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PROTECTION BY A TAURINE SUPPLEMENTED DIET FROM LEAD-INDUCED DEFICITS OF LONG-TERM POTENTIATION/DEPOTENTIATION IN DENTATE GYRUS OF RATS *IN VIVO*

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Abstract—Previous studies have demonstrated that synaptic plasticity, which includes long-term potentiation (LTP) and depotentiation (DP) in hippocampus, is important for learning and memory. The purpose of this study is to evaluate the effect of taurine via drinking water on the lead-induced impairments of LTP and DP in rat dentate gyrus (DG) *in vivo*. The experiments were carried out in four groups of rats (control, lead-exposed, control and lead-exposed with a taurine-supplement diet, respectively). The input–output (I/O) function, excitatory postsynaptic potential (EPSP) and population spike (PS) amplitude were measured in the DG area of adult rats (60–90 days) in response to stimulation applied to the lateral perforant path. The results show that: 1. chronic lead exposure impaired LTP/DP measured on both EPSP slope and PS amplitude in DG area of the hippocampus; 2. in control rats, taurine had no effect on LTP/DP; 3. the amplitudes of LTP/DP of lead-exposed group were significantly increased by applying taurine. These results suggest that dietary taurine supplement could protect rats from the lead-induced impairments of synaptic plasticity and might be a preventive medicine to cure the cognitive deficits induced by lead. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: lead, taurine, LTP, DP, hippocampus.

Lead is one of the most important neurotoxic metals in the environment. Chronic lead exposure during early developmental period is known to be associated with cognitive and neurobehavioral dysfunction in children and animals (Cory-Slechta, 1990; Bellinger et al., 1991). Functional impairment of developing brain has been reported at blood lead level as low as 100–150 μg/l in children and <200 mg/dl in rodents (Bellinger et al., 1991). It has been shown that lead alters the release of neurotransmitters such as dopamine, norepinephrine and acetylcholine by interfering with calcium metabolism or synaptic function (Rius et al., 1988; Goldstein, 1992), impairs cerebral growth, alters the number of synapses per neuron, and causes hypomyelination of the nervous system. Lead also has deleterious effects on many enzymes, such as protein kinase C (PKC), calmodulin, tyrosine hydroxylase, and choline acetyltransferase, as well as on brain energy metabolism (Levin et al., 1987; Markovac and Goldstein, 1988; Ronnback and Hansson, 1992).

It has been shown that lead concentrates in hippocampus (Walsh and Tilson, 1984) in which long-term potentiation (LTP) and long-term depression (LTD) underlie the neurobiological basis of learning and memory. Morphometric analyses following developmental lead exposure indicate a decrease in hippocampal pyramidal neurons spine density (Kiraly and Jones, 1982), a reduction in the dendritic fields of dentate granule cells (Alfano and Petit, 1982), and a decreased number of mossy fiber terminals (Campbell et al., 1982). Our previous studies indicated that lead seriously damaged LTP and LTD in CA1 and dentate gyrus (DG) area of hippocampus *in vivo* and *in vitro* (Ruan et al., 1998; Sui et al., 2000). In hippocampus, lead blocks calcium channels and also alters the calcium-mediated second messenger process. In addition, lead directly inhibits both *N*-methyl-D-aspartate receptor (NMDAR)-mediated calcium signaling (Alkondon et al., 1990) and the expression of *N*-methyl-D-aspartate (NMDA) receptor gene (NR1 and NR2) (Lau et al., 2002), which is necessary for the induction of LTP and LTD. Application of low-frequency stimulation after LTP was induced results in a reversal of induced LTP, known as depotentiation (DP). It is uncertain that DP utilizes the same mechanism as LTD. However, DP has a number of similarities with LTD. Both are induced by low-frequency stimulations (1–5 Hz), require NMDAR activation, and are Ca^{2+} dependent processes.

Taurine, 2-amino-ethanesulfonic acid, is one of the most abundant amino acids in mammals (Jacobsen and Smith, 1968). The physiological role of taurine has received considerable attention since the reports that cats fed with a taurine deficient diet developed central retinal degeneration (Hayes et al., 1975). Now taurine has been shown to be involved in many important physiological functions, such as a trophic factor in the development of the central nervous system (CNS), maintaining the structural integrity of the membrane, regulating calcium binding and transporting, and as a neuroprotector against L-Glu-induced neurotoxicity. Recently, del Olmo et al. (2000b) reported that taurine could induce a long-lasting potentiation in the CA1 area of rat hippocampus slice, because of the enhancement of both synaptic efficacy and axon excitability, which challenged the proposal that taurine is an inhibitory neurotransmitter. These actions of

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Abbreviations: CNS, central nervous system; DG, dentate gyrus DP, depotentiation; EPSP, excitatory postsynaptic potential; HFS, high frequency stimulus; I/O, input/output; IPI, interpulse interval; LFS, low frequency stimulus; LLP_{tau}, taurine-induced long-lasting potentiation; LTD, long-term depression; LTP, long-term potentiation; LVACC, low-voltage activated calcium channel; NMDA, *N*-methyl-D-aspartate; PCB, phencyclidine-binding; PKC, protein kinase C; PPF, paired-pulse facilitation; PPR, paired-pulse ratio; PS, population spike.

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taurine suggest that it is a neuron protector in many neuron-damage conditions and play an important role in learning and memory, which is proved by many ethological studies. The purpose of this experiment was to investigate whether taurine was an effective drug to reduce lead-induced deficits of LTP/DP.

EXPERIMENTAL PROCEDURES

Experimental animals and treatment

The experiments were performed on four groups of adult Wistar rats (postnatal days 60–90, male and female): Control, control with a taurine diet, lead-exposed and lead-exposed with a taurine diet. In the present protocol, the offspring were exposed to lead and/or taurine only via their mother's milk. After pups' delivery, the dam had access either to 20 ml tap water (control group) or to water with 0.3% lead acetate added and/or 0.625% taurine (Huxtable, 1982) depending on the group. After weaning until the experiment, the rats had access to the same solution as their mother. Extracellular recording measured in DG area of hippocampus were carried out in 60–90 days old animal. No more than two animals per litter were utilized for a given experimental measure. The chemicals used in this experiment were all bought from Sigma (St. Louis, MO, USA). All experiments conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23) revised 1996. The number of animals and their suffering were minimized to the least.

Hippocampus lead determination

Lead concentration in the hippocampus was estimated on the animals used for electrophysiology. After decapitation of the animals, the hippocampi were isolated, and digested with an organic tissue solubilizer. The lead concentration in the hippocampus was measured by PlasmaQuad 3 inductive coupled plasma mass spectroscopy (VG Elemental Ltd, UK).

Stimulation and recording

In each recording session, rats were anesthetized with urethane 1.8 g/kg and the head was fixed in a stereotaxic headholder. 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, 4.5 mm lateral to the midline, 2.8–3.0 mm ventral). A 2 M NaCl-filled glass pipette recording electrode (3–5 μm tip diameter, 1-3 MΩ resistance) was lowered into the DG (coordinates: 3.8 mm posterior to bregma, 2.5 mm lateral to the midline) until maximal response to prefrontal path stimulus was observed (3.0–3.5 mm ventral).

Input/output function (I/O)

I/O curves were generated by increasing 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 0.125 Hz and three responses at each current intensity were averaged.

Paired-pulse ratio (PPR)

PPR was evaluated by increasing the interpulse intervals (IPIs) by steps (IPIs, 10–300 ms). The stimulus current intensity was adjusted at intensity yielding 50% of the maximal amplitude of population spike (PS). Stimulus pairs were delivered at 0.125 Hz and three responses were averaged at each IPI.

LTP and DP

In this study, both LTP and DP were recorded in each animal. First, LTP was evoked. The stimulus intensity selected for baseline measurements was adjusted to yield about 40% of excitatory postsynaptic potential (EPSP) maximal amplitude. After 10 min recording with stimulus applied at 8 s intervals, a high frequency stimulus was applied (HFS: 250 Hz, 1 s). Posttetanic recordings were performed for 1 h with single pulse applied at a frequency of 0.125 Hz. Between LTP and DP recordings, the animal had 1 h for break. After 10 min baseline recording, DP was induced by applying the low frequency stimulation (LFS: 1 Hz, 15 min) after 10 min of baseline recording. 0.125 Hz pulses were then applied for 45 min. At the end of each recording session, small electrolytic lesions (10 mA, 10 s) were made for histological verification of the tip position of the electrodes. Then hippocampus was isolated for measuring lead concentration.

Data analysis

The EPSP slope was measured on the rising phase of EPSP by measuring the amplitude at a fixed latency (0.5 ms) from EPSP onset. The PS amplitude was measured by averaging the distance from the negative peak to leading and following positive peak, mean values of LTP and DP were obtained across time points in every 5 min and are presented as the mean±S.E.M. Mean values were normalized to pre-tetanus baseline values. The amplitudes of LTP were calculated by averaging the percentage of post-tetanus data in 1 h compared with pre-tetanus baseline data, and the amplitudes of DP were calculated by averaging the percentage of post-HFS data in 45 min compared with pre-LFS baseline data. Comparisons between control and lead-exposed rats, control and control+taurine rats, lead-exposed and lead-exposed+taurine rats were analyzed by two-way ANOVA with Tukey test. Probabilities less than 0.05 were considered significant.

RESULTS

Lead concentration in hippocampus

Lead concentrations in the hippocampus of the four groups are listed in Table 1. Significant difference was found between control and lead-exposed groups ($P<0.05$). There is no significant difference between control and control+taurine groups ($P>0.05$). Rats in lead-exposed+taurine group had a lower lead concentration in hippocampus than that of lead-exposed group ($P<0.05$), but still higher than that of control

Table 1. Lead concentration in hippocampus

	Control ($n=7$)	Lead-exposed ($n=8$)	Control+tau ($n=7$)	Lead+tau ($n=8$)
Lead concentration in hippocampus (μg/g)	0.245±0.014	1.511±0.015[a]	0.254±0.019	0.922±0.071[b]

[a] Significantly different from controls ($P<0.05$).
[b] Significantly different from lead-exposed group ($P<0.05$) and controls ($P<0.05$).