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MATERNAL HIGH-FAT DIET ALTERS ANXIETY BEHAVIOR AND GLUCOCORTICOID SIGNALING IN ADOLESCENT OFFSPRING

A. SASAKI, W. DE VEGA, S. SIVANATHAN, S. ST-CYR AND P. O. MCGOWAN*

Centre for Environmental Epigenetics and Development, Department of Biological Sciences, University of Toronto, Room SW548, Scarborough Campus, 1265 Military Trail, Toronto, ON M1C 1A4, Canada

Department of Cell and Systems Biology, University of Toronto, 1265 Military Trail, Toronto, ON M1C 1A4, Canada

Abstract—Maternal obesity and overconsumption of saturated fats during pregnancy have profound effects on offspring health, ranging from metabolic to behavioral disorders in later life. The influence of high-fat diet (HFD) exposure on the development of brain regions implicated in anxiety behavior is not well understood. We previously found that maternal HFD exposure is associated with an increase in anxiety behavior and alterations in the expression of several genes involved in inflammation via the glucocorticoid signaling pathway in adult rat offspring. During adolescence, the maturation of feedback systems mediating corticosteroid sensitivity is incomplete, and therefore distinct from adulthood. In this study, we examined the influence of maternal HFD on several measures of anxiety behavior and gene expression in adolescent offspring. We examined the expression of corticosteroid receptors and related inflammatory processes, as corticosteroid receptors are known to regulate circulating corticosterone levels during basal and stress conditions in addition to influencing inflammatory processes in the hippocampus and amygdala. We found that adolescent animals perinatally exposed to HFD generally showed decreased anxiety behavior accompanied by a selective alteration in the expression of the glucocorticoid receptor and several downstream inflammatory genes in the hippocampus and amygdala. These data suggest that adolescence constitutes an additional period when the effects of developmental programming may modify mental health trajectories.

*Correspondence to: P. McGowan, Department of Biological Sciences, University of Toronto, Room SW548, 1265 Military Trail, Toronto, ON M1C 1A4, Canada. Tel: +1-416-208-5153; fax: +1-416-287-7676.

E-mail address: patrick.mcgowan@utoronto.ca (P. O. McGowan).

Abbreviations: ANOVA, analysis of variance; CD11b, cluster of differentiation molecule 11B; CHD, control house chow diet; GR, glucocorticoid receptor; HFD, high-fat diet; HPA, hypothalamic–pituitary–adrenal; IkBa, I-kappa-B-alpha; IL-1Ra, interleukin-1 receptor antagonist; IL-6, interleukin-6; MKP-1, mitogen-activated protein kinase phosphatase-1; MR, mineralocorticoid receptor; NFkB, nuclear factor kappa beta; PD, postnatal day; qRT-PCR, quantitative reverse transcriptase-polymerase chain reaction.

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Key words: glucocorticoid receptor, inflammatory, anxiety behavior, maternal, obesity, gene expression programming.

INTRODUCTION

Epidemiological studies suggest that maternal obesity and overconsumption of saturated fats during pregnancy have profound effects on offspring health, ranging from metabolic to behavioral disorders in later life (Godfrey et al., 2010). High-fat exposure during development has been linked with adverse health outcomes such as diabetes and coronary heart disease (Marx, 2002; Kahn et al., 2006; Van Gaal et al., 2006; Bersamin et al., 2008). Obesity has also been associated with an increased risk of developing behavioral disorders related to anxiety in humans (Boksa, 2004; Desai et al., 2009; Rofey et al., 2009; Peleg-Raibstein et al., 2012).

The influence of maternal high-fat diet (HFD) exposure on the development of brain regions implicated in anxiety behavior is not well understood. Non-human primates show increased fear response when faced with novelty after being developmentally exposed to HFD (Sullivan et al., 2010). Rodent studies have observed increased anxiety behavior in adult offspring perinatally exposed to HFD, accompanied by increased inflammation (Bilbo and Tsang, 2010; Peleg-Raibstein et al., 2012; Sasaki et al., 2013). In humans, maternal obesity predicts child obesity, and child obesity is associated with inflammation at an early age and with anxiety disorders (Weiss et al., 2004; Whitaker, 2004; Boney et al., 2005; Desai et al., 2009; Rofey et al., 2009).

Environmental factors during development have been shown to affect the long-term activity of the hypothalamic–pituitary–adrenal (HPA) axis, a primary mediator of the response to stress. Studies examining maternal care in early postnatal life and prenatal maternal stress have found long-term effects on offspring anxiety behavior and on the expression of HPA axis-associated genes in the brain (Meaney, 2001; Welberg and Seckl, 2001; McGowan et al., 2008; Brunton, 2010). These factors modify offspring HPA axis, in part, through changes in corticosteroid receptors in limbic regions such as the hippocampus and amygdala, which regulate circulating corticosterone levels during basal and stress conditions (Vazquez, 1998; Welberg and Seckl, 2001; Brunton, 2010). In addition to its role in inhibiting the stress response, as a transcription factor, the glucocorticoid receptor (GR) can influence downstream inflammatory processes, which are also affected

by altered HPA activity (Smoak and Cidlowski, 2004; Sorrells et al., 2009). Glucocorticoids have both pro- and anti-inflammatory roles in the brain (Sorrells et al., 2009). The expression of the inflammatory genes nuclear factor kappa beta (NFkB), interleukin-6 (IL-6), and cluster of differentiation molecule 11B (CD11b), has been previously linked to chronic alterations in glucocorticoid signaling (Sorrells et al., 2009) and maternal HFD exposure (Bilbo and Tsang, 2010; Sasaki et al., 2013). Likewise, the anti-inflammatory genes I-kappa-B-alpha (IkBa), mitogen-activated protein kinase phosphatase-1 (MKP-1), and interleukin-1 receptor antagonist (IL-1Ra) are known negative regulators of inflammatory response that are modified through chronic changes in GR, NFkB, and IL-6 signaling as well as in the context of maternal HFD exposure (Sorrells et al., 2009; Sasaki et al., 2013).

We previously examined the effects of perinatal HFD exposure on adult (postnatal day (PD) 90) anxiety behavior (Sasaki et al., 2013). Rats perinatally exposed to HFD exhibited an increase in anxiety behavior in the Open Field, Light–dark transition, and Elevated Plus Maze tasks. Females exposed to HFD appeared particularly vulnerable, as they showed an increase in the expression of corticosteroid receptors in the amygdala in association with increased anxiety. Male and female adult offspring showed alterations in the expression of several genes involved in inflammation via the glucocorticoid signaling pathway. It is known that the developing adolescent brain differs from the adult brain in terms of structure and function (Vazquez, 1998; Spear, 2000). Anxiety behavior and responses to stress in adolescent animals are distinct from those in adult animals, likely reflecting incomplete maturation of feedback systems mediating corticosteroid sensitivity (McCormick et al., 2008). To our knowledge, the influence of maternal HFD on adolescent anxiety behavior and related gene expression has not been examined.

In this study, we examined the effects of perinatal HFD exposure on adolescent (PD35) offspring anxiety behavior. Corticosteroid receptor and downstream inflammatory pathway genes were examined in the hippocampus and amygdala, as these two components of the limbic system are implicated in anxiety behavior and interact with the HPA axis to mediate the response to psychosocial stress.

EXPERIMENTAL PROCEDURES

Animals

Adult male and female Long Evans rats (7 weeks old) were obtained from Charles River Canada (St. Constant, QC). Rats were housed in same-sex pairs, maintained on a 12:12-h light–dark cycle (lights on from 7:00 AM to 7:00 PM), and had *ad libitum* access to food and water. All experimental protocols were approved by the Local Animal Care Committee at the University of Toronto Scarborough and were in accordance with the guidelines of the Canadian Council on Animal Care.

Diets

Female breeders were given access to either a HFD ($n = 10$) or control house chow diet (CHD, $n = 10$). The HFD (5.24-kcal/g) was obtained from Research Diets, Inc. (New Brunswick, NJ, USA: cat. No. D12492) and contained (by kcal): 60% fat, 20% protein, and 20% carbohydrate. The CHD (3.02 kcal/g) was obtained from Purina Lab Diets (St. Louis, MO, USA: cat. No. 5001) and contained (by kcal): 13.5% fat, 28.5% protein, and 58% carbohydrate. A comparison between similar formulations of HFD and CHD has been used to examine diet-induced obesity in several previous studies (e.g., (El-Haschimi et al., 2000; De Souza et al., 2005; Dunn and Bale, 2009; Tamashiro et al., 2009; Purcell et al., 2011; Sasaki et al., 2013). Dams remained on their respective diets for 4 weeks prior to mating and throughout pregnancy and lactation. Upon weaning at PD21, all offspring were given *ad libitum* access to CHD.

Subjects and general procedures

After mating, female breeders were housed individually. There were no significant differences in litter size or sex ratio among the diet groups. Offspring remained undisturbed until PD21 when they were housed in same-sex pairs. Body weights were measured at the start of the behavioral assays during mid-adolescence between PD35 and PD45 (Tirelli et al., 2003). A subset of male and female offspring (1–2 offspring/sex/litter) was used for behavioral assays. The offspring were run in squads, resulting in the following total numbers of offspring tested on the Light–Dark and Elevated Plus Maze tasks (HFD females $n = 19$; HFD males $n = 13$; CHD females $n = 19$; CHD males $n = 19$). Only the second squad was run on the Open Field task (HFD females $n = 13$; HFD males $n = 7$; CHD females $n = 13$; CHD males $n = 13$). Brains from $n = 6$ offspring per sex and per diet group were collected for gene expression analysis after completion of behavioral testing (~PD45). The order of behavioral testing for each sex and diet condition was counterbalanced within each task. All behavioral tasks were run in a dimly lit room (33.7 lux) that was illuminated through a single light bulb placed over the apparatus. After each behavioral test, the apparatus was cleaned using a 70% ethanol solution and allowed to air dry to remove or homogenize odorants. All behavioral testing and sacrifices occurred at the mid-point of the light phase of the circadian cycle (11 AM–3 PM) to control for potential confounding circadian effects.

Light–dark transition

The Light–dark transition task consisted of an opaque white Plexiglas box (light zone) connected to an opaque black box (dark zone) through a small (12×12 cm) opening to allow passage between the chambers. Both boxes were 30×30 cm. The rat was placed in the dark box at the beginning of each trial and allowed to explore the boxes for a period of 5 min. The task measured the duration and frequency of entries within the light zone