Neuroscience 162 (2009) 924–932

EARLY LIFE EXPOSURE TO A HIGH FAT DIET PROMOTES LONG-TERM CHANGES IN DIETARY PREFERENCES AND CENTRAL REWARD SIGNALING

S. L. TEEGARDEN,[1] A. N. SCOTT AND T. L. BALE*

Department of Animal Biology, School of Veterinary Medicine, 201E Vet, 6046, University of Pennsylvania, 3800 Spruce Street, Philadelphia, PA 19104-6046, USA

Abstract—Overweight and obesity in the United States continues to grow at epidemic rates in large part due to the overconsumption of calorically-dense palatable foods. Identification of factors influencing long-term macronutrient preferences may elucidate points of prevention and behavioral modification. In our current study, we examined the adult macronutrient preferences of mice acutely exposed to a high fat diet during the third postnatal week. We hypothesized that the consumption of a high fat diet during early life would alter the programming of central pathways important in adult dietary preferences. As adults, the early-exposed mice displayed a significant preference for a diet high in fat compared to controls. This effect was not due to diet familiarity as mice exposed to a novel high carbohydrate diet during this same early period failed to show differences in macronutrient preferences as adults. The increased intake of high fat diet in early exposed mice was specific to dietary preferences as no changes were detected for total caloric intake or caloric efficiency. Mechanistically, mice exposed to a high fat diet during early life exhibited significant alterations in biochemical markers of dopamine signaling in the nucleus accumbens, including changes in levels of phospho–dopamine and cyclic AMP-regulated phosphoprotein, molecular weight 32 kDa (DARPP-32) threonine-75, ΔFosB, and cyclin-dependent kinase 5. These results support our hypothesis that even brief early life exposure to calorically-dense palatable diets alters long-term programming of central mechanisms important in dietary preferences and reward. These changes may underlie the passive overconsumption of high fat foods contributing to the increasing body mass in the western world.

Key words: dopamine, striatum, macronutrient, development.

The obesity epidemic in the United States continues to grow, with recent statistics indicating that over 60% of American adults are currently overweight or obese (Ogden et al., 2006). Another, equally important trend is the increasing rate of childhood obesity (Ogden et al., 2002). Children in western societies, in addition to an increased sedentary lifestyle, are exposed to a wide variety of foods high in fat and calories that contribute to the development of obesity. Obese children are more likely to become obese adults, perhaps in part because of the persistence of habits and programming of dietary preferences developed during childhood (Serdula et al., 1993).

Studies have shown that exposure to certain taste stimuli during infancy and early childhood can alter dietary preferences in children years later (Johnson et al., 1991; Kern et al., 1993; Liem and Mennella, 2002; Mennella and Beauchamp, 2002). However, the mechanisms whereby such long-term effects occur have not been elucidated. Therefore, we examined the effects of early life exposure to a high fat diet on adult macronutrient preferences in mice. Mice were exposed to a high fat diet for 1 week, from postnatal days 21–28 (P21-28), the time during which they begin to consume solid food and are no longer dependent on the dam for nutrition. At weaning, mice were returned to standard house chow and examined for macronutrient choice preference and caloric intake on a chronic high fat diet as adults. Based on previous studies showing an effect of palatable diets on brain reward centers and changes in dopamine (DA) signaling (Teegarden and Bale, 2007; Teegarden et al., 2008), we also examined biochemical markers in the ventral striatum of these mice. We hypothesized that exposure to and withdrawal from a high fat diet during early life would lead to an increased preference for diets high in fat in adulthood via changes in reward circuitry that promote intake of energy-dense, palatable food.

EXPERIMENTAL PROCEDURES

Animals and early diet exposure

Mice were generated on a mixed C57Bl/6:129 background as part of our in-house breeding colony. These mice have been on a mixed background population for more than 10 years (Bale et al., 2000), with introduction of a new gene pool every two years by breeding with an F1 C57Bl/6: 129 cross. At 3 weeks of age, litters were exposed to the high fat diet (Research Diets, New Brunswick, NJ, USA) for 1 week. The high fat diet contained 4.73 kcal/g and consisted of 44.9% fat, 35.1% carbohydrate, and 20% protein. Control litters remained on standard house chow (Purina Laboratory Diet, St. Louis, MO, USA). House chow contained 4.00 kcal/g and consisted of 12% fat, 60% carbohydrate, and 28% protein. This time period for diet exposure was selected as by 3 weeks of age, offspring are consuming solid food and are not dependent on the mother for nutrition. After weaning, all mice (n=16 control, 14 early high fat exposed) were maintained on house chow until 3 months of age. All studies were conducted according to protocols approved by the University of Pennsylvania Institutional Animal Care and Use Committee, and all procedures were performed in

[1] Current address: Department of Psychiatry, School of Medicine, 2401 TRL, University of Pennsylvania, 125 S. 31st Street, Philadelphia, PA 19104, USA.
*Corresponding author. Tel: +1-215-898-1369; fax: +1-215-573-5187.
E-mail address: tbale@vet.upenn.edu (T. L. Bale).
Abbreviations: Cdk5, cyclin-dependent kinase 5; DA, dopamine; DARPP-32, dopamine and cyclic AMP-regulated phosphoprotein, molecular weight 32 kDa; E, embryonic day; P, postnatal day; Thr, threonine.

0306-4522/09 $ - see front matter
doi:10.1016/j.neuroscience.2009.05.029

accordance with institutional guidelines. The authors further certify that the studies were carried out in accordance with the National Institute for Health Guide for the Care and Use of Laboratory Animals and that all efforts were made to minimize the number of animals used and their suffering.

Macronutrient choice preference

In order to examine how early exposure to a macronutrient-enriched diet would affect adult food preferences, 3 month-old mice were examined for macronutrient choice preference over 10 days. Mice were allowed to habituate to individual housing for 1 week prior to choice preference. Pre-weighed pellets of high fat, high carbohydrate, and high protein diets (Research Diets, New Brunswick, NJ, USA) were placed on the floor of the cage. Mice and food pellets were weighed daily. High carbohydrate diet contained 3.85 kcal/g consisting of 10% fat, 70% carbohydrate and 20% protein. High protein diet contained 4.29 kcal/g and consisted of 29.5% fat, 30.5% carbohydrate, and 40% protein. The high fat diet used was identical to that used for the early exposure.

In order to control for the effects of diet familiarity on macronutrient preferences, we also examined separate litters exposed to the high carbohydrate diet (Research Diets, as described above), again from 3 to 4 weeks of age and tested for macronutrient choice preference as adults ($n=6$).

Adult chronic high fat diet exposure

Following macronutrient choice preference, a subset of mice ($n=7$ control, $n=9$ early high fat exposure) were exposed to the high fat diet alone for 15 weeks in order to examine the consumption and effects of chronic high fat diet and the possible development of obesity in mice that had been exposed to this diet during early life. Mice were weighed weekly during this period, and 24-h food intake was measured during a 1 week period following 6 weeks of chronic exposure. At the end of the chronic high fat diet period, mice were sacrificed by decapitation following brief isoflurane anesthesia, and adipose tissue, plasma, and brains were collected for analysis.

Adiposity and plasma leptin

At sacrifice, mice were weighed and brown adipose tissue and reproductive and renal white adipose tissue depots were removed and also weighed. Trunk blood was collected in tubes containing 50 mM EDTA and centrifuged for 10 min at 5000 rpm and 4 °C to separate plasma. Plasma was stored at −80 °C until assayed. Leptin levels were determined by radioimmune assay (Linco Research, St. Charles, MO, USA). Fifty microliters of plasma was used per sample, and all samples were run in duplicate. The sensitivity of the assay was 0.2 ng/ml, and the intra- and interassay coefficients of variance were 7.2% and 7.9% respectively.

Biochemical analyses

At sacrifice, the brain was rapidly removed, the ventral striatum (approximately 0.5–1.75 mm from bregma, at a depth of 3.5–5.5 mm) was dissected (Teegarden and Bale, 2007), and the tissue immediately frozen in liquid nitrogen. Western blots ($n=4$ control, $n=5$ early high fat exposure) were performed as previously described using a phosphatase inhibitor cocktail (P2850; Sigma, St. Louis, MO, USA) to preserve phosphorylation state (Bale et al., 2003; Teegarden and Bale, 2007). Antibodies used were FosB (1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA), cyclin-dependent kinase 5 (Cdk5) (1:500; Santa Cruz Biotechnology), phospho–dopamine and cyclic AMP-regulated phosphoprotein, molecular weight 32 kDa (DARPP-32) threonine (Thr) 75 (1:200; Cell Signaling Technology, Danvers, MA, USA), phospho-DARPP-32 Thr 34 (1:500; PhosphoSolutions, Aurora, CO, USA), total DARPP-32 (1:500; R&D Systems, Minneapolis, MN, USA), and mu opioid receptor (1:500; Abcam, Cambridge, MA, USA). ΔFosB was distinguished from full-length FosB by weight (Nestler et al., 2001). All blots were stripped and reprobed for β-actin for normalization (1:1000; Sigma, St. Louis, MO, USA). Blots were analyzed using IPLab software (Teegarden and Bale, 2007). Optical density values for target proteins were divided by values for β-actin within each sample to correct for loading error.

Statistics

All data were analyzed using a Student's *t*-test with early diet treatment as the independent variable. All data are presented as the mean±SEM.

RESULTS

Macronutrient choice preference

In order to determine how early diet exposure affected adult dietary preferences, mice exposed to a high fat diet from 3 to 4 weeks of age were examined for macronutrient choice preference for 10 days beginning at 3 months of age. Preference for the high fat diet (reported as the percent of total calories consumed as high fat diet; Fig. 1A) was significantly greater in mice that had been exposed to the high fat diet during early life ($P<0.05$). Preference for the high protein diet was not significantly altered by early diet exposure ($P=0.17$). Mice previously exposed to the high fat diet consumed significantly less of the high carbohydrate diet than controls ($P<0.05$). Average daily caloric intake between control and early high fat exposed mice was not different (Fig. 1B). When daily intake was expressed as grams of food consumed, there were again no significant differences between groups (control=3.29±0.13 g/day, early high fat exposed=3.15±0.14 g/day).

Average body weights were not significantly different between treatment groups before or after macronutrient choice preference (Fig. 1C). Caloric efficiency was calculated as weight gained (g)/calories consumed (kcal) over the course of the experiment. There was no difference in caloric efficiency between groups while on macronutrient choice preference (Fig. 1D). This suggests that while early exposure to a high fat diet increases adult preference for a high fat diet, it does not lead to changes in overall caloric intake or efficiency.

In order to control for effects of diet familiarity on long-term diet preference, a separate cohort of mice received the high carbohydrate diet from 3 to 4 weeks of age. These mice showed no changes in macronutrient preferences for high carbohydrate or high fat diets relative to controls (Fig. 1E), supporting the powerful effect specific to a high fat diet on brain systems governing food preferences.

Chronic high fat diet

Mice were exposed to a chronic high fat diet and food intake, body weight, adiposity and plasma leptin levels were measured. There were no significant differences in average daily food intake, final body weight, or caloric efficiency during high fat diet exposure (Fig. 2A–C). There were no differences in the relative amounts of body fat between groups after 3 months on high fat diet (Fig. 2D). Further, there were no differences between groups in plasma leptin levels following chronic high fat diet (Fig. 2E).