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Lead-induced effects on learning/memory and fear/anxiety are correlated with disturbances in specific cholinesterase isoform activity and redox imbalance in adult brain

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HIGHLIGHTS

- Pb caused significant deficits on mice learning/memory ability and increased anxiety.
- Pb inhibited the AChE G4 and G1 activities in all brain regions tested.
- Pb increased lipid peroxidation and decreased GSH levels in all brain regions.
- Coefficients between behavior, AChE activity and redox balance were brain region-specific.

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ABSTRACT

The aim of the present study was to investigate whether the underlying mechanism of lead (Pb)-induced effects on learning/memory and fear/anxiety behavior involves changes either on AChE G4 (most abundant in brain) or on G1 isoform activity, and/or to a putative local disruption of oxidant/antioxidant balance. Adult male mice were randomly divided into two groups (18 animals/group): a vehicle group [500 ppm (mg/L) CH_3COONa/day for 4 weeks in their drinking water] and a Pb-treated group [500 ppm $Pb(CH_3COO)_2$/day for 4 weeks in their drinking water]. At the end of the treatment period, mice were subjected to the behavioral tasks. Learning/memory was tested by step-through passive avoidance test, whereas fear/anxiety was studied using the elevated plus-maze and thigmotaxis tests. Pb levels in mice brain were determined using atomic absorption spectrometry. AChE activity was determined colorimetrically, and GSH and MDA levels fluorometrically in whole brain minus cerebellum, cerebral cortex, midbrain, hippocampus, striatum and cerebellum. The possible correlations between learning/memory or fear/anxiety behavior with the AChE activity and/or the lipid peroxidation levels and GSH content were also examined. Pb consumption caused significant deficits on mice learning/memory ability and increased anxiety. The consumption of the Pb solution inhibited the activity of the two AChE isoforms in all brain regions tested. Moreover, Pb exposure increased lipid peroxidation and decreased GSH levels in all brain regions examined. Spearman correlation analysis revealed that the coefficients between the particular behaviors, AChE activity and redox balance were brain region- and AChE isoform-specific.

Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; BuChE, butyrylcholinesterase; GSH, glutathione; SOD, superoxide dismutase; EPM, elevated plus-maze; TT, thigmotaxis test; IL, initial latency; STL, step-through latency; AAS, atomic absorption spectrometry; ICP-MS, inductively coupled plasma-mass spectrometry; SS, salt soluble; DS, detergent soluble; MDA, malonyldialdehyde; VDCC, voltage-dependent calcium channel; NMDA, N-methyl-D-aspartate; ROS, reactive oxygen species.

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1. Introduction

Lead (Pb) is a neurotoxic heavy metal associated with cognitive decline in human and animals. Pb crosses the blood–brain barrier and accumulates in the brain and its concentration does not decrease even if the blood level of Pb declines [1,2]. Lead is thought to exert its neurotoxic effects through causing variable changes in several neurotransmitter systems [3].

Acetylcholinesterase (AChE) rapidly hydrolyzes the neurotransmitter acetylcholine (Ach) at brain cholinergic synapses as well as at neuromuscular junctions [4]. Its functions are, at least in part, specific to the three C-terminal variants of AChE, which are produced by alternative splicing of the single *ACHE* gene [5]; the "synaptic" or S variant, which is also called "tailed" [6], the "erythrocytic" or E variant [7] and the "readthrough" or R variant [8]. Membrane-bound AChE-S tetramers are traditionally designated as G4, while AChE-R monomers and AChE-S homodimers are designated as G1 and G2, respectively [9].

The implication of the cholinergic system in learning and memory processes is well established [10–13] as well as in the pathophysiology of anxiety disorders [14]. Particularly, recent studies demonstrate AChE-R (G1) involvement in both cognitive status of rodents, in terms of fear learning under stress conditions [15] and in anxiety behavior [9]; Meshorer et al. [16] demonstrated that neuronal hypersensitivity under stress involves neuritic replacement of AChE-S by AChE-R isoform. However, there are no evidences correlating lead-induced cognitive deficits and its anxiogenic effects [17] with changes in AChE isoform activity; Nehru and Sidhu [18] reported that exposure of 1-month old rats to different dose levels of lead diminishes total AChE activity in brain and may be involved to the also observed significant reduction of both short-term memory and locomotor activity. A proposed underlying molecular mechanism involved in lead-induced neurotoxicity is the disruption of the prooxidant/antioxidant balance [19], which can lead to brain injury via oxidative damage to critical biomolecules, such as lipids, proteins and DNA. In particular, it has been proposed that unbalanced accumulation of oxidatively modified proteins in brain potentiates neurodegeneration and impairs cognitive functions [20]. Pb-exposed animals exhibit age-dependent and brain specific alterations in antioxidant enzyme activities and changes in the concentrations of some antioxidant molecules [21,22]. In addition, *in vitro* and *in vivo* experiments in rat brains indicate that intense lipid peroxidation may contribute to lead-induced neurotoxicity [2,23].

In this context, the aim of the present study was to investigate whether the underlying mechanism of the Pb-induced effects on adult (3 month-old) male mice learning/memory and fear/anxiety behavior involves changes either on AChE G4 (the most abundant in brain) or on G1 isoform activity, given the fact that the ratio of these activities has been proposed to be an index of neurodegenerative processes [24], and/or to a putative local disruption of oxidant/antioxidant balance. Towards this, learning/memory was tested by step-through passive avoidance test, whereas fear/anxiety was studied using the elevated plus-maze (EPM) and thigmotaxis test (TT). Pb levels in brain were determined using atomic absorption spectrometry. The activity of AChE isoforms was determined colorimetrically in whole brain (minus cerebellum), cerebral cortex, midbrain, hippocampus, striatum and cerebellum. In addition, we estimated the levels of lipid peroxidation and reduced glutathione, as indices of the oxidative status in the same brain regions, after the consumption of Pb solution. Finally, we extended our research by examining the possible correlation of the observed behavioral changes to the biochemical parameters.

2. Materials and methods

2.1. Animals

Male, Balb-c mice (30–40 g BW), 3–4 month-old, were kept in polyacrylic cages and housed in a room under controlled temperature of 24–26 °C, relative humidity of 50–60% and within 12 h light–dark cycle. Food and water were available *ad libitum*. The treatment protocol followed is according to Bennet et al. [25]. Particularly, the adult mice were randomly divided into two groups (18 animals/group): a vehicle group, which consumed sodium acetate solution [500 ppm (mg/L) CH_3COONa/day for 4 weeks] in their drinking water and a Pb-treated group, which consumed lead solution (500 ppm $Pb(CH_3COO)_2$/day for 4 weeks) in their drinking water. A few drops (0.5 mL) of acetic acid were added in the lead acetate solution in order to avoid precipitation. The same volume of acetic acid was added in the sodium acetate solution. The consumption of food and solutions was recorded daily and mice body weight (BW) were measured every 7 days. After behavioral testing, mice were sacrificed with cervical dislocation. Cerebral cortex, hippocampus, striatum, midbrain, and cerebellum were excised, cleaned with isotonic saline, weighted, and kept at −20 °C until further use. For comparison reasons, another double set of Pb-treated and control animals was used to excise whole brain minus cerebellum. All procedures were in accordance with the Greek National Laws (Animal Acb PD 160/91).

2.2. Behavioral testing

2.2.1. Learning/memory — Step-through passive avoidance task

At the end of the treatment period, step-through passive avoidance test was performed on two consecutive days in order to examine memory consolidation. According to previously described procedures [26], a two-compartment (white/dark, separated by a guillotine door) passive avoidance apparatus was used. The dark compartment was equipped with a grid floor. The test was slightly modified.

Roughly, on day 26, the first day of the test, each mouse was placed in the illuminated compartment and left for 100 s to habituate the apparatus (habituation trial). One hour after the habituation trial, in the acquisition trial, each mouse was placed in the illuminated chamber and the guillotine door was opened. Once the animal crossed all four paws in the dark chamber a foot shock (25 V, 3 mA, 5 s) was applied. The initial latency (IL) required to enter the dark compartment was measured (max time allowed 120 s). The animals that did not enter the dark chamber were eliminated from the experiment. Twenty four hours later, on day 27, the retention trial was performed. Each mouse was placed in the illuminated compartment of the apparatus, the door was opened and the step-through latency time (STL) until the mouse enters the dark chamber was recorded (maximum time allowed 300 s). During these sessions, no electric shock was applied. All training and testing sessions were carried out during the light phase (08:00–14:00).

2.2.2. Anxiety-like behavior

In order to assess the effects of lead intake on anxiety-like behavior, we used two behavioral tests, based on the animals' fear/anxiety for the unknown environment (open field and height), despite its tendency to explore it.

2.2.2.1. Thigmotaxis test (open field test). Thigmotaxis refers to the preference of mice to walk near the walls of an open field devise. The experimental device was first described by Simon et al. [27].

At day 26, mice were kept for 1 h in a slightly illuminated room to habituate. Then, each mouse was gently placed into the device and recorder for 10 min. Thigmotaxis time, which is the time spent close to the walls of the apparatus (<5 cm) and the entries of the mouse in the open field of the device were recorded and used as an index of anxiety. All training and testing sessions were carried out during the light phase (08:00–14:00).

2.2.2.2. Elevated plus maze test. At day 27, we assessed the effects of lead intake on anxiety-like behavior using the elevated plus-maze test [28]. The duration of the test was 10 min. The devise used for this test is cross-shaped, with two open and two closed (with walls) arms. On