaH_2PO_4, and 10 mM glucose) at room temperature for at least 1 h.

2.4.2. Whole-cell recordings

Experiments were performed in a recording chamber on the stage of an Axioskop 2 FS microscope with infrared differential interference contrast (DIC) optics for visualizing whole-cell patch-clamp recordings. Excitatory postsynaptic currents (EPSCs) were recorded from pyramidal neurons in the CA1 region using an Axon 200B amplifier (Axon Instruments, CA, USA), and stimulations were delivered using a bipolar tungsten stimulating electrode which was placed on Schaffer collateral-commissural fibers in the CA3 stratum radiatum. Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated EPSCs were induced by repetitive stimulations at 0.02 Hz and neurons were voltage clamped at −70 mV. After obtaining stable EPSCs for at least 10 min, LTP was induced by 80 pulses at 2 Hz paired with postsynaptic depolarization at +30 mV (we called pairing training). The recording pipettes (3–5 MΩ) were filled with solution containing (mM) 145 K-gluconate, 5 NaCl, 1 $MgCl_2$, 0.2 EGTA, 10 HEPES, 2 Mg-ATP, and 0.1 Na_3-GTP (adjusted to pH 7.2 with KOH). Picrotoxin (100 μM) was always present to block gamma-aminobutyric acid (GABA) A receptor-mediated inhibitory synaptic currents. Access resistance was 15–30 MΩ and monitored throughout the experiment. Data were discarded if access resistance changed more than 15% during an experiment. Results are expressed as means ± SEM. Statistical comparisons were performed using the Student's *t* test.