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PRENATAL FAT-RICH DIET EXPOSURE ALTERS RESPONSES OF EMBRYONIC NEURONS TO THE CHEMOKINE, CCL2, IN THE HYPOTHALAMUS

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Abstract—Maternal consumption of a high-fat diet (HFD) during pregnancy is found to stimulate the genesis of hypothalamic orexigenic peptide neurons in the offspring, while HFD intake in adult animals produces a systemic low-grade inflammation which increases neuroimmune factors that may affect neurogenesis and neuronal migration. Building on this evidence and our recent study showing that the inflammatory chemokine, CCL2, stimulates the migration of hypothalamic neurons and expression of orexigenic neuropeptides, we tested here the possibility that prenatal exposure to a HFD in rats affects this chemokine system, both CCL2 and its receptors, CCR2 and CCR4, and alters its actions on hypothalamic neurons, specifically those expressing the neuropeptides, enkephalin (ENK) and galanin (GAL). Using primary dissociated hypothalamic neurons extracted from embryos on embryonic day 19, we found that prenatal HFD exposure compared to chow control actually reduces the expression of CCL2 in these hypothalamic neurons, while increasing CCR2 and CCR4 expression, and also reduces the sensitivity of hypothalamic neurons to CCL2. The HFD abolished the dose-dependent, stimulatory effect of CCL2 on the number of migrated neurons and even shifted its normal stimulatory effect on migrational velocity and distance traveled by control neurons to an inhibition of migration. Further, it abolished the dose-dependent, stimulatory effect of CCL2 on neuronal expression of ENK and GAL. These results demonstrate that prenatal HFD exposure greatly disturbs the functioning of the CCL2 chemokine system in embryonic hypothalamic neurons, reducing its endogenous levels and ability to promote the migration of neurons and their expression of orexigenic peptides.

Key words: prenatal high-fat diet, hypothalamus, C–C chemokine ligand 2 (CCL2), enkephalin, galanin, migration.

INTRODUCTION

The ingestion of a diet rich in fat is known to increase caloric intake that promotes obesity (Dourmashkin et al., 2006). This effect can be attributed to changes in the hypothalamus, a region of the brain that is important in controlling consumption and energy homeostasis. Two highly expressed neuropeptides, enkephalin (ENK) and galanin (GAL), are found to be stimulated by the ingestion of a high-fat diet (HFD) (Akabayashi et al., 1994; Chang et al., 2007) and are believed to contribute to the overconsumption of this diet, with central injection of these peptides found to preferentially stimulate the consumption dietary fat (Kyrkouli et al., 1990; Naleid et al., 2007). This effect of a HFD in adult animals is similarly evident with prenatal exposure to this diet, which predisposes the offspring to overconsuming dietary fat (Chang et al., 2008) and increases fat mass and body weight (Reynolds et al., 2014). These offspring and embryos exhibited increased expression and number of ENK and GAL neurons in the hypothalamus (Chang et al., 2008). While the molecular mechanisms involved in these changes are not known, recent studies suggest that neuroinflammatory systems may play a role.

The ingestion of a HFD and dietary obesity result in a state of systemic low-grade inflammation (Milanski et al., 2009; Takanabe-Mori et al., 2010). This state is characterized by an increase in the production of immune factors, such as the cytokines, interleukin 1-beta and tumor necrosis factor-alpha, and a superfamily of small chemotactic cytokines, also known as chemokines (Barbarroja et al., 2010; Gregor and Hotamisligil, 2011). Exposure to this diet during the prenatal period additionally increases placental inflammation (Reynolds et al., 2015). Neuroinflammatory mediators, in addition to recruiting immune cells to fight infection, have more recently been shown to affect neuronal function and development. In particular, the chemokine, C–C chemokine ligand 2 (CCL2), also called monocyte chemotactic protein-1 (MCP-1), along with its receptor, C–C chemokine receptor type 2 (CCR2) (Craig and Loberg, 2006), has been singled out as an important regulator of neuronal function and development (Banisadr et al., 2005; Edman et al., 2008; Chintawar et al., 2009; Poon et al., 2014). This chemokine, classically known to attract monocytes (Yoshimura et al., 1989; Rollins, 1996) and stimulate migration of these cells during an immune response (Lu et al., 1998), is increased in the brain, uterus and

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Abbreviations: CCL2, C–C chemokine ligand 2; CCR2, C–C chemokine receptor type 2; ENK, enkephalin; GAL, galanin; HFD, high-fat diet.

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placenta during pregnancy (Meng et al., 1999; He et al., 2007), and its receptors that bind CCL2, both CCR2 and the less studied CCR4, are also expressed in the brain (Banisadr et al., 2002, 2005) and found in hippocampal neurons (Meucci et al., 1998). In the hypothalamus, CCL2 is shown to co-localize with the neuropeptides, melanin-concentrating hormone and arginine vasopressin (Banisadr et al., 2005). This chemokine also modulates the activity of neurons in the brain (Gosselin et al., 2005; Guyon et al., 2009a,b) and has been linked to the migration of neurons into damaged brain areas (Liu et al., 2007; Deng et al., 2008). In addition, our recent *in vitro* study of hypothalamic neurons cultured from embryonic day 19 (E19) embryos from dams consuming a low-fat chow diet showed that CCR2 co-expresses with ENK and that the colocalization of this chemokine receptor and orexigenic peptide is increased in embryos exposed to a HFD (Poon et al., 2014). Additionally, in normal chow-exposed neurons, CCL2 produced a dose-dependent, stimulatory effect on the migration of neurons and on mRNA expression of ENK and GAL in the hypothalamus (Poon et al., 2014). While reducing or knocking out CCL2 in adult mice is shown to trigger symptoms of increased ingestion and obesity (Inouye et al., 2007), there is other evidence showing that pharmacological blockade of the CCR2 receptor has the opposite effect of improving symptoms of obesity (Rull et al., 2010). These mixed findings leave unclear as to the specific functions of this chemokine system in dietary obesity and its specific relation to the orexigenic peptide neurons in the embryo when exposed to a fat-rich diet.

Thus, the objectives of the present study were to investigate whether prenatal exposure to a HFD affects the CCL2/CCR2 system and its normal functioning in relation to the expression of orexigenic peptides and the migratory behavior of neurons in the hypothalamus. To examine this after removing the endogenous CCL2 stimuli, we extracted and cultured hypothalamic neurons from embryos that were prenatally exposed to a HFD as compared to a low-fat chow diet and determined first whether this HFD exposure affects the expression of CCL2, CCR2, and CCR4 in the hypothalamus and then whether this diet alters the actions of exogenous CCL2 on the migratory behavior of these hypothalamic neurons and on the gene expression of the orexigenic peptides, ENK and GAL.

EXPERIMENTAL PROCEDURES

Animals

Timed-pregnant Sprague–Dawley rats were acquired from Charles River Laboratories (Hartford, CT, USA) on embryonic day 5 (E5). All experimental procedures were performed according to institutionally approved protocols as specified in the National Institutes of Health Guide for the Care and Use of Laboratory Animals and also with approval of the Rockefeller University Animal Care and Use Committee. The dams were individually housed in a fully accredited AAALAC facility (22 °C, with a 12:12-h light–dark cycle with lights off at 12 pm).The rats were split into two groups of eight dams each and maintained *ad libitum* from E7 on either a high-fat diet (HFD; 5.02 kcal/g) with 50% fat or a standard lab chow (3.36 kcal/g) with 13% fat (Purina, St. Louis, MO, USA), as previously described (Dourmashkin et al., 2006; Chang et al., 2008; Poon et al., 2012). In the HFD group, standard lab chow was available for the first 3 days (until E9) before being completely removed, in order for the HFD group to overcome neophobia and adapt to the HFD. Over the course of the experiments, food intake was measured three times per week, and body weight was recorded weekly. There was no difference between the HFD and chow dams in their daily caloric intake during pregnancy (70–90 kcal). Dams were sacrificed on embryonic day 19 (E19), as previously described (Poon et al., 2012). The whole hypothalamus was extracted and dissociated for plating into cell culture, as previously described (Poon et al., 2012, 2013), or placed in either RNA later for mRNA purification. Whole hypothalamus was used since individual regions of the hypothalamus are not fully differentiated at this age.

Diet

The standard lab chow diet (3.36 kcal/g) consisted of 13% fat and was acquired from Purina (St. Louis, MO, USA), and the HFD used in this report has been described in detail in previous publications (Dourmashkin et al., 2006; Chang et al., 2008; Poon et al., 2012). Briefly, the HFD consisted of 50% fat composed of 75% lard (Armour Star, Peoria, IL, USA) and 25% vegetable oil (Crisco, Orrville, OH, USA), of 25% carbohydrate composed of 30% dextrin (ICN Pharmaceuticals, Costa Mesa, CA, USA), 30% cornstarch (ICN Pharmaceuticals, Costa Mesa, CA), and 40% sucrose (Domino Foods Inc., Yonkers, NY, USA), and of 25% protein from casein (Bio-Serv, Frenchtown, NJ, USA), and it was supplemented with minerals (USP XIV Salt Mixture Briggs; ICN Pharmaceuticals, Costa Mesa, CA) and vitamins (Vitamin Diet Fortification Mixture; ICN Pharmaceuticals, Costa Mesa, CA). This diet is nutritionally complete and is found to have no detrimental effects on the health of the animals.

Cell culture

Hypothalami from E19 embryos were micro-dissected and dissociated, as previously described (Poon et al., 2012, 2013). The cells (1 million/mL) were resuspended in Neurobasal Media containing B27 supplement (Life Technologies, Grand Island, NY, USA) and cultured in either a 6-well plate (BD Biosciences, Sparks, MD, USA), in a cell culture insert (BD Biosciences, Sparks, MD), or on a glass bottom dish (Greiner Bio-One, Monroe, NC, USA). As previously measured (Poon et al., 2014), $>95\%$ of the cells were found to be positive for NeuN, a neuronal marker, with the astrocyte marker, GFAP, undetectable. Neurons were then treated with 0, 50, 100, or 200 ng/mL of CCL2, concentrations that are within the EC_{50} range (Matsushima et al., 1989; Carr et al., 1994; Kao et al., 2012), as previously described (Poon et al., 2014). For each experiment, the hypothalami of embryos were taken from 8 dams consuming either the low-fat chow diet ($n = 4$) or the HFD ($n = 4$), and their