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THE HYPOTHALAMIC TRANSCRIPTIONAL RESPONSE TO STRESS IS SEVERELY IMPAIRED IN OFFSPRING EXPOSED TO ADVERSE NUTRITION DURING GESTATION

N. M. GRISSOM, R. GEORGE AND T. M. REYES *

University of Cincinnati, College of Medicine, Department of Psychiatry and Behavioral Neurosciences, 2120 East Galbraith Road, A-129, Cincinnati, OH 45237-1625, United States

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Abstract—Gestation is a time of profound vulnerability, as insults during pregnancy increase the lifelong risk of morbidity for the offspring. Increasingly, maternal diet is recognized as a key factor influencing the developing fetus. Poor-quality maternal diets, whether they provide an excess or an insufficiency of nutrients, lead to overt gestational growth disturbances in the offspring, and elevated risk for a common cluster of metabolic and mental disorders. Metabolic disturbances, particularly a substantially increased risk of obesity, have been linked in both maternal overnutrition and maternal undernutrition with abnormal development of the offspring hypothalamus, which serves a vital role in the central regulation of feeding. Additionally, the hypothalamus also coordinates physiological responses to stressors, and may thus play a role in vulnerability to psychiatric disease in these offspring. We examined hypothalamic molecular and endocrine responses to a psychological stressor (restraint) and a physiological stressor (lipopolysaccharide; LPS) in adult offspring from dams fed a high-fat diet or a low-protein diet during gestation and lactation. Targeted gene expression in the hypothalamus for 26 genes of interest sorted via hierarchical clustering revealed that the vast majority of these transcripts were substantially upregulated by both stressors. In contrast, offspring of maternal high-fat and low-protein diets mounted essentially no gene expression response to either stressor. However, male and female offspring of all conditions showed elevated hypothalamic-pituitary-adrenal glucocorticoid responses to both stressors, though the recovery of corticosterone responses after stress termination was significantly impaired in offspring of poor-quality maternal diets. Overall, it appears that the ability of the hypothalamus to respond in the immediate aftermath of stressful experiences is severely impaired in offspring of poor-quality maternal diets, regardless of whether the diet provided insufficient nutrients or excessive nutrients.

Key words: maternal diet, HPA, hypothalamus, transcription, gene expression, stress.

INTRODUCTION

Gestational insults to the developing fetus can increase the lifelong risk of morbidity, and a well-known factor influencing the quality of gestation is the diet and nutrient balance consumed by the mother. Poor-quality maternal diets, including those with an insufficiency of protein or an excess of fat can have a profound influence on the health and progression of a pregnancy and fetal development, increasing the rates of complications, preterm birth, and infants born small or large for gestational age (SGA or LGA, respectively, Grissom and Reyes, 2012a). Gestational size at birth is a biomarker of an at-risk pregnancy and as a result has been heavily studied epidemiologically. Significantly, both SGA and LGA are associated with similar disease profiles, with both conditions significantly increasing the risk of obesity and metabolic disorders in later life (Chiavaroli et al., 2009; Ornoy, 2011), as well as a cluster of psychiatric diseases, including neurodevelopmental disorders (autism, attention-deficit/hyperactivity disorder, and schizophrenia) and anxiety (Van Lieshout and Boyle, 2011; Grissom and Reyes, 2012a; Krakowiak et al., 2012; Moore et al., 2012). In an effort to identify mechanisms linking gestational growth disturbance to altered brain development, our lab examines mouse offspring exposed to a gestational low-protein diet and born small for gestational age (Vucetic et al., 2010c; Whitaker et al., 2011; Grissom et al., 2015), as well as offspring exposed to a gestational high-fat diet and born large for gestational age (Vucetic et al., 2010a; Grissom et al., 2015). Previously, we have identified altered gene expression related to dopamine, opioid, and epigenetic function in mesocorticolimbic structures, as well as numerous behavioral abnormalities (hyperactivity, altered response to cocaine and sucrose, executive function deficits) (Vucetic et al., 2010b, 2010c; Grissom and Reyes, 2012a; Grissom et al., 2015). Interestingly, when these effects have been directly compared, they have largely

*Corresponding author. Tel: +1-513-558-4338; fax: +1-513-297-0966.
E-mail address: reyesta@ucmail.uc.edu (T. M. Reyes).

Abbreviations: HDAC, histone deacetylase; HPA, hypothalamic–pituitary–adrenal; LGA, large for gestational age; LPS, lipopolysaccharide; SGA, small for gestational age; PVN, paraventricular nucleus; ZT, zeitgeiber time.

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been in opposing directions (Grissom and Reyes, 2012a; Grissom et al., 2015). These data suggest that developing mesocorticolimbic structures are particularly vulnerable to gestational insults, but do not fully explain the striking similarities in metabolic and psychiatric profiles of offspring affected by very different insults.

In the present manuscript, we focus on the hypothalamus, a brain structure that is critical in maintaining homeostasis, and whose dysfunction has been linked to both obesity, as well as neuropsychiatric disorders. As noted before, the risk of obesity faced by offspring born either too small or too large is mediated in part by altered development of energy balance circuits within the hypothalamus, including the paraventricular nucleus (PVN) (Chang et al., 2008; Page et al., 2009; Giraudo et al., 2010; Gout et al., 2010; Carmody et al., 2011; Stevens et al., 2011). Additionally, the PVN is also known to regulate circulating glucocorticoid release via the hypothalamic-pituitary-adrenal (HPA) axis, an essential system for regulating responses to stressful and anxiety-provoking environments (Herman et al., 2002; Grissom and Bhatnagar, 2009). Disruptions in HPA activity, both basally and in response to stressful events, have been linked with both mood and neurodevelopmental disorders (Laviola et al., 2002; Young et al., 2004; Thomson and Craighead, 2008; Freitag et al., 2009; Isaksson et al., 2012, 2013). Thus, the hypothalamus is poised to regulate not only energy balance but also emotional states. Fittingly, gestational growth abnormalities and poor-quality maternal diets have previously been linked with elevated HPA tone (Lesage et al., 2001; D'Asti et al., 2010; Li et al., 2013) and dysregulated stress responses (Vieau et al., 2007; Naef et al., 2013; Sasaki et al., 2013; Zhang et al., 2013; Nemoto et al., 2014) However, it is not known whether these problems with endocrine stress response reflect dysregulated stress-induced transcription in the hypothalamus (Reyes et al., 2003). In the following experiments, we examine whether the stress-responsive functions of the hypothalamus, including both gene expression within the region as well as HPA activity, are impacted by poor-quality maternal diets, and whether the response pattern dissociates by the type of acute stressor (physiological vs. psychological) (Herman et al., 2003) or maternal diet. We find that both a maternal low-protein and a maternal high-fat diet during gestation lead to remarkably similar disruptions in molecular and endocrine stress responses driven by both kinds of stress, indicative of a common mechanism by which very different maternal insults can lead to a similar risk of offspring morbidity.

EXPERIMENTAL PROCEDURES

Animals and stress paradigms

All animals were cared for according to the guidelines of the University of Pennsylvania Institutional Animal Care and Use Committee. The offspring used in behavioral testing were (C57BL/6 J X DBA/2 J) F1 mice. Maternal diets were given at the start of pregnancy as previously published (Fig. 1a; see Vucetic et al., 2010a, 2010b; Whitaker et al., 2011; Grissom and Reyes, 2012a; Grissom et al., 2015 for information on maternal and offspring phenotypes). All diets were from TestDiet, Richmond, IN, USA; control: Test Diet 5755, 4.09 kcal/g with 18% of total energy calories from protein, 22% from fat, and 60% from carbohydrate; low protein: Test Diet 5769, 4.13 kcal/g with 8.5% of total energy calories from protein, 22% from fat, and 69.5% from carbohydrate; high fat: Test Diet 58G9, 5.21 kcal/g with 18% of total energy calories from protein, 60% from fat, and 22% from carbohydrate). Diets were continued through lactation, as this period covers rodent brain development on par with the human third trimester. On p21, the offspring were weaned onto *ad libitum* standard chow (Lab Diet 5001, St. Louis, MO, USA), and housed in groups of 5. Only one animal per litter was used in any specific experiment. Experimentation commenced in young adulthood, when animals were between 12 and 20 weeks of age. All animals were housed in a 12:12 L:D cycle (Fig. 1b).

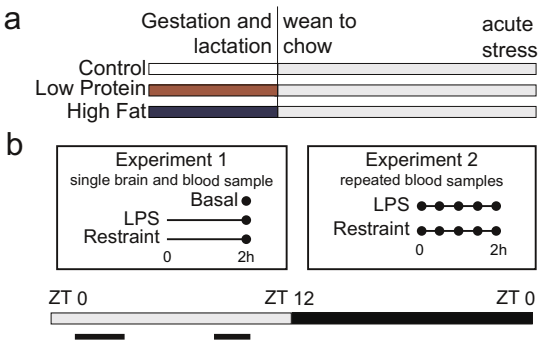

Fig. 1. Experimental design. (a) All animals used in these experiments were bred from dams which began one of three maternal diets at the start of pregnancy (see Experimental Procedures 2.1 for details) – a control diet, a low-protein diet, or a high-fat diet. All diets were maintained until weaning, at which time all offspring transitioned to colony chow and were allowed to develop undisturbed into adulthood. Animals were adults when tested under acute stress. (b) Design of the two stress experiments. In Experiment 1, brains and blood samples were collected from offspring of each of the three maternal diets 2 h after the start of either the physiological stressor LPS, or the psychological stressor restraint. These samples were collected late in the light period (zeitgeiber time 7–9), during the mounting glucocorticoid levels in anticipation of waking at the onset of the dark period at ZT 12. In Experiment 2, repeated blood samples were collected from the tail of animals after a single injection of LPS or during and after a 15-minute restraint. These samples were collected earlier in the light period (ZT 2–5), when basal glucocorticoid secretion is at its trough.

Stress and sample collection

Experiment 1: Gene expression and single-timepoint corticosterone. For the first experiment designed to examine gene expression and corticosterone levels, animals were sacrificed either under basal conditions or 2 h after the onset of either a physiological stressor (lipopolysaccharide (LPS) administration, IP), or a psychological stressor (15 min restraint) (Li et al., 1996) (Fig. 1b). Samples were collected between zeitgeiber time (ZT) 7 and 9 (several hours before lights off, Fig. 1b)