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Hormone levels in neonatal hair reflect prior maternal stress exposure during pregnancy

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ABSTRACT

Hormones present in hair provide summative information about endocrine activity while the hair was growing. Therefore, it can be collected from an infant after birth and still provide retrospective information about hormone exposure during prenatal development. We employed this approach to determine whether a delimited period of maternal stress during pregnancy affected the concentrations of glucocorticoids and gonadal hormones in the hair of neonatal rhesus monkeys. Hair from 22 infant monkeys exposed to 5 weeks of gestational disturbance was compared to specimens from 13 infants from undisturbed control pregnancies. Using an LC/MS/MS based technique, which permitted seven steroid hormones to be quantified simultaneously, we found 2 hormones were significantly different in infants from disturbed pregnancies. Cortisol and testosterone levels were lower in the hair of both male and female neonates. Maternal hair hormone levels collected on the same day after delivery no longer showed effects of the disturbance earlier during pregnancy. This study documents that a period of acute stress, lasting for 20% of gestation, has sustained effects on the hormones to which a developing fetus is exposed.

1. Introduction

It is well established that stress during pregnancy can impact the developing fetus. Both physical and psychological insults have been associated with lasting effects, including altered neuroendocrine and immune function, emotional and attentional problems, and a greater risk for obesity in adulthood (Davis et al., 2011; Entringer, 2013; Laplante et al., 2004; O'Connor et al., 2002, 2013; Pluess et al., 2010). One of the primary processes underlying these relationships is a re-programming of the fetal hypothalamic–pituitary–adrenal (HPA) axis by the placental transfer of maternal glucocorticoids (Coe and Lubach, 2005; Glover et al., 2009; Jensen Pena et al., 2012; Sarkar et al., 2008; Walsh et al., 1979). A plethora of studies in humans and animal models have provided support for this view that maternal hormone responses can act on the regulatory set points of the fetal endocrine system (Baibazarova et al., 2013; Buss et al., 2012; Coe et al., 2002; Kapoor and Matthews, 2005; La Marca-Ghaemmaghami et al., 2015), but it has been difficult to directly assess the extent of change in the fetal compartment. The capacity to measure the hormone content of infant hair, which had grown during fetal period, now provides that opportunity.

The use of hair to analyze hormones is a novel, non-invasive method for obtaining information about the endocrine system over an extended period of time while the hair had been growing. The hypothesized way that hormone is incorporated into hair is largely through passive diffusion from systemic circulation during the formation of the hair shaft, although some have argued that the follicle itself can synthesize and regulate hormones, and secretions from sweat and sebum may play a role (Davenport et al., 2006; Grass et al., 2015; Sauve et al., 2007; Stalder and Kirschbaum, 2012). Therefore, in contrast to point measurements, such as from serum, saliva and urine, hair provides information about the cumulative exposure to hormones representative of weeks to months, depending on the length of the hair.

We have previously shown that hair from newborn rhesus monkeys can be used to measure steroid hormones, which reflects their prior fetal experiences since the hair started growing approximately two months before term (Kapoor et al., 2014; Schultz, 1937). Using this technique, we found that parity affected hormone levels, with higher glucocorticoids, testosterone, and estrogen in the hair obtained from infants of primiparous mothers. It seemed reason-

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able, therefore, to investigate whether a similar analysis of neonatal hair would permit one to detect the influence of maternal stress during pregnancy.

The majority of studies that have analyzed hair hormone levels, both in human and animal research, have measured only cortisol. We developed innovative mass spectrometry methods permitting the simultaneous determination of seven hormones (Kapoor et al., 2014). This refinement is significant for two reasons. First, it is often informative to assess cortisone in addition to cortisol when assessing hair (Ito et al., 2005; Keckeis et al., 2012). Second, a comprehensive panel is especially important when investigating prenatal stress, because it is known that other hormones synthesized by the adrenal and gonads can be affected (DiPietro et al., 2011; Kapoor and Matthews, 2008, 2011; Kim et al., 2015; Pepe and Albrecht, 1995; Sharp et al., 2014). For example, prenatal stress in some animal species has been shown to affect sexual differentiation by influencing the organizational actions of androgens and estrogens.

The stress condition in the present study involved acute manipulations of the gravid female on a daily basis for 20% of pregnancy. The disturbance was then stopped before the final weeks of pregnancy to determine if there were persistent effects on fetal endocrine activity. We employed a brief acoustical startle paradigm known to elevate maternal cortisol levels for several hours. Previous studies showed that prenatal manipulations had a number of persistent effects on infant physiology and brain development after birth (Coe et al., 2003). Its advantages are that: 1 it is reliable, eliciting a cortisol response in all pregnant females, 2 both the stressor and reaction are delimited; a moderate elevation in cortisol is induced by a brief 10-min event, and 3 it is temporally defined and finite, with the stressful period resolving immediately at the end of 5 weeks of daily manipulation. During the first week after delivery, hair samples were collected from the mother and the newborn infant to assay the hormone panel, which included glucocorticoids, androgens, estrogens, and progesterone.

2. Methods

2.1. Animals

Mother and infant rhesus monkeys (*Macaca mulatta*) were evaluated from a large, long-established breeding colony at the Harlow Primate Laboratory. All were descendants of monkeys originally imported from India, >14 generations earlier in the 1950s and 1960s (Price and Coe, 2000). The adult females were laboratory-reared, multiparous adults, 5.8–15.4 years of age. Each was bred with one male for 4–7 days to verify paternity and date of conception. Pregnancy was confirmed by implantation bleeding and the cessation of menstruation. Females were housed under standardized conditions in similar size, stainless steel cages until the birth of their infants (Coe and Shirtcliff, 2004). The monkeys were continuously in visual and auditory communication with other animals, either in pairs or partitioned with an open mesh panel from one other female. The diet is based on a commercial biscuit with known nutritional constituents (PMI International, 5LFD) plus fruits and vegetables provided as part of an enrichment husbandry program to give additional stimulation, along with foraging devices and other manipulanda. All 35 infants, 13 controls (4 female and 9 male) and 22 prenatally stressed (12 female, 10 male), were born naturally at term. To determine typical level of glucocorticoids in the hair of a non-pregnant female for comparison, hair specimens were obtained from 7 females and analyzed with the same assay methods. All experimental and husbandry procedures were approved by the Institutional Care and Animal Use Committee at UW-Madison.

2.2. Prenatal stress

The prenatal stress condition involved a controlled disturbance induced with an acoustical startle protocol (Coe et al., 2003; Shirtcliff et al., 2013). Briefly, 5 days a week for 5 weeks, or 20% of the 24-week pregnancy, the gravid female was relocated to a darkened room where a computer program randomly broadcast 3 loud noises (1 s, 110 dB) during a 10-min period. This protocol induces a transient activation of the maternal HPA axis (Clarke and Schneider, 1993; Rendina et al., in press). The stress induction occurred during one of three 5-week periods during pregnancy, either early (weeks 7–11, $n = 6$), mid (weeks 12–16, $n = 9$) or late in pregnancy (weeks 17–21, $n = 7$). For the current analyses, hair hormone data from the 3 periods are considered together because there was not a significant effect of gestational stage on either the dam or infant. The stress manipulations were stopped 3–13 weeks before the end of gestation, ensuring that hormone differences in infant hair reflected hormonal changes that continued after the gestational disturbance. Maternal age was similar between the females assigned to the Control and Stress conditions (11.38 ± 4.7 years and 12.95 ± 3.5 years, respectively). To verify that the disturbance protocol elicited HPA activation, small blood samples (1 mL) were obtained at 1, 3 and 5 weeks after the startle protocol. Blood samples were obtained from the Control females at the same time of day and same 2-week intervals in order to determine basal levels of cortisol and to control for the handling procedures required for the blood sampling. No sedation was employed, and samples were collected via saphenous venipuncture in a specially designed apparatus for blood collection. Cortisol levels were determined for 28 of the females from both Control and Stress conditions via radioimmunoassay (MP Biomedicals).

2.3. Hair collection

Hair specimens were collected 2–4 days after delivery. Adult female monkeys at the HPL are trained to leave their cages and transfer into a smaller apparatus for brief immobilization, where the infants could be manually removed for approximately 15 min between 0900 h and 1100 h. The mothers were not sedated for this procedure, nor were they sedated during pregnancy for the blood collection. Hair from the mother was shaved with commercial grooming clippers from the upper back region; infant hair was obtained from the posterior region of the neck, with some from the back of the head and the upper shoulders. The mother and infant were then reunited, and returned to their cage. Hair samples were placed into small aluminum foil pouches and stored at room temperature until ground and assayed.

2.4. Analytical method

Hair washing, steroid extraction and LC/MS/MS method were conducted as previously described (Kapoor et al., 2014). Briefly, hair was washed twice with 2-propanol and dried. Hair was ground to a fine powder using a Retsch ball mill (Verder Scientific, Newtown, PA) and carefully weighed into 50 mg aliquots in culture tubes. For steroid extraction, methanol, Sorenson buffer and internal standard mixture were added to the ground hair and the tubes were incubated at 30 °C for 16 h. After incubation, tubes were centrifuged and the supernatant was run through solid-phase extraction followed by liquid–liquid extraction with ethyl acetate. Samples were re-suspended in 40 μL of 20:80 methanol/water for liquid-chromatography/tandem mass spectrometry.

All samples were analyzed on a QTRAP 5500 quadrupole linear ion trap mass spectrometer (AB Sciex, Concord, ON, Canada) equipped with an atmospheric pressure chemical ionization source. The system included two Shimadzu LC20ADXR pumps and a