NeuroToxicology 54 (2016) 65–71

Contents lists available at ScienceDirect

NeuroToxicology

Full length article

Sex-dependent effects of lead and prenatal stress on post-translational histone modifications in frontal cortex and hippocampus in the early postnatal brain

Jay S. Schneider[a,*], David W. Anderson[a], Sarah K. Kidd[a], Marissa Sobolewski[b], Deborah A. Cory-Slechta[b]

[a] Thomas Jefferson University, Dept. of Pathology, Anatomy and Cell Biology, Philadelphia, PA, USA
[b] Dept. of Environmental Medicine, University of Rochester School of Medicine, Rochester, NY, USA

ARTICLE INFO

Article history:
Received 1 December 2015
Received in revised form 18 February 2016
Accepted 23 March 2016
Available online 24 March 2016

Keywords:
H3K9/14Ac
H3K9Me3
Hippocampus
Frontal cortex
Lead
Prenatal stress

ABSTRACT

Environmental lead (Pb) exposure and prenatal stress (PS) are co-occurring risk factors for impaired cognition and other disorders/diseases in adulthood and target common biological substrates in the brain. Sex-dependent differences characterize the neurochemical and behavioral responses of the brain to Pb and PS and sexually dimorphic histone modifications have been reported to occur in at-risk brain regions (cortex and hippocampus) during development. The present study sought to examine levels and developmental timing of sexually dimorphic histone modifications (i.e., H3K9/14Ac and H3K9Me3) and the extent to which they may be altered by Pb ± PS. Female C57/Bl6 mice were randomly assigned to receive distilled deionized drinking water containing 0 or 100 ppm Pb acetate for 2 months prior to breeding and throughout lactation. Half of the dams in each group were exposed to restraint stress (PS, three restraint sessions in plastic cylindrical devices 3×/day at for 30 min/day (1000, 1300, and 1600 h)) from gestational day 11–19 or no stress (NS). At delivery (PND0) and postnatal day 6 (PND6), pups were euthanized and frontal cortex and hippocampus were removed, homogenized, and assayed for levels of H3K9/14Ac and H3K9Me3. Sex-dependent differences in both levels of histone modifications as well as the developmental trajectory of changes in these levels were observed in both structures and these parameters were differentially affected by Pb ± PS in a sex and brain-region-dependent manner. Disruptions of these epigenetic processes by developmental Pb ± PS may underlie some of the sex-dependent neurobehavioral differences previously observed in these animals.

1. Introduction

Many aspects of both normal and pathological brain functioning exhibit important yet poorly understood sex differences (Wizemann and Pardue, 2001; McCarthy et al., 2012). Sex differences have been described for various cognitive functions, as well as for anxiety and stress responses, outcomes from traumatic brain injury, risk and outcomes from various neurodegenerative diseases, and the risk for developing various emotional and affective disorders (see McCarthy et al., 2012 for review). Sex-dependent differences also characterize the response of the brain to the neurotoxicant lead (Pb) and to prenatal stress (PS). Developmental Pb exposure and PS are important co-occurring risk factors for a variety of adverse health outcomes in adulthood as well as for various cognitive/behavioral problems during childhood, with effects expressed differently in males and females (Virgolini et al., 2008a,b; Cory-Slechta et al., 2010, 2012; Giesbrecht et al., 2015; Wainstock et al., 2015; Zohar et al., 2015).

The precise molecular mechanisms underlying the effects of developmental Pb exposure and of PS on the brain are not yet completely known, nor are the molecular underpinnings for the sex-related differences in the responses of the brain to such risk factors. However, it seems likely that epigenetic mechanisms are involved (Auger and Auger, 2011). Epigenetic outcomes resulting from environmental perturbations are frequently found to be sex-dependent, particularly in relation to neuroendocrine-acting systems. These sex differences likely reflect reported sex differences in such aspects of epigenetic programming as levels of the DNA methyltransferases (DNMTs) (McCarthy et al., 2009; Kolodkin and Auger, 2011) or DNA methylation patterns, and expression of methyl-binding proteins and co-repressor proteins (McCarthy

* Corresponding author at: Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, 1020 Locust Street, JAH 521, Philadelphia, PA 19107, USA.
E-mail address: jay.schneider@jefferson.edu (J.S. Schneider).

http://dx.doi.org/10.1016/j.neuro.2016.03.016
0161-813X/

et al., 2009). The existence of such sex differences early in development has been suggested to be critical to brain differentiation (Auger and Auger, 2011). These differences also establish a different biological substrate for each sex on which environmental stressors, including chemical exposures, then act.

In the mammalian brain, chromatin-level modifications play pivotal roles in coordinating developmental programs. Gene regulation during development is regulated by the binding of a variety of regulatory proteins to gene promoter regions and through epigenetic modification of chromatin by post-translational histone modifications and DNA methylation. The role of epigenetics in determining sex differences in the brain (McCarthy et al., 2009) can also be significantly influenced by the period of development during which these environmental factors act. For example, higher levels of DNMT3a mRNA expression in female amygdala were reported at postnatal day 1, but had disappeared by postnatal day 10, with these changes related to sex differences in steroid exposure levels (Kolodkin and Auger, 2011). These dynamics in the developmental trajectory could influence the extent to which environmental risk factors affect the epigenome in a sex-dependent manner.

Epigenetic alterations may occur in the germline as well as in somatic cells (Skinner et al., 2008), both of which are modifiable by environmental stimuli such as steroid hormones and endocrine-disrupting chemicals (McCarthy et al., 2009; Gore, 2008) and potentially, by environmental factors such as developmental Pb exposure and PS. It is possible that an interaction between genes and sex hormones during development may make a variety of brain regions, including the frontal cortex and hippocampus, which are particularly sensitive to the effects of Pb and PS (Cory-Slechta et al., 1998, 1999; Berger et al., 2002; Barros et al., 2004; Virgolini et al., 2008a; Martinez-Tellez et al., 2009; Rossi-George et al., 2011; Neal et al., 2012; Stansfield et al., 2012; Baranowska-Bosiacka et al., 2013; Hu et al., 2014; Li et al., 2014, 2015) differentially susceptible to epigenetic alterations, such as post-translational histone modifications, that are associated with modulation of transcriptional regulation.

As sexually dimorphic histone modifications have been reported to occur in cortex and hippocampus during development (Tsai et al., 2009), the present study examined the extent to which these histone modifications may be influenced by Pb and/or PS and thus contribute to the sex-dependent differences in the patterns of effects observed in response to these environmental risk factors alone and in combination (Cory-Slechta et al., 2004b, 2008, 2010; Virgolini et al., 2006, 2008a,b; Rossi-George et al., 2011). The goal of the present study was to examine levels and developmental timing of sexually dimorphic histone modifications, H3K9/14Ac (associated with gene activation) and H3K9Me3 (associated with gene silencing) (Tsai et al., 2009) and the extent to which they may be altered by Pb and/or PS (Pb ± PS). Modifications of these histone marks by Pb ± PS could have wide-ranging effects on gene transcription as well as later behavioral/cognitive functions.

2. Materials and methods

2.1. Animals and lead exposure and prenatal stress

The use of animals was in compliance with NIH Guidelines for the Care and Use of Laboratory Animals and the study was approved by the institutional animal care and use committee at the University of Rochester School of Medicine. Two-week-old female C57/Bl6 mice (Jackson Laboratories, Bar Harbor ME) were randomly assigned to receive distilled deionized water drinking containing 0 or 100 ppm Pb acetate for 2 months prior to breeding and throughout lactation. Standard rat chow diet (Lab Diet, Laboratory Rodent Diet) was provided *ad libitum*. Female mice were mated with males (1:1) for 4 to 5 days to cover the duration of an estrous cycle. Gestational day 1 (GD1) was designated as the second day after pairing.

Pregnant females in the 0 and 100 ppm Pb-treated groups were weighed and further randomly subdivided to a non-stress (NS) or prenatal stress (PS) condition. Half of the dams in each Pb group were exposed to restraint stress (PS) 3×/day at for 30 min/day (1000, 1300, and 1600 h) from gestational day 11–19 or no stress (NS). The stress procedure consisted of three 30-min restraint sessions in plastic cylindrical devices, a protocol previously verified to elevate corticosterone levels (Cory-Slechta et al., 2004a). Non-stressed dams were left undisturbed in their home cages. This yielded 4 treatment groups/sex: 0-NS, 0-PS, 100-NS and 100-PS.

2.2. Blood collection for lead determinations

Blood Pb measurements were determined from trunk blood of PND5-6 pups and from dams (collected at weaning so as not to produce maternal stress) by anodic stripping voltammetry using the Lead Care II system with a detection limit of 3.3 μg/dl.

2.3. Measurement of post-translational histone modifications

At delivery (PND0) and postnatal day 6 (PND6), pups were euthanized and frontal cortex and hippocampus were removed. Genomic DNA was extracted from tail snips and the sex of the pups was confirmed by *Jarid 1C* (X-chromosome-specific gene)/*Jarid 1D* (Y-chromosome-specific gene) PCR (Clapcote and Roder, 2005). To measure H3K9/14Ac and H3K9Me3, hippocampus and prefrontal cortex from each animal was homogenized in hypotonic lysis buffer (10 mM HEPES, 1.5 mM MgCl2 and 10 mM KCl, pH 7.9) with 1x HALT protease inhibitor followed by addition of 1 M HCl. Samples were incubated at 4 °C for 30 min and then vortexed, centrifuged for 10 min at 15,000 rpm, and supernatant containing acid soluble proteins was transferred to fresh tubes at 4 °C and protein was quantified using the BCA reaction (Pierce Inc.). Samples (10 μg) were separated on 4–12% Bis-Tris gels (Invitrogen Inc.) run for 1 h at 200 V and then transferred to 0.2 μm nitrocellulose membranes using semi-dry transfer (Bio-rad Inc.) for 15 min/membrane at 15 V. Membranes were washed in tris-buffered saline (TBS) containing tween-20 (TBS-T) and blocked in 5% milk for 1hr at room temperature. Primary antibody, rabbit anti-acetyl H3K9/14 at 1:10,000 (Millipore), anti-H3K9 Me3 at 1:5000 (Millipore) or rabbit anti-β-actin at 1:2000 (Imgenex), was added for 1 h at room temperature. Following multiple washes in TBS-T, secondary antibody (horseradish peroxidase conjugated goat anti rabbit at 1:20,000; Thermo Fisher Scientific) was added for 1 h. Membranes were washed repeatedly in TBS-T and exposed to chemiluminescent developing reagent (Pierce Pico or Dura, Thermo Fisher Scientific). Densitometry was used to quantify the amount of H3K9/14Ac and H3K9Me3 relative to β-actin for each sample (MCID™ Basic V 7.2, Interfocus Imaging LTD). Relative optical density was normalized to β-actin.

2.4. Statistical analyses

Statistical analyses were carried out using between factor ANOVAs. Specifically, initial overall analyses included sex, Pb treatment, PS and day (PND0 and PND6) for each region (hippocampus and frontal cortex) and histone marker separately. Because of the extensive effects of sex and interactions of sex with other variables, subsequent analyses were undertaken using Pb, PS and day separately for each histone marker in each brain region for each sex. This allowed the assessment of impact of