Early post-natal, low-level lead exposure increases the number of PSA-NCAM expressing cells in the dentate gyrus of adult rat hippocampus

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Abstract

Although lead is widely known as a potent neurotoxin, the effect of lead exposure on the expression of the polysialic acid linked neural cell adhesion molecule (PSA-NCAM) remains unclear. We exposed Wistar rat pups to 0.2% lead acetate from postnatal day (PND) 1 to PND 30. This exposure protocol resulted in pup blood lead levels, which increased to 29.3 ± 5.0 mg/dl on PND 15, and subsequently rose to 34.2 ± 5.8 mg/dl at weaning. Corresponding brain tissue lead levels were 456 ± 23 ng/g on PND 15 and 781 ± 87 ng/g on PND 30. Animals were sacrificed on PND 80, when the blood and brain lead concentrations did not differ from those of the control group. Lead exposure induced a significant increase in the total number of PSA-NCAM expressing cells, compared to the control group (p < 0.01), and did not change the proportion of cells co-expressing PSA-NCAM with glial or neuronal markers (calbindin, TuJ1, GFAP). These results suggest that early post-natal lead exposure induces persistent changes in the number of PSA-NCAM expressing cells, which could be, at least, partly the basis of impairments in the learning and memory formation, which follows low-level lead exposure.

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1. Introduction

Lead (Pb++) is widely known as a potent neurotoxin. Several prospective studies in humans, as well as animal experiments, have correlated developmental low-level lead exposure with enduring neurobehavioral impairments (see Lidsky and Schneider, 2003, for review). Developmental lead exposure has been correlated with a lowered IQ, impaired neuro-psychological functioning, impaired academic achievement, attention deficit disorder and hyperactivity in children, whilst mentioning the most predominant features of lead toxicity (Lidsky and Schneider, 2003). Furthermore, it has been repeatedly demonstrated, that the effects of lead persist long after the exposure has ceased (Winneke et al., 1977; Needleman and Gatsonis, 1990). Despite intensive research, no consensus on the mechanisms of the long-term neurotoxicity of the chemical has resulted.

Current evidence suggests that deficits in cognitive function, and altered behavior following developmental low-level lead exposure, are at least partly inflicted by disturbances in the formation and regulation of the cell recognition system mediated synaptic function (Regan and Keegan, 1990). Cell recognition systems involve neuronal cell adhesion molecules (NCAM). NCAM is a member of the immunoglobulin super family of adhesion molecules (Edelman, 1986) and plays a major role in cell-to-cell and cell to extra cellular matrix interactions (see Crossin and Krushel, 2000, for review). Adhesive properties of NCAM can be regulated through the addition of long linear homopolymers of alpha-2,8-linked sialic acid residues (polysialic acid or PSA) (Rutishauser, 1996), which attenuates NCAM mediated cell interaction and thereby creates plasticity in the positioning and movements of the cells and/or their processes (Bruses and Rutishauser, 2001; Rutishauser, 1996). NCAM and PSA-NCAM could also affect intracellular signaling through different signaling cascades (Crossin and Krushel, 2000; Doherty and Walsh, 1996; Muller et al., 2000). PSA-NCAM is strongly expressed during neural
development and generally down-regulated in the adult, however, it remains prominent in some brain areas that exhibit physiological plasticity (Seki and Arai, 1993).

It has been found that the molecule has an important part to play in neurodevelopmental processes (Edelman, 1984), as well as during adulthood in the maintenance of neural plasticity (Seki and Arai, 1993), and activity induced plasticity in defined brain regions’ (Kiss and Rougon, 1997), learning and memory consolidation (Cremer et al., 2000).

A limited number of studies have investigated the effects of early post-natal low-level lead exposure on the number of PSA-NCAM positive cells in the granular cell layer (GCL) of the hippocampus. It has been found that lead chloride significantly stimulates Golgi sialyltransferase activity from PND 16 onwards in vitro (Breen and Regan, 1988) and chronic low-level lead exposure antagonizes the physiological decrease in the NCAM polysialylation state during brain maturation (Cookman et al., 1987). On the other hand, it has been shown that that low-level lead exposure does not induce any significant change in the basal sialylation state in the dentate gyrus of hippocampus (Murphy et al., 1995; Murphy and Regan, 1999).

To examine the long-term effects of early post-natal low-level lead exposure on the number of PSA-NCAM expressing cells in the dentate gyrus of rat hippocampus, we exposed rat pups to low-level lead and examined the effects of lead in the adulthood (PND 80) on the total number of PSA-NCAM expressing cells in the dentate gyrus as well as changes in the proportion of cells co-expressing PSA-NCAM with glial or neuronal markers.

2. Materials and methods

All experimental procedures were approved by the Ethical Committee of Tartu University, Tartu, Estonia and were carried out by individuals who held an appropriate license.

All animals were housed under standard housing conditions. Food and water ad libitum.

2.1. Lead exposure protocol

The lead administration was performed according to the protocol published previously (Murphy and Regan, 1999). Wistar rat pups were cycled to eight per litter at birth and exposed to 0.2% lead acetate via their dams’ drinking water from postnatal day (PND) 1 to 21 and directly via drinking water from weaning until PND 30. This exposure protocol resulted in pup blood lead levels that increased to 29.3 ± 5.0 μg/dl by postnatal day (PND) 15 and subsequently rise to 34.2 ± 5.8 μg/dl at weaning. Corresponding brain tissue lead levels were 456 ± 23 ng/g on PND 15 and 781 ± 87 ng/g on PND 30. At postnatal day 30, lead was removed from the drinking water, and the animals were allowed to attain adulthood (PND 80), at which time blood lead levels were 6.5 ± 1.2 μg/dl and brain tissue lead levels 6 ± 1 ng/g. Mean blood lead levels of the control group were 4.2 ± 1.7 μg/dl and brain tissue lead levels 6 ± 2 ng/g.

Concentration of lead was measured by the ETAAS PU9100X atomic absorption spectrometer with a Philips HGA/P3105 graphite furnace and deuterium background corrector.

This exposure protocol had no effect on body weight gain during the exposure period or thereafter.

2.2. Tissue processing and immunohistochemistry

On PND 80 all animals were sacrificed and brain tissue processed for immunohistochemistry.

Lead-treated and control animals were deeply anesthetized with chloral hydrate (350 mg/kg) and transcardially perfused with normal saline followed by 4% paraformaldehyde in PBS. Immunohistochemistry was performed on free-floating coronal sections (40 μm) as described previously (Zharkovsky et al., 2003; Nacher et al., 2001). The sections were blocked in 2% normal goat serum in PBS containing 0.3% Triton X-100 for 1 h. This was followed by 48 h of incubation at 4 °C with mouse monoclonal antibodies against PSA-NCAM (1:400, AbCys, France) diluted in blocking buffer. After being washed in PBS, sections were incubated in biotinylated goat anti-mouse antibody (1:50, Vector Laboratories, UK) diluted in blocking buffer. After being washed in PBS, sections were incubated in biotinylated goat anti-mouse antibody (1:50, Vector Laboratories, UK) diluted in blocking buffer for 1 h. PSA-NCAM positive cells were visualized using the standard immunoperoxidase method (ABC system and diaminobenzidine as chromogen, Vector Laboratories).

The number of PSA-NCAM expressing cells was estimated using a modified version of the fractionator method (West, 1993; Madsen et al., 2003), counting all PSA-NCAM positive cells on every 20th section in a subgranular zone of dentate gyrus, starting randomly and multiplied by 20 to get the total number of PSA-NCAM expressing cells per animal.

Mean ± S.E.M. were calculated and lead-exposed animals were compared to controls using the Student’s t-test.

Double immunofluorescent labeling was performed using a mixture of mouse anti PSA-NCAM primary antibody with one of the following primary antibodies: rabbit anti-GFAP (1:1000, marker for astrocytes, DAKO, Denmark), mouse anti-TuJ1 (1:100, Covance, USA), rabbit-anti calbindin (1:1000, marker for mature neurons, Chemicon). Secondary antibodies were goat anti-mouse Texas Red (Jackson Im., USA) and goat anti-rabbit Alexa-488 (Molecular Probes).

Between four to six sections from each animal were analyzed for co-expression of PSA-NCAM and neuronal or glial markers.

Fluorescent signals were detected with a confocal microscope MRC-1024 (Olympus/BioRad, Germany) equipped with an argon–krypton laser. 3D images were constructed from a series (12–15) of scans at 2 μm intervals and further analyzed for co-localization signals.

The data were expressed as a percentage of PSA-NCAM-positive cells (80–100 PSA-NCAM expressing cells counted per animal) that expressed the phenotype marker TuJ1, calbindin or GFAP.

3. Results

PSA-NCAM immunohistochemistry exteriorized a population of cells preferably located in the subgranular zone of the
dentate gyrus, with occasional cells presented in the granular cell layer and hilus. PSA-NCAM positive cells extended neurites towards the GCL region. Almost no PSA-NCAM positive cells were found in CA1 and CA3 regions of the hippocampal formation. Quantitative analysis revealed that chronic post-natal low-level lead exposure induced a significant mean increase in the number of PSA-NCAM immunoreactive cells in the subgranular zone of the hippocampus compared to the control group. In control animals the number of PSA-NCAM positive cells was 27,540 ± 3024 and in lead-exposed animals 47,540 ± 3575 (t = 4.271; d.f. = 8; *p < 0.01; Fig. 1), respectively.

To obtain more information about the phenotype of PSA-NCAM expressing cells we performed double immunohistochemistry using antibodies against PSA-NCAM and neuronal or glial markers. Double-labeling immunofluorescence labeling of PSA-NCAM and a marker for young immature neurons, TuJ1, revealed that most of PSA-NCAM positive cells co-localized with TuJ1. The proportion of cells co-expressing PSA-NCAM and TuJ1 did not differ, however, between lead-exposed and control animals. Double-labeling immunofluorescence, using antibodies against GFAP and PSA-NCAM or calbindin and PSA-NCAM, showed that both markers never colocalized within the same cell in this region (Table 1; Fig. 2).

4. Discussion

In this paper we report that early post-natal low-level lead exposure induces a significant increase in the number of PSA-NCAM immunoreactive cells in the granular cell layer of the adult rat dentate gyrus. Our results are in a logical correlation with some previous reports (Cookman et al., 1987; Breen and Regan, 1988).

It should be emphasized that the observed increase in the number of PSA-NCAM-positive cells was found in 80-days-old animals, whereas the lead exposure was terminated at PND 30.
not fully excluded, such a possibility seems unlikely, because it is observed in Alzheimer’s disease (Arendt, 2000). Although functional significance of the persistent increase in PSA-NCAM-positive cells remains unknown, our results suggest, that early post-natal low-level lead exposure induces persistent changes in the number of PSA-NCAM expressing cells, which could at least partly attribute to cognitive dysfunction observed in adulthood.

References


