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Toxicology

The effects of early life lead exposure on the expression of P2X7 receptor and synaptophysin in the hippocampus of mouse pups

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ABSTRACT

The present study was undertaken to investigate the effects of maternal lead exposure on expression of P2X7 receptor and synaptophysin in the hippocampus of mice offspring. Lead exposure initiated from beginning of gestation to weaning. Lead acetate administered in drinking solutions was dissolved in distilled deionized water at the concentrations of 0.1%, 0.5% and 1% groups, respectively. On the 21st postnatal day, the Pb levels were also determined by graphite furnace atomic absorption spectrometry. The expression of P2X7 receptor and synaptophysin in hippocampus was examined by immunohistochemistry and Western blotting. The lead levels in blood and hippocampus of all lead exposure groups were significantly higher than that of the control group ($P<0.05$). Compared with the control group, the expression of P2X7 receptor was increased in lead exposed groups ($P<0.05$), but the expression of synaptophysin was decreased ($P<0.05$). The high expression of P2X7 receptor and low expression of synaptophysin in the hippocampus of pups may contribute to the neurotoxicity associated with maternal Pb exposure.

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Introduction

Lead (Pb) has been widely used in many products; for example, leaded gasoline, lead-based paint, and cans containing foods or alcoholic beverages. Exposure to high levels of lead causes various public health problems, particularly among young children, because of its effects on the blood and brain, including disruption of nervous system communication [9]. The heavy metals lead (Pb) pose potential risks to sustainability of environment and thus to our future generations. Research on lead neurotoxicity has been of interest for many years [5,16], but its effects on the P2X7 receptor and synaptophysin in brain are rarely reported.

P2X receptors are a family of purinoceptors which are ligand-gated membrane ion channels activated by extracellular adenosine 5′-triphosphate. P2X receptors consist of seven isoforms designated P2X1–P2X7 [28]. They are widely distributed in most types of cells in nearly every origin and involved in many actions, such as synaptic transmission in the peripheral and central nervous systems, contraction of smooth muscle, platelet aggregation, macrophage activation, cell death and immunomodulation [33]. A unique feature of the P2X7 receptor is that its activation can result in the formation of large plasma membrane pores that allow not only the flux of ions, but also of hydrophilic molecules of up to 900 Da. Recent studies indicate that P2X7-mediated signaling can trigger apoptotic cell death after ischemia and during the course of certain neurodegenerative disorders. Within the central nervous system (CNS), functional P2X7 receptors are found on microglia and Schwann cells, as well as on astrocytes [27]. Although the existence of P2X7 receptors on peripheral or central neurons has been controversial owing to the poor selectivity of antibodies targeting the rat P2X7 receptor, recent developments have yielded small-molecule receptor antagonists with greatly improved activity at rat P2X7 receptors [19]. In rat peripheral sensory ganglia, P2X7 receptors appear to be selectively localized to glial cells [34]. The best characterized activity of the P2X7 receptor is its role in interleukin-1β (IL-1β) release from macrophages and microglia primed with substances such as bacterial endotoxin (lipopolysaccharide, LPS).

Synaptophysin is a major integral membrane protein of synaptic vesicles, involved in neurotransmitter release and synaptic vesicle cycle. The protein is widely distributed at synapses throughout the nervous system and is used for specific marker of synaptic vesicles/synaptic terminals [31]. Recent research indicates that

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synaptophysin is closely related to synaptic remodeling and cognitive process and plays an important role in synaptic plasticity [26]. Several studies indicate that synaptophysin regulates endo- and exo-cytosis of neurotransmitters and participates in recycling of synaptic vesicles. It is also suggested that synaptophysin plays a role in formation and stabilization of synapses and provided a molecular marker for the synapse [18]. Altered synaptophysin levels have been described in the brain tissue of several neurodegenerative diseases. Synaptophysin expression is significantly reduced in frontal cortex and hippocampus in patients with AD and is negatively correlated with the degree of dementia and with the pathological severity of AD [17]. Synaptophysin and other synaptic vesicle proteins together with proteins belonging to postsynaptic densities such as PSD-95 have been implicated in mechanisms of activity-dependent neuroplasticity underlying learning and memory formation [31]. It has been suggested that synaptophysin plays a role in formation and stabilization of synapses [29] and thus it is considered a reliable marker that can be used to determine distribution and density of synapses [14]. All these processes are part of cellular mechanisms underlying various forms of synaptic plasticity including learning and memory formation [20].

The developing brain is experiencing intensive cellular proliferation, differentiation, and synaptogenesis. It is very sensitive to environmental hazard at this stage. It is known that early life Pb exposure could cause severe consequences for brain development. Both P2X7 receptor and synaptophysin have an important role in the survival of the nervous system, and hippocampus is a key point in nervous. However, the underlying mechanism of Pb exposure on brain development is still unclear. In order to discover the potential mechanism, this study will test the expression of P2X7 receptor and synaptophysin in hippocampus of Pb exposed mouse pups to know whether these proteins are the media between Pb exposure and brain damage.

Materials and methods

Animals and treatment

All procedures involving animals were carried out in strict accordance with the International Standards of Animal Care Guidelines and were approved by the Local Care of Experimental Animals Committee. Forty pregnant Kunming female mice (SCXK 2005-0001) were ordered from Henan Laboratory Animal Center. They were randomly divided into four groups and caged individually. The control group mice were served with distilled water without lead. Lead acetate was dissolved into distilled at three different concentration levels, 0.1%, 0.5% and 1% mass fraction for low, moderate and high concentration, respectively. About dose selection, we are according to the LD50, preliminary experiment and related references [7,23] to develop a low dose, medium dose and high dose. Up to 1% Pb in drinking water is really high, is also the highest lead exposure dose. We know lead neurotoxicity, reproductive toxicity and developmental toxicity, with the increase of lead exposure dose, eating and drinking seems no abnormalities, no obvious lead poisoning symptoms and signs, but the fertility rate and farrowing rate in mice declined. Lead exposure started from beginning of gestation and lasted until weaning (the 21st postnatal day) by drinking lead containing water [32]. The animals were maintained on a 12–12 h light/dark cycle with food and water available *ad libitum.* All experiments were carried out in accordance with the regulations of the Zhengzhou University Committee on Ethics in the Care and Use of Laboratory Animals. At birth, all litters (both male and female) were culled to eight pups.

Determination of lead concentrations in blood and hippocampus samples

Sample collection

The blood samples were collected from the tail of the pups on the 21st postnatal day after wiping the skin to remove the contaminated lead. Then, the pups were anesthetized under ether inhalation and the brain was dissected. The hippocampus were isolated.

Sample preparing for assay

Total of 100 μL of blood was mixed well with 3.9 mL of 0.5 N nitric acid containing 0.01% Triton X-100. The clear solution after digestion was collected for further analysis of lead content. The isolated hippocampus tissue from each sample was digested in a mixture of 0.5 N nitric acid, 0.5 N perchloric acid and 0.01% Triton X-100 to make 1:10 (w/v) of diluted homogenate before being heated in a DGT100 microwave oven. On completing the digestion, samples were made up to volumes of 25 mL with double-deionized water.

The measurement of Pb concentration in samples was performed as described [10]. A 20 μL aliquot of each sample was injected into the graphite furnace atomic absorption spectrometry (HITACHI) by an autosampler and 20 μL of 0.2% magnesium nitrate was added as modifier. Pb concentration was measured at 283.3 nm by a programmed heating procedure for blood and brain Pb. Determination condition: wavelength 283.3 nm, lamp current 9.0 mA, slit 1.3 nm, argon (Ar, 200 mL/min). Atomization conditions: 85 °C – 10 s, 95 °C – 10 s, 120 °C – 20 s, 400 °C – 20 s, and 2100 °C – 3 s, 2300 °C – 3 s. Pure argon (Henan Yuanzheng Science and Technology Development Limited Company, Zhengzhou) was used as the purge and protecting gas. The analysis was carried out in duplicate and the mean peak height value was taken for calculation. For the standard curve, 1×10^6 μg/L Pb chloride in 0.3 M nitric acid was diluted to 5×10^3, 1×10^4 and 2×10^4 μg/L with 0.2% nitric acid containing 0.01% Triton X-100. To examine the recovery rate, standard Pb solution was added (final concentration: 1×10^4 μg/L) to blood and brain samples as internal standard. The recovery rates for blood and brain homogenate were 97.6% and 98.6%, respectively.

Sample preparing and immunohistochemistry

Three pups from each group were selected for histological study [11]. The selected pups were anaesthetized by overdose with sodium nembutal (35 mg/kg, i.p.). The whole brain was dissected. The fixed brain samples were cut at 5 μm thickness starting at 3 mm posterior to the anterior pole. The tissue slides were treated by microwave in 10 mM citrate buffer (pH 6) for 3 min and followed by blockade of non-specific binding by incubation in 0.1 M PBS containing 3% normal goat serum. Sections were then subsequently incubated with primary antibodies overnight at room temperature. The primary antibodies were rabbit anti-P2X7 (1:500, Santa Cruz) and anti-synaptophysin (1:500, Santa Cruz). After extensive rinsing steps in 0.1 M PBS, the sections were incubated in biotinylated goat anti-rabbit antibody (1:1000, Santa Cruz) for 1 h at room temperature and followed by using the vector ABC system. Subsequent incubation in diaminobenzidine was performed for visualization of the reaction product. For negative controls, the primary antibody was omitted.

Protein isolation and Western blotting

The dissected hippocampus samples were homogenized in lysis buffer containing 2% SDS, 10% glycerol, 2% 2-mercaptoethanol, 0.002% bromphenol blue in 75 mM Tris–HCl [10]. The samples were heated at 95 °C for 10 min before separating on 10% Tris/glycine/SDS acrylamide gels. The proteins were subsequently

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