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ScienceDirect
Neurotoxicology and Teratology 29 (2007) 385–393
NEUROTOXICOLOGY AND TERATOLOGY
www.elsevier.com/locate/neutera

Developmental lead (Pb) exposure reduces the ability of the NMDA antagonist MK-801 to suppress long-term potentiation (LTP) in the rat dentate gyrus, in vivo

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Available online 19 January 2007

Abstract

Chronic developmental lead (Pb) exposure increases the threshold and enhances decay of long-term potentiation (LTP) in the dentate gyrus of the hippocampal formation. MK-801 and other antagonists of the *N*-methyl-D-aspartate (NMDA) glutamate receptor subtype impair induction of LTP. In addition, Pb exposure reduces presynaptic glutamate release and is associated with alterations in NMDA receptor expression. This study examined LTP in Pb-exposed animals challenged with a low dose of MK-801 to assess the sensitivity of this receptor to inhibition. Pregnant rats received 0.2% Pb acetate in the drinking water beginning on gestational day 16, and this regimen was continued through lactation. Adult male offspring maintained on this solution from weaning were prepared with indwelling electrodes in the perforant path and dentate gyrus. Several weeks later, input/output (I/O) functions were collected in awake animals before and after saline or MK-801 administration (0.05 mg/kg, s.c.). LTP was induced using suprathreshold train stimuli 60 min post-drug. Post-train I/O functions were reassessed 1 and 24 h after train delivery. Upon full decay of any induced LTP, drug conditions were reversed such that each animal was tested under saline and MK-801. I/O functions measured 1 and 24 h after train induction as well as immediate post-train responses revealed significant LTP of comparable magnitude that was induced in both control and Pb-exposed animals tested under saline conditions. In contrast, MK-801 reduced LTP in control but not in Pb-exposed animals. The broadening of the excitatory postsynaptic potential evident in responses evoked by train stimuli is NMDA-dependent. Pb exposure attenuated the MK-801-induced reduction in area of this NMDA component by ~50%. These findings are consistent with other neurochemical and behavioural observations and suggest that up-regulation of postsynaptic NMDA receptors produces subsensitivity to the inhibitory effects of MK-801 on hippocampal LTP following chronic developmental Pb exposure.

Keywords: Dentate gyrus; Long-term potentiation; NMDA; MK-801; Glutamate; Lead; Pb; In vivo; Hippocampus; Rat; Neurotoxicity; Learning and memory

1. Introduction

Developmental lead (Pb) exposure has long been associated with cognitive dysfunction, particularly in young children, yet the mechanisms whereby this metal produces these deficits remain unclear [5,22]. One of the primary cellular actions of Pb^{2+} is to block voltage-gated calcium channels and reduce evoked transmitter release [14,38,41]. We have previously demonstrated dose-dependent reductions in glutamate release in animals exposed to Pb throughout development using in vivo microdialysis in the hippocampus [29,31–33]. Nonetheless, due to the importance of *N*-methyl-D-aspartate (NMDA) subtype glutamate receptors in cognitive function and synaptic plasticity [10,39], investigation of the postsynaptic locus of Pb action has also received considerable attention.

Long-term potentiation (LTP) is an electrophysiological model of activity-dependent plasticity that embodies the cellular components of information storage at the synaptic level and is widely accepted as a primary mechanism of memory [6,10,40]. Long-lasting increases in synaptic strength induced by LTP require increases in both presynaptic neurotransmitter release and enhancement of postsynaptic glutamate receptor function. It

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doi:10.1016/j.ntt.2007.01.006

is well established that activation of NMDA receptors is essential for the induction of LTP at both hippocampal CA1 and dentate gyrus sites [10,39]. Chronic developmental exposure to Pb from birth increases the threshold for hippocampal LTP induction, reduces the magnitude of potentiation, and accelerates its decay [2,17,19–21]. Several reports of acute exposure to Pb^{2+} in cell culture or cortical tissue indicate disruption of the NMDA receptor ionophore [1,8,24,50]. Alterations in the expression of NMDA receptor subunits in the hippocampus of Pb-exposed animals have also been reported [23,27,43], suggesting that interaction of Pb^{2+} at this postsynaptic site may also contribute to the impairment of LTP. However, a direct assessment of the effects of chronic developmental Pb exposure on NMDA receptor function and its relationship to LTP has not been conducted.

The present study was designed to address this deficiency by examining the functional impact of developmental Pb exposure on NMDA receptor function. It was reasoned that if Pb reduces LTP by interfering with NMDA-mediated neurotransmission, Pb-exposed animals would be more susceptible to LTP disruption by antagonists that act at this receptor complex. To this end, control and developmentally Pb-exposed animals were challenged with a low dose of the NMDA receptor antagonist MK-801. Experiments were conducted in conscious animals equipped with chronic indwelling electrodes such that each animal was tested for LTP under saline and MK-801 conditions and thus could serve as its own control. Contrary to expectation, a decrease in sensitivity to the NMDA blocking properties of MK-801 rather than an increase was observed in Pb-exposed animals.

2. Methods

2.1. Subjects

Female Long Evans rats were obtained from Charles River (Raleigh, NC). On gestational day 16 (GD16) dams were placed on 0.2% Pb acetate in the drinking water, while controls were administered distilled water. Both groups of animals were maintained on NIH-07 chow (Ziegler Bros., Gardners, PA) to insure consistent levels of mineral intake. Previous work from our laboratory had revealed maximal effects on LTP and glutamate release at this concentration [21,32]. At parturition, litters were culled to 8 pups, retaining the maximal number of males per litter. On postnatal day 21 offspring were weaned, housed in a colony room on a 12:12 h light/dark schedule, and permitted free access to food and the same water given their dam. Animals were group housed (1–2/cage) until surgery and individually housed thereafter in plastic cages with woodchip bedding. Subjects from exposed dams were maintained on a 0.2% Pb-treated water supply throughout the experiment. Only one animal/litter is represented in the data reported.

2.2. Surgical procedures

As adults (3–6 months of age), animals were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) followed by atropine sulfate (0.2 mg/kg, i.p.). Bipolar twisted stainless steel wire electrodes (250-μm diameter wire insulated with Teflon except for the cut tips), crimped onto gold-plated Amphenol pins, were lowered into the angular bundle of the perforant path according to flat skull stereotaxic coordinates (7.2 mm posterior to bregma, 4.1 mm lateral to the midline). A bipolar recording electrode with a 0.5-mm tip separation was lowered into the ipsilateral dentate gyrus 3.5 mm posterior to bregma and 2.2 mm lateral to the midline. Optimal ventral placement was achieved through electrophysiological monitoring of the response evoked in the dentate gyrus following single pulse perforant path stimulation. Electrode pins were inserted into a 9-pin connector which was anchored to the skull with stainless steel screws and was secured with dental cement. The animals were grounded through a screw electrode inserted in the skull overlying anterior neocortex. Immediately following surgery, all animals received an i.m. injection of penicillin G (100,000 units). Animals were permitted a minimum of 2 weeks to recover from surgery prior to the commencement of testing.

2.3. Baseline stimulation and recording procedures

Following recovery, animals were administered biphasic square wave stimulus pulses (0.1 ms duration) using a Grass S-88 stimulator with PSIU-6 constant current converters. Animals were acclimated to the testing box for a minimum of 15 min prior to commencement of the recording session. All I/O functions were collected while the animal was in a quiet alert state. Animals with maximum postsynaptic potential response amplitudes of less than 3 mV were excluded from the study. No group differences in exclusion on this criterion were evident. Ten stimulus intensities (20–1500 μA) ranging from those subthreshold for population spike (PS) elicitation to those producing maximal PS amplitude were selected for each rat to provide a normalized range of PS amplitudes. Over several weeks, daily test sessions recorded baseline I/O functions by averaging 10 responses evoked at each of the ten stimulus intensities. Signals were amplified and digitally sampled at a frequency of greater than 25 kHz for a 30-ms epoch. Five points were selected on each averaged waveform to estimate the slope of the excitatory postsynaptic potential (EPSP) and the population spike (PS) amplitude as previously described [15].

2.4. I/O functions and drug challenge

Following the recording of stable baseline I/O functions on 3 consecutive days (PS amplitudes that did not vary more than 10% over days), animals were administered a pre-drug baseline I/O followed by an injection of saline (1 ml/kg, s.c.) or MK-801 (0.05 mg/kg, s.c.). A second I/O function was collected 20 min later (post-drug I/O). Immediately after collection of the post-drug I/O function, and corresponding to 1 h after drug administration, LTP was induced as described below. At 1 and 24 h later, additional I/O functions were collected to evaluate the magnitude and persistence of LTP. Animals were monitored for several weeks until any LTP induced by this initial treatment had