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Hypothalamic orexigenic peptides are overexpressed in young Long–Evans rats after early life exposure to fat-rich diets

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Abstract

Nutritional factors have a critical influence during prenatal life on the development and regulation of networks involved in body weight and feeding regulation. To establish the influence of the macronutrient type on feeding regulatory mechanisms and more particularly on stimulatory pathways (galanin and orexins), we fed female rats on either a high-carbohydrate (HC), a high-fat (HF), or a well-balanced control diet during gestation and lactation, and measured peptide expression in the hypothalamus and important hormones (leptin, insulin) in their pups at weaning. HF weanlings were 30% lighter than control and HC pups ($P < 0.001$). They were characterized by reduced plasma glucose and insulin levels ($P < 0.01$ or less). Their galanin and orexin systems were upregulated as shown by the significant augmentation of mRNA expression in the paraventricular nucleus and lateral hypothalamus, respectively. Inhibitory peptides like corticotropin-releasing hormone and neurotensin were not affected by this dietary treatment during early life. There was, therefore, a more intense drive to eat in HF pups, perhaps to compensate for the lower body weight at weaning. HF diets during early life had meanwhile some positive consequences: the lower metabolic profile might be beneficial in precluding the development of obesity and metabolic syndrome later in life. This is however valid only if the orexigenic drive is normalized after weaning.

Keywords: Orexins; Galanin; Leptin; Corticotropin-releasing hormone; Neurotensin; Fetal origin; Insulin; Metabolic syndrome; Diet composition

More and more palatable (high-fat, high-carbohydrate) food is available in our societies, and in parallel the prevalence of obesity is rapidly increasing, especially in young people [1,2]. Nutritional factors have a critical influence during prenatal life on the development and regulation of pathways and networks involved in body growth. These factors are particularly important for the development of the central nervous system both from the quantitative and qualitative points of view.

In laboratory rats, global undernutrition during gestation leads to the development of delayed-onset hyperphagia and obesity in the male offspring [3–5]. Protein-calorie malnutrition in prenatal life can induce insulin resistance in the adult offspring [6]. Perinatal hyperinsulinism or insulin deficiency induced in pregnant rat dams are also predisposing factors for the development of overweight and diabetes in the offspring at adulthood [7,8]. Overnutrition during the first days of life has similar long-term effects on body-weight regulation [9].

These early periods of life correspond largely to the period of neuronal differentiation and of central nervous system maturation. This is the case for some hypothalamic neuropeptides that are involved in the regulation of feeding behavior, including neuropeptide Y (NPY), galanin (GAL), and the orexins (OX) [10–17]. These peptides stimulate food ingestion [18–20] and also influence dietary preferences. NPY preferentially stimulates carbohydrate intake [21] whereas GAL and OX orient the food choice towards fats [22–24]. Each dietary preference can be associated with a specific neuropeptidergic profile [25]. Conversely, ingestion of high-fat (HF) or high-carbohydrate (HC) diets

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can influence the hypothalamic levels of these peptides [26–29]. Fat-to-carbohydrate ratio appears to be a critical factor in this influence.

The differentiation of these systems takes place in part during the last week of gestation in the rat, with development continuing during the first days of life until weaning. During this period, hypothalamic systems are very sensitive to maternal factors, including both metabolic factors and nutritional environment. As indicated above, most experiments have studied the quantitative aspects of food intake either overnutrition through litter size manipulation [30] or food restriction [31]. Concerning the impact of macronutrients, protein-energy malnutrition has also been studied because of the importance of this problem in developing countries [32,33]. The direct impact of the other macronutrients (carbohydrates and fats) on the central feeding regulatory systems has been less studied. From a more general point of view, it is known that carbohydrates must provide a minimum of energy for a pregnancy to go to full term [34]. Offspring of dams fed 32% fat diet during gestation are lighter at birth and fatter at weaning than controls [35]. At adulthood, both fats and carbohydrates can induce obesity and insulin resistance ([26]; see [36,37] for review).

We have previously shown that the offspring of dams fed on unbalanced (HF or HC) diets during the gestation and lactation periods exhibit persistent alterations in the functioning of the NPY system in adulthood [38–40]. The changes in sensitivity to NPY injection and an increased peptide release after a glucoprivic challenge are associated with delays in the establishment of dietary preferences [39,40].

To further determine the influence of these types of diets on feeding stimulatory pathways, we fed female rats on either an HC, an HF, or a well-balanced control (C) diet during gestation and lactation, and measured in their pups at weaning the expression of peptides that are more linked to fat intake, such as galanin and orexins [23,41]. These measures were associated with the measurement of some other important metabolic factors, including insulin, leptin, or blood glucose but also to anorexigenic peptides such as neurotensin (NT) and corticotropin-releasing hormone (CRH) [42,43]. The latter is also involved in the regulation of the hypothalamic–pituitary axis and therefore is linked to stress [44].

Materials and methods

This experiment was conducted according to the guidelines edited by the Society For Neuroscience for the use and care of animals in neuroscience research.

Animals and protocol

Female Long–Evans non-primiparous rats weighing about 250 g and male Long–Evans rats were obtained from CERJ (Centre d'Elevage R. Janvier; Le Genest, St-Isle, France). All rats were placed in a temperature-regulated room with an automatic 12 h light/12 h dark rhythm with chow and tap water ad libitum. After 1 week of adaptation to the conditions of the vivarium, they were fed ad libitum either the C, HF, or HC diets for 2 weeks to control their good adaptation to these new diets. They were then weighed and put in a breeding cage (3 females for 1 male).

From day 1 of gestation, the dams ($n = 42$) were installed in individual plastic cages for the whole duration of the gestation and suckling periods. At birth, the litter size was adjusted to 8–10 pups with pups of the same dietary group in order to avoid any litter size effect. The dams were fed their respective diets until weaning of the pups. Female weanlings were discarded and randomly chosen male rats were weighed and killed by decapitation 3 h after the beginning of the light period. Food was withdrawn from the dark-to-light transition until sacrifice in order to have all the animals in the same basal metabolic state without a long, stressful fast. Trunk blood and the brain were sampled.

Diets

The three diets contained the same quantity of protein (18% by weight) to avoid any confounding of results. Protein content was sufficient to allow a normal pregnancy. Wheat starch and sucrose (2/3–1/3) were the carbohydrates used for the three diets. Margarine was the only lipid source; it was used instead of oil to provide a consistent texture in the HF diet. The three diets were supplemented with 0.2% methionine. About 73.7% and 13.3% of the available energy resulted from lipids in the HF and HC diets, respectively. The detailed composition of the diets has been previously described [39].

Samples and assays

All biochemical measurements were made at the beginning of the light period because physiologically, it is a period of relative inactivity for feeding behavior. We could therefore easily avoid potential interference of food ingestion with some peptides like leptin or insulin, which are sensitive to the feeding state. In addition, this choice also avoided interference with daily rhythms of peptides like galanin and leptin, which peak in the dark period [45,46].

Blood. Trunk blood was sampled in tubes containing aprotinin and EDTA. It was centrifuged at 2500*g* for 20 min at 4 °C. The plasma was divided in aliquots and stored at −20 °C for the determination of plasma glucose, immunoreactive insulin (IRI), and leptin.

IRI was measured by a single antibody-charcoal radioimmunoassay technique using commercially available kits (INSIK, CIS, Gif sur Yvette, France) and rat insulin (Novo, Copenhagen) as standard. Leptin was measured with rat leptin RIA kit (Linco Research, St. Charles, USA). Plasma glucose was measured by an enzymatic technique using a commercially available kit (Boehringer–Mannheim, Meylan, France).

Brain. After sampling, the brains were immediately frozen and stored at −80 °C until processed for mRNA expression measurement ($n = 6$–8 per group) and peptide determination ($n = 10$–13 per group).

Gene expression for galanin, orexin, and CRH was measured using in situ hybridization. The protocol used has been previously published [47,48] and is described briefly below. mRNA levels were quantified in 20 μm coronal sections. The CRF probe was generated from a rat cDNA generously provided by Dr. K. Mayo. Galanin and orexin probes were generated as described previously [47,48]. Hypothalamic sections were collected onto slides, with adjacent sections on consecutively numbered slides. This permitted a number of mRNAs of interest to be localized and quantified in brain sections that were representative of different hypothalamic regions. Slides were fixed, acetylated, and hybridized overnight at 58 °C using ^{35}S-labeled cRNA probes ($1–2 \times 10^7$ cpm/ml). Autoradiographic images (Hyperfilm β-max; Amersham) were quantified using the Image-Pro Plus system. Data were manipulated using a standard curve generated from ^{14}C autoradiographic micro-scales (Amersham), and the integrated intensity of the hybridization signal computed.

For the peptide content determination, serial brain sections of 300 μm were cut, and discrete hypothalamic sites were micropunched with needles of various diameters, as previously detailed [49]. The arcuate (ARC), the paraventricular (PVN), and the ventromedial (VMN) nuclei were micropunched, as well as the lateral hypothalamus (LH). Bilateral tissue samples