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Chronic low-level Pb exposure during development decreases the expression of the voltage-dependent anion channel in auditory neurons of the brainstem

John M. Prins, Diane M. Brooks, Charles M. Thompson, Diana I. Lurie *

Center for Structural and Functional Neuroscience, Center for Environmental Health Sciences, Department of Biomedical & Pharmaceutical Sciences, College of Health Professions and Biomedical Sciences, The University of Montana, Missoula, MT 59812, United States

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ABSTRACT

Lead (Pb) exposure is a risk factor for neurological dysfunction. How Pb produces these behavioral deficits is unknown, but Pb exposure during development is associated with auditory temporal processing deficits in both humans and animals. Pb disrupts cellular energy metabolism and efficient energy production is crucial for auditory neurons to maintain high rates of synaptic activity. The voltage-dependent anion channel (VDAC) is involved in the regulation of mitochondrial physiology and is a critical component in controlling mitochondrial energy production. We have previously demonstrated that VDAC is an in vitro target for Pb, therefore, VDAC may represent a potential target for Pb in the auditory system. In order to determine whether Pb alters VDAC expression in central auditory neurons, CBA/CaJ mice (n = 3–5/group) were exposed to 0.01 mM, or 0.1 mM Pb acetate during development via drinking water. At P21, immunohistochemistry reveals a significant decrease for VDAC in neurons of the Medial Nucleus of the Trapezoid Body. Western blot analysis confirms that Pb results in a significant decrease for VDAC. Decreases in VDAC expression could lead to an upregulation of other cellular energy producing systems as a compensatory mechanism, and a Pb-induced increase in brain type creatine kinase is observed in auditory regions of the brainstem. In addition, comparative proteomic analysis shows that several proteins of the glycolytic pathway, the phosphocreatine circuit, and oxidative phosphorylation are also upregulated in response to developmental Pb exposure. Thus, Pb-induced decreases in VDAC could have a significant effect on the function of auditory neurons.

1. Introduction

Lead (Pb) is a naturally occurring toxic heavy metal that has been widely distributed throughout the environment due to its extensive use in a variety of industrial procedures and products. Environmental Pb enters biological systems through ingestion and respiration (Toscano and Guilarte, 2005) and continues to be a serious problem in many parts of the U.S. The U.S. Centers for Disease Control and Prevention have determined that blood Pb levels of 10 μg/dl should prompt public health actions, however recent studies in humans and animals have shown that the neurotoxic effects of Pb occurs at even lower blood Pb levels (Gilbert and Weiss, 2006). Low-level Pb exposure is a risk factor for learning disabilities and attention deficit hyperactivity disorder (ADHD) (Lidsky and Schneider, 2003; Braun et al., 2006). Many children with these behavioral syndromes also demonstrate deficits in auditory temporal processing, suggesting a disturbing link between developmental Pb exposure, behavioral dysfunction and auditory temporal processing (Gray, 1999; Otto and Fox, 1993; Lurie et al., 2006; Breier et al., 2003; Montgomery et al., 2005).

Auditory temporal processing involves the processing of central auditory neuronal signals in time and space, allowing the listener to resolve complex sounds and to recognize specific signals within a noise background. Children exposed to Pb show decreased performance in tests requiring appropriately timed reactions and demonstrate increased latencies in brainstem auditory evoked potentials (Finkelstein et al., 1998; Holdstein et al., 1986). In animals, chickens exposed to low levels of Pb show deficits in backward masking, a test of central auditory temporal processing (Gray, 1999). We have found that mice exposed to low levels of Pb demonstrate alterations of two measures of central auditory brainstem function, the brainstem conduction time and gap encoding in the inferior colliculus (Jones et al., 2008). Taken together, these studies suggest that the auditory system is a target for Pb.

* Corresponding author at: Department of Pharmaceutical Sciences, School of Pharmacy and Allied Health Sciences, Skaggs Building Room 383, The University of Montana, Missoula, MT 59812, United States. Tel.: +1 406 243 2103; fax: +1 406 243 5228.
E-mail address: Diana.lurie@umontana.edu (D.I. Lurie).

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We have recently demonstrated that the voltage-dependent anion channel (VDAC) is a novel target for Pb in CNS neurons in vitro (Prins et al., 2010). VDAC is an ion channel located in the mitochondrial outer membrane that plays a central role in regulating energy metabolism in neurons by maintaining cellular ATP levels and regulating calcium buffering (Shoshan-Barmatz et al., 2006; Shoshan-Barmatz and Gincel, 2003). In vitro exposure to lower levels of Pb results in decreased VDAC transcription and expression in two different neuronal cell lines (Prins et al., 2010). Further, the decrease in VDAC is correlated with a decrease in cellular ATP levels, suggesting a connection between decreased VDAC expression and decreased cellular ATP levels. It is not known whether developmental Pb exposure results in loss of VDAC protein in brainstem auditory neurons in vivo. Because auditory neurons have high and fluctuating energy requirements (Trussell, 1999; Hiel et al., 1996), a decrease in VDAC expression could alter the function of auditory neurons by disrupting energy buffering systems within these neurons.

In order to determine if VDAC represent a potential target for Pb in central auditory neurons, the current study examines the expression of VDAC in the brainstem following developmental Pb exposure. We find that chronic low-level Pb exposure during development results in the decreased expression of VDAC in the murine auditory brainstem. Immunohistochemical analysis of the auditory brainstem nucleus, the Medial Nucleus of the Trapezoid Body (MNTB), demonstrates that neurons in the MNTB show a significant decrease in VDAC staining following Pb exposure. In addition, western blot analysis of a ventral brainstem region (VBS) containing several auditory nuclei, including the MNTB, and the medial and lateral superior olivary nuclei, reveal a significant decrease in VDAC expression.

A comparative proteomic analysis of the VBS was then conducted to determine if other energy pathways, such as the glycolytic pathway, were upregulated in response to the Pb-induced decrease in VDAC. If decreased VDAC levels result in the decreased production of ATP, then one would expect to see an increase in other cellular energy producing systems to compensate. We found that several proteins of the glycolytic pathway, the phosphocreatine circuit, and oxidative phosphorylation were upregulated in response to developmental Pb exposure.

2. Experimental procedures

2.1. Chronic Pb exposure

Breeding pairs of CBA/CaJ mice were obtained from The Jackson Laboratory (Bar Harbor, Maine) and maintained in microisolator units in the University of Montana specific pathogen free animal facility. Cages, bedding, and food were sterilized by autoclaving and mice were handled with aseptic gloves. Mice were allowed food and water ad libitum. All animal use was in accordance with NIH and University of Montana IACUC (Institutional Animal Care and Use Committee) guidelines. Thirteen breeding pairs of CBA mice were randomly assigned to three groups having unlimited access to water (pH 3.0) containing 0 mM (control), 0.01 mM (low), or 0.1 mM (high) Pb acetate. Breeding pairs were given leaded water when they were paired so that offspring were exposed to Pb throughout gestation and through the dam's milk until postnatal day 21 (P21) when mice were sacrificed.

2.2. Blood lead levels

Blood was collected from deeply anesthetized mice by retro-orbital puncture. Blood Pb levels were measured by the Montana Health Department in Helena, MT. It should be noted that the means for the no Pb group include values of <1.0 which were included in the data set as equal to 1.0 (data not shown).

2.3. Antibodies

The polyclonal antibody against VDAC was raised by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to the amino terminus of human VDAC 1 and purified using protein A and peptide affinity chromatography (#4866, Cell Signaling Technology, Beverly, MA). VDAC detects endogenous levels of total VDAC protein that is ubiquitously expressed and located in the outer mitochondrial membrane. The staining is in mouse brainstem is virtually identical to that observed in the rat cerebellum (Shoshan-Barmatz et al., 2004) and hippocampus (Jiang et al., 2007). Preadsorption with the VDAC protein (2 μg/100 μl) eliminates all immunoreactivity.

The polyclonal antibody against brain type Creatine Kinase (CKB) was raised against a KLH conjugated synthetic peptide selected within amino acid 200–300 of human CKB (ab38211, Abcam, Cambridge, MA). The CKB antibody recognizes a single band at approximately 43 kDa in our Western blots, similar to that seen in Balasubramani et al. (2006).

The polyclonal antibody against p44/42 MAPK(Erk1/2) was raised against a synthetic peptide (KLH-coupled) derived from a sequence in the C-terminus of rat p44 MAP kinase (Cell Signaling, Danvers, MA; product #9102). The p44/42 MAPK antibody recognizes a double band in our western blots similar to that seen in Numakawa et al. (2007).

2.4. Immunohistochemistry

At P21, mice from the three treatment groups ($n = 5$–6 per Pb treatment group) were deeply anesthetized and perfused transcardially with 4% Na-periodate-lysine-paraformaldehyde fixative (PLP, final concentrations 0.01 M sodium periodate, 0.075 M lysine, 2.1% paraformaldehyde, 0.037 M phosphate). The tissue was then processed, paraffin embedded, and immunostained as previously described (Jones et al., 2008). For light microscopy, a "one out of six" series of 10 μm sections of the auditory brainstem was immunostained for VDAC. The standard peroxidase anti-peroxidase procedure using the Vector ABC kit was used with appropriate secondary antibodies (Vector Laboratories, Burlingame, CA) and visualized using 3-3′ diaminobenzidine (DAB, Sigma) in Tris buffer with 0.001 M imidazole and 0.1% hydrogen peroxide as the chromagen. Sections were then rinsed in water, dehydrated, and coverslipped using DPX mounting media (BDH Limited, Poole, UK). The VDAC antibody concentration used for immunohistochemistry was 1:1000 overnight at 4 °C. (#4866; Cell Signaling Technology, Beverly, MA). Antibody control sections were run as described above but the VDAC antibody incubation was omitted from the procedure. Sections were also run with VDAC-specific blocking peptide (#1711B, Cell Signaling Technologies, Beverly MA) to evaluate the specificity of the antibody. Sections run with VDAC pre-incubated with blocking peptide were negative for signal (data not shown).

2.5. Tissue analysis

VDAC stained brainstem sections from 5 to 6 mice per Pb treatment group were viewed with a Nikon Eclipse E800 microscope and a black and white Cohu (San Diego, CA) video camera connected to a PowerMac computer. Six to twelve sections per mouse were chosen from the center of each region of interest and then slides were blinded as to treatment group. Analysis of VDAC immunostaining was performed using NIH Image V1.61 as follows. Briefly, images from each region analyzed were captured