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Levothyroxine rescues the lead-induced hypothyroidism and impairment of long-term potentiation in hippocampal CA1 region of the developmental rats

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ABSTRACT

Lead (Pb) exposure during development has been associated with impaired long-term potentiation (LTP). Hypothyroidism happening upon subjects with occupational exposure to Pb is suggestive of an adverse effect of Pb on thyroid homeostasis, leading to the hypothesis that Pb exposure may alter thyroid hormone homeostasis. Hippocampus is one of the targets of Pb exposure, and is sensitive to and dependent on thyroid hormones, leading us to explore whether levothyroxine (L-T_4) administration could alter the thyroid disequilibrium and impairment of LTP in rat hippocampus caused by Pb exposure. Our results show that Pb exposure caused a decrease in triiodothyronine (T_3) and tetraiodothyronine (T_4) levels accompanied by a dramatic decrease of TSH and application of L-T_4 restored these changes to about control levels. Hippocampal and blood Pb concentration were significantly reduced following L-T_4 treatment. L-T_4 treatment rescued the impairment of LTP induced by the Pb exposure. These results suggest that Pb exposure may lead to thyroid dysfunction and induce hypothyroidism and provide a direct electrophysiological proof that L-T_4 relieves chronic Pb exposure-induced impairment of synaptic plasticity.

Introduction

Lead (Pb), a common heavy metal, is one of the major environmental toxicants (Lewin et al., 2000). Environmental and occupational Pb exposure continues to be among the most significant public health problems with neurological, hematological, renal and gastrointestinal manifestations (Gurer-Orhan et al., 2004). Developmental exposure to Pb can negatively interfere with anthropometric measurements of newborn and fetal maturation (Lamb et al., 2008; Zentner et al., 2006). The World Health Organization (WHO) and the U.S. Centers for Disease Control and Prevention (CDC) recommended that child blood Pb levels (BLLs) not exceed 10 μg/dL (CDC, 1991; WHO, 1995). However the BLLs lower than 10 μg/dL are associated with alterations in the neurocognitive, motor, auditory, and visual systems of children (Furman and Laleli, 2001; Sanin et al., 2001). However, the potential mechanisms whereby prenatal and early-life exposure to Pb hinders development in children are still not fully understood.

It has been shown that thyroid hormones are essential for the maturation and development of the central nervous system of mammals (Sethi and Kapil, 2004). Furthermore, the maturation and development of hippocampus are dependent upon thyroid hormone (Madeira et al., 1992). Hippocampus comprises three specific subregions, each shows different ontogenetic profiles and displays distinct vulnerability to deficiencies in thyroid hormones (Iniguez et al., 1996). The dendritic spine density of CA1 pyramidal cells in the hippocampus of animals was modulated by thyroid hormone treatment (Gould et al., 1990). Meanwhile, both acute and chronic 0.2% Pb acetate exposure during development impairs the induction, magnitude, and LTP (long-term potentiation) duration in both area CA1 and dentate gyrus of rat hippocampus (Chen et al., 2007; Gilbert et al., 1999). Improvement in cognitive function has been reported in subclinical hypothyroid subjects or euthyroid rats on L-T_4 replacement therapy (Jensovsky et al., 2002; Smith et al., 2002). Alzoubi et al. have shown that thyroxine treatment reverses hypothyroidism-induced impairment of hippocampus-dependent cognition, late LTP (L-LTP), and normalized the electrophysiological and molecular effects of hypothyroidism on the CA1 region (Alzoubi et al., 2009).

Hypothyroidism happening upon subjects with occupational exposure to Pb is suggestive of an adverse effect of Pb on thyroid homeostasis, by interfering with the uptake and concentration of iodine in the thyroid gland (Robins et al., 1983). Pb exposure also greatly reduced the uptake of T_4 from the blood into the choroid plexus in vivo (Zheng et al., 2003). Some investigators reported that Pb impaired thyroid function with or without TSH elevation (Robins et al., 1983; Singh et al., 2000). On the other hand, the lack of significant alterations in the levels of these hormones has also been reported in a few studies about Pb exposure (Siegel et al., 1989;

Abbreviations: L-T_4, levothyroxine(L-thyroxine); EPSPs, excitatory postsynaptic potentials; LTP, long-term potentiation; T_3, triiodothyronine; T_4, tetraiodothyronine; TSH, thyroid stimulating hormone; PTU, propylthiouracil; ACSF, artificial cerebrospinal fluid; HFS, high-frequency stimulation; ip, intraperitoneally; BLLs, blood lead levels.

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Schumacher et al., 1998). There is no investigation of Pb exposure on thyroid functions of rats in the literature. We designed this experiment to establish the relationship between thyroid dysfunction and Pb exposure, then to test whether L-T_4 administration could alter the thyroid dysfunction and impairment of LTP in the hippocampus of Pb-exposed rats.

Material and methods

Experimental animals. Wistar rats were obtained from the Laboratory Animal Center, University of Science and Technology of China, P. R. China and were maintained with the highest standards of animal care and housing, according to the National Institute of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number and suffering of the animals used.

The present protocol for chronic exposure to lead has been described previously (Wang et al., 2008a, 2008b). Female Wistar rats were fed distilled water and standard laboratory chow to ensure consistent levels of mineral intake prior to mating. During gestation the rats were maintained at a controlled ambient temperature and relative humidity of 24 ± 1 °C and 50 ± 10%, respectively. On parturition day, the dams were randomly divided into three experimental groups: (1) control group which was allocated to four subgroups: one control subgroup and three control + L-T_4 subgroups; (2) propylthiouracil (PTU)-exposed group which was also allocated to four subgroups: one PTU subgroup and three PTU + L-T_4 subgroups; and (3) Pb-exposed group which was divided into four subgroups: one Pb-exposed subgroup and three Pb-exposed + L-T_4 subgroups. The pups were exposed to Pb only via the milk of dams whose drinking water contained 0.2% (1090 ppm) lead acetate from parturition to weaning and the PTU-exposed dams were administered PTU via the milk of dams whose drinking water contained 15 ppm PTU beginning on GD18 and continuing throughout lactation until postnatal day 21 (P21) (Gilbert, 2004), while the control dams remained on distilled water throughout the lactation period. Litters were culled to eight pups with both sexes in each group. The day of birth was considered P1. L-T_4 (Sigma-Aldrich, USA) was dissolved using 0.04 M NaOH and the final solution was prepared with saline solution for all treatments. From P14–21, the offspring of three control + L-T_4 subgroups, three Pb-exposed + L-T_4 subgroups and three PTU-exposed + L-T_4 subgroups were injected intraperitoneally (ip) daily with different amounts of L-T_4 (i.e., 20 μg/kg, 50 μg/kg and 100 μg/kg, respectively). The animals in the control subgroup, Pb-exposed subgroup and PTU-exposed subgroups were injected with 0.9% saline chloride. The injection volume of all groups amounted to 4 mL/kg body weight. At P21, offspring were weaned, housed in a colony room with a 12:12 light: dark schedule and permitted free access to food and distilled water. Electrophysiology recordings were carried out between P23 and P30. A total of 80 control, 64 PTU-exposed rats and 64 Pb-exposed rats were used in the experiment. In all the groups, equal numbers of females and males were used for the experiments. No more than two animals per litter were used for a given experimental measure.

Blood and hippocampus lead determinations. In order to characterize the exposure protocols, blood and hippocampus Pb determinations were measured from littermates of the animals used for electrophysiology. Blood samples (2 mL per animal) were collected in heparinized syringes and analyzed by a PerkinElmer 600 atomic absorption spectrometer (AAS). After decapitation, the two hippocampi were collected. Pb concentrations were measured by a plasmaQuad3 plasma mass spectrograph (VG Elemental, UK) after the tissues were digested with an organic tissue solubilizer. Added concentrated HNO_3 2 mL, stamped to heat the sample solution with low-temperature to nearly dry. Additional added HNO_3 1 mL and 5 drops of concentrated $HClO_4$, stamped to heat. Plus the HNO_3 4 drops and a small amount of water after cooling, volume to 5.00 mL. The system can automatically complete all steps.

Assessment of hormone levels. After the last administration of L-T_4, the relative animals were given rest. Thyroid hormone levels were determined in blood sampled from animals at the same time of day at PN22. Blood samples (3 mL per animal) were collected in syringes via cardiac puncture of anesthetized rats, and immediately centrifuged at 12,000 rpm for 10 min. The serum was quickly frozen at −80 °C for later analyses. Plasma thyroid hormone levels were measured with a chemiluminescence immunoassay (Immuno chemiluminescence assay, ICMA). Apparatus and reagents used in the assay came from the DPC (Diagnostic Products Co., Immulite 2000, Los Angeles, CA, USA). All samples were run in duplicate and the intra- and interassay variations were less than 10%. The detection range of the total T_3, T_4 and TSH was 0.15–12.3 nmol/L, 3.9–387 nmol/L and 0.004–150 μIU/mL respectively.

T_3 or T_4 was measured by direct chemiluminescent competitive immunoassay technology. T_3 or T_4 in the blood and T_3 or T_4 analogues covalently combination with paramagnetic ion in the solid-phase reagents compete with a number of mouse monoclonal anti-T_3 or T_4 antibody present in the labeled reagent with acridine (acridinium) ester flag. TSH was measured by direct chemiluminescent technology, double-immunoradiometric assay, using two constant number antibodies. The first antibody is the mouse monoclonal TSH antibody present in the labeling reagent with acridine (acridinium) ester of labeled. Second antibody present in the solid phase reagent, is polyclonal sheep TSH antibodies covalently combination with paramagnetic ion. The system can automatically complete all steps.

Slice preparations and recordings. The rats were decapitated without anesthesia between postnatal days 23 and 30. The brains were quickly removed and immersed in cold (nearly 0 °C) oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 124, KCl 5, NaH_2PO_4 1.25, $NaHCO_3$ 26, $MgCl_2$ 1.25, $CaCl_2$ 2.5 and dextrose 10, pH = 7.30–7.45. The hippocampus in one hemisphere was dissected free and transverse slices (400–500 μm) were cut using a sliding microtome. The slices were incubated for at least 1 h in ACSF at room temperature. Then one hippocampal slice was transferred to the recording chamber (BSC-HT Med. Sys., USA) in which it was continuously perfused at a rate of 1 mL/min with 30–32 °C ACSF saturated with 95% O_2/5% CO_2. Electrophysiological recording was conducted with only one slice per animal. After 1 h equilibration in the slice chamber, field excitatory postsynaptic potentials (fEPSPs) were recorded. One bipolar stimulating electrode was located in the Schaffer/commissural fibers. The other recording electrode, a glass micropipette (resistance: 1–3 MΩ, tip diameter: 3–5 μm) filled with ACSF, was positioned in the dendrites of CA1 pyramidal cell. fEPSPs were evoked by using 0.2–0.3 mA stimuli of 0.2 ms duration at 0.05 Hz. After recording the baseline responses for 15 min, LTP was induced by applying a high-frequency stimulation (HFS) of 100 Hz for 1 s. Testing with single shocks was repeated for at least 45 min after HFS. LTP was presented as the increase in EPSP slopes in relation to the baseline response (100%) after tetanic stimulus application, and its amplitude was the mean of relative EPSP slopes in 25–40 min.

Data analysis. Data were recorded using Igor Pro 4.05 software (Wave Metrics Inc, OR, USA) and analyzed with Origin 8.0 (OriginLab Corporation, MA, USA). The slope of fEPSP of each experiment was normalized based on the average slope over the 15 min period before HFS. Group was defined as between-subject factor while time was defined as within-subject factor. All values are given as the mean ± S.E.M, and n represents the number of the animals that were sampled. Comparisons in the analysis of LTP were analyzed by two-way variance (ANOVA) with Tukey test. The other statistical analyses were