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Prenatal arsenic exposure alters the programming of the glucocorticoid signaling system during embryonic development

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ABSTRACT

The glucocorticoid system, which plays a critical role in a host of cellular functions including mood disorders and learning and memory, has been reported to be disrupted by arsenic. In previous work we have developed and characterized a prenatal moderate arsenic exposure (50 ppb) model and identified several deficits in learning and memory and mood disorders, as well as alterations within the glucocorticoid receptor signaling system in the adolescent mouse. In these present studies we assessed the effects of arsenic on the glucocorticoid receptor (GR) pathway in both the placenta and the fetal brain in response at two critical periods, embryonic days 14 and 18. The focus of these studies was on the 11β-hydroxysteroid dehydrogenase enzymes (11β-HSD1 and 11β-HSD2) which play a key role in glucorticoid synthesis, as well as the expression and set point of the GR negative feedback regulation. Negative feedback regulation is established early in development. At E14 we found arsenic exposure significantly decreased expression of both protein and message in brain of GR and the 11β-HSD1, while 11β-HSD2 enzyme protein levels were increased but mRNA levels were decreased in the brain. These changes in brain protein continued into the E18 time point, but mRNA levels were no longer significantly altered. Placental HSD11B2 mRNA was not altered by arsenic treatment but protein levels were elevated at E14. GR placental protein levels were decreased at E18 in the arsenic exposed condition. This suggests that arsenic exposure may alter GR expression levels as a consequence of a prolonged developmental imbalance between 11β-HSD1 and 11β-HSD2 protein expression despite decreased 11HSDB2 mRNA. The suppression of GR and the failure to turn down 11β-HSD2 protein expression during fetal development may lead to an altered set point for GR signaling throughout adulthood. To our knowledge, these studies are the first to demonstrate that gestational exposure to moderate levels of arsenic results in altered fetal programming of the glucocorticoid system.

1. Introduction

Early developmental and adult exposure to arsenic is associated with a multitude of health problems, including cognitive (Rodriguez-Barranco et al., 2013; Rosado et al., 2007; von Ehrenstein et al., 2007; Wasserman et al., 2004), cardiovascular (McClintock et al., 2014; Moon et al., 2012; Wu et al., 2014), metabolic (Chen et al., 2009; Joshi and Shrestha, 2010; Lu et al., 2014) and metastatic (Ferreccio et al., 2013; Mostafa and Cherry, 2013; Wong et al., 1998; Xie et al., 2014) disorders that are manifested across the lifespan. The developmental origins of health and disease (DOHaD) theory (Gluckman et al., 2007; McMullen and Mostyn, 2009; Wadhwa et al., 2009) proposes that susceptibilities to chronic diseases are, in part, determined by physiologic changes initiated in utero in response to an altered prenatal environment. The theory posits that an adverse intra-uterine environment programs the development of the fetus to accommodate for this toxic environment by altering gene expressions. These adaptations are likely through epigenetic modifications. One system, the glucocorticoid signaling pathway has been demonstrated to be programmed in response to prenatal environments (Bolten et al., 2013; Conradt et al., 2013; Khulan and Drake, 2012; Lukaszewski et al., 2013; Reynolds, 2013) and these programming changes have been observed into adulthood (Goldstein et al., 2014; Khalife et al., 2013), including the transmission of the altered programming across generations (Luo et al., 2014). Further, there is support for the suggestion that altered glucocorticoid receptor (GR) programming could impact adult health (Brunton and Russell, 2011; Harris and Seckl, 2011; Merlot et al., 2008; Sarkar et al., 2008).

While there are many systems affected by arsenic, few have been implicated to play a role in all the various health problems associated with exposure. The GR signaling system, has been linked with cognitive deficits (Rodriguez et al., 2011), cardiovascular (Santos and Joles, 2012), metabolic (Sarr et al., 2012; Spencer, 2012), immune (Bellavance and

Abbreviations: GR, glucocorticoid receptor; HSD, hydroxysteroid dehydrogenase; E14, embryonic day 14; E18, embryonic day 18; GD, gestational day; MMA, methylarsonic acid; DMA, dimethylarsinic acid; PNL, post nuclear lysate; Nuc, nuclear fraction

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Rivest, 2014) and metastatic diseases (Kitchin and Wallace, 2008; Schmitz et al., 2009). Several studies have demonstrated that arsenic produces specific disruptions in glucocorticoid transcriptional activity and steroid receptor function (Ahir et al., 2013; Barr et al., 2009; Bodwell et al., 2004; Davey et al., 2007; Gosse et al., 2014; Hamilton et al., 1998; Kaltreider et al., 2001; Shaw et al., 2007). Many of these studies have utilized cell lines or cultured cells, which provide a great deal of mechanistic information regarding the impact of toxins on specific cellular regulators but precludes the ability to assess the impact of arsenic on developmental programming including the interaction between maternal and fetal physiology.

Our own work using an in vivo mouse model exposed prenatally to 50 ppb arsenic in drinking water has found alterations in the levels of glucocorticoid signaling (Goggin et al., 2012; Martinez-Finley et al., 2009; Martinez et al., 2008), in the hippocampus from adolescent offspring. While it is clear that arsenic, both in vivo and in vitro, produces significant changes in the glucocorticoid system, the impact of arsenic on the glucocorticoid system in both the developing fetus and the placenta have not been explored. Fetal levels of glucocorticoid are regulated by both the fetal and the placental tissues through the developmentally regulated isozymes, 11β-hydroxysteroid dehydrogenases (11β-HSD) 1 and 2, which interconvert active and inactive cortisol (corticosterone). In the mouse, placental expression of 11β-HSD2 is elevated until about embryonic day 13 (E13), when it serves to inactivate maternal corticosterone and protects the developing fetus from the high glucocorticoid activation. Expression of 11β-HSD1 begins around E14 and peaks at E18 (Brown et al., 1996; Speirs et al., 2004). 11β-HSD1 is a reductase, which activates corticosterone and regenerates the glucocorticoid increasing fetal corticosterone levels. The developmental expression and levels of these isozymes are seen as being a critical regulation point for hypothalamic–pituitary–adrenal (HPA) axis negative feedback (Brunton and Russell, 2011; Chapman et al., 2013; Reichardt and Schutz, 1996). Maternal exposure to arsenic may alter the timing of 11β-HSD isozyme expression levels in an attempt to adapt to the influence of arsenic on GR signaling. Additionally, arsenic readily crosses the placenta (Caumette et al., 2007; Fei et al., 2013; He et al., 2007; Vahter, 2009) and might directly alter expression and levels of the GR signaling complex within the developing fetal brain. Little is known about the impact of arsenic in utero on the developing fetal brain and placenta.

In the present study, we evaluated the effect of 50 ppb (50 μg/L) arsenic on the glucocorticoid signaling system in both the fetal brain and placenta at two development time-points (E14 and E18) to identify critical shifts in the expression of signaling components which might impact the GR programming and later adult stress responding. The embryonic period from E14 to E18 is critical for the expression shift between 11β-HSD2 and 11β-HSD1 and for development of the negative feedback system for the centrally regulated HPA axis. If prenatal arsenic alters the level and/or the timing of the expression of these proteins which regulate the glucocorticoid response, then it may alter the central regulation of HPA axis programming and affect stress responding throughout life.

2. Materials and methods

2.1. Prenatal arsenic exposure paradigm

All procedures were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee at the University of New Mexico. Animals were maintained in a 22 °C vivarium on a reverse light/dark cycle with lights on at 2000 and ad libitum access to water and food. Exposure to arsenic was performed as previously described during all three trimesters of development (Martinez et al., 2008; Tyler et al., 2014). Briefly, singly housed female C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) were acclimated to drinking 50 ppb arsenic-laced water (sodium arsenate, Na_2 $HAsO_4$-$7H_2O$ Sigma-Aldrich, St. Louis, MO) for seven to ten days prior to mating. Dams were offered ad lib access to TekLad 2020X rodent chow and either 50 ppb arsenic in water or tap water alone during breeding and pregnancy until fetuses and placentas and brains were removed at gestational day (GD) 14 or GD 18. The day of pregnancy was designated as GD1. All tissue was collected early in the lights off period, between 0800 h and 1000 h. For all of the analyses, there were no conditions where brains or placentas were pooled; only one brain or one placenta was used in the preparation. Male fetuses were selected from within the middle of the uterus. Only one embryo brain or its associated placenta was used from each dam per treatment condition. Due to the different tissue preparations required for protein versus mRNA analyses, different embryo brains and their associated placentas were represented in the Western and the qPCR assays.

2.2. Assessment of As5, As3, MMA and DMA

Levels of arsenic species were determined in whole brain from E14 and E18 arsenic and control fetuses using HPLC–ICP-MS. Samples were taken from 8 separate dams with one embryo per dam analyzed per group. Samples were kept frozen until sample analysis time. The brain tissue was thawed, homogenized and deproteinated by extraction into an equal volume of mobile phase (2 mM octanesulfonic acid and 2 mM malonic acid, pH 7). Control tissue blanks were included in the analysis. Samples were vortexed until dissolved and filtered (0.45 μm filter). Samples were diluted 1:1 in deionized water and loaded into sealed autosampler vials and maintained at 20 °C. The analytical mobile phase was buffered at pH 4 and brought to volume in 2% HPLC grade methanol. A PerkinElmer Flexar HPLC was coupled with a NexION 300D ICP/MS through injection valve. The ICP/MS mode setting was a Dynamic Reaction Cell (DRC) using oxygen (O2 = 0.5; q = 0.5) with a dwell time of 500 ms (2 pts/s). The HPLC separation used a C18 Shiseido Capcell Pak MG (5 μm × 4.6 mm × 25 cm) with a mobile phase of 2 mM octanesulfonic acid + 2 mM malonic acid + 2% MeOH, pH 4.0 (adjusted with 10% NH_4OH) at a 1.5 mL/min flow rate, 1000–1600 psi, and a column temperature of 50 °C. Samples were injected at a 50 μL volume. The outlet of the HPLC column was connected directly to an injection valve that is also connected to a glass Meinhard nebulizer leading to the glass cyclonic spray chamber of ICP/MS, allowing continuous transportation of the determinants to the argon plasma of ICP-MS. Retention time for the As species was determined using mixed standards of 10 μg/L arsenic (III) oxide (iAsIII), arsenic (V) oxide (iAsV), dimethylarsinic acid (DMA) (all from Sigma-Aldrich Chemical Co), and methylarsonic acid (MMA) (Wako Pure Chemical Industries, Ltd). Peaks of different As species were identified by comparison with the retention times of individual standard compounds Calibration standards for Arsenite (As3), Arsenate (As5), Monomethyl Arsenic (MMA), and Dimethyl Arsenic (DMA) were prepared similar to the tissue samples using the buffered mobile phase. The detection limit was 0.010 μg/L for iAsIII DMA, MMA and iAsV. The system was calibrated using a blank and four point calibration standards. Samples were analyzed in automated mode. Chromatogram retention times were adjusted, peaks were identified and the calibration curves linearity was verified for each species. The analytical data were reprocessed and validated.

2.3. Maternal plasma corticosterone levels

Pregnant dams at GD14 and GD18 were sacrificed by decapitation and trunk blood was collected using Safe-T-Fill EDTA capillary tubes (Ram Scientific Inc. #077051) and plasma was separated by centrifugation at 1000 ×g for 10 min at 4 °C, and stored at −80 °C until use in the corticosterone assay. Corticosterone (CORT) levels in plasma were determined using the DetectX Corticosterone EIA Kit (Arbor Assays, Ann Arbor, MI; #K014-H1) per the manufacturer's instructions. Optical