Peptides 43 (2013) 146–154

Contents lists available at SciVerse ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

The hypothalamic POMC mRNA expression is upregulated in prenatally undernourished male rat offspring under high-fat diet

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ARTICLE INFO

Article history:
Received 25 January 2013
Received in revised form 13 March 2013
Accepted 13 March 2013
Available online 20 March 2013

Keywords:
Fetal
Maternal undernutrition
Appetite regulation
Microarray
Arcuate nucleus

ABSTRACT

Epidemiological studies demonstrated that adverse environmental factors leading to intrauterine growth retardation (IUGR) and low birth weight may predispose individuals to increased risk of metabolic syndrome. In rats, we previously demonstrated that adult male IUGR offspring from prenatal 70% food-restricted dams throughout gestation (FR30) were predisposed to energy balance dysfunctions such as impaired glucose intolerance, hyperleptinemia, hyperphagia and adiposity. We investigated whether postweaning moderate high-fat (HF) diet would amplify the phenotype focusing on the hypothalamus gene expression profile. Prenatally undernourished rat offspring were HF-fed from weaning until adulthood while body weight and food intake were measured. Tissue weights, glucose tolerance and plasma endocrine parameters levels were determined in 4-month-old rats. Hypothalamic gene expression profiling of adult FR30 rat was performed using Illumina microarray analysis and the RatRef-12 Expression BeadChip that contains 21,792 rat genes. Under HF diet, contrary to C animals, FR30 rats displayed increased body weight. However, most of the endocrine disorders observed in chow diet-fed adult FR30 were alleviated. We also observed very few gene expression changes in hypothalamus of FR30 rat. Amongst factors involved in hypothalamic energy homeostasis programming system, only the POMC and transthyretin mRNA expression levels were preferentially increased under HF diet. Both elevated gene expression levels may be seen as adaptive mechanisms counteracting against deleterious effects of HF feeding in FR30 animals. This study shows that the POMC gene expression is a key target of long-term developmental programming in prenatally undernourished male rat offspring, specifically within an obesogenic environment.

1. Introduction

In addition to lifestyle and dietary factors, increasing evidence suggests that the origin of some metabolic disorders, which manifest in adult life, may have their roots during development. Indeed, epidemiological studies demonstrated that adverse environmental factors leading to intrauterine growth retardation (IUGR) and low birth weight may predispose individuals to development of pathologies related to the metabolic syndrome [12,33]. As illustrated by the Dutch Famine Study, fetuses exposed to famine during early pregnancy displayed a higher energy intake and adiposity in adulthood [48]. Originally called Barker hypothesis or fetal programming, these observations have led to the developmental origin of health and disease (DOHaD) hypothesis [4,52]. This concept states that an adverse perinatal environment programs or imprints the development of several tissues. It then may permanently determine physiological responses and ultimately produce energy balance dysfunction and diseases later in life. In addition, postnatal hypercaloric nutrition, and more specifically rapid catch-up growth, are important accelerators in the etiology of adult-onset disease in human born with low birth weight [19,28]. Thus, the perinatal perturbation of fetus/neonate nutrient supply has been proposed to be a major determinant, especially the degree of mismatch between the pre-and postnatal environments [23].

To get insights into the underlying mechanisms, numerous animal models have been developed to promote intrauterine fetal programming, especially maternal undernutrition [56]. These studies confirmed that impaired fetal development has long-term metabolic consequences sensitizing the offspring to long-lasting

Abbreviations: DOHaD, developmental origin of health and disease; C, control; FR30, offspring from 70% food-restricted pregnant dams; IUGR, intrauterine growth retardation; MPU, maternal prenatal undernutrition; LP, low protein diet; WAT, white adipose tissue; BAT, brown adipose tissue; NPY, neuropeptide Y; POMC, propiomelanocortin; CART, cocaine-and-amphetamine-regulated transcript; AgRP, agouti-related peptide; Ob-Rb, long-form of the leptin receptor; InsR, insulin receptor; Ttr, transthyretin; OGTT, oral glucose tolerance; PND, postnatal day; PVN, paraventricular nucleus; DIO, diet-induced obesity.

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0196-9781/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.peptides.2013.03.013

perturbation of energy balance regulation. In particular, the hypothalamus–adipose axis is a key target of developmental programming by maternal nutritional manipulation [10].

In adult, the pivotal role of the hypothalamus, especially the arcuate nucleus (Arc), for the maintenance of energy homeostasis controlling the nutritional status and energy storage level, is well established [51]. Peripheral hormones and energetic substrates act on the Arc by modulating the expression and release of hypothalamic orexigenic peptides such as neuropeptide Y (NPY)/agouti-related peptide (AgRP) and anorexigenic peptides such as α-melanocyte-stimulating hormone (α-MSH, a neuropeptide derived from proopiomelanocortin (POMC) processing in the hypothalamus)/cocaine- and amphetamine-regulated transcript (CART). Then, the Arc drives other hypothalamic areas such as ventromedial, dorsomedial, and paraventricular nuclei (PVN) (considered as satiety centers) as well as the lateral hypothalamic area (considered as hunger center) [2]. Several hypothalamic nuclei, especially the PVN may, in turn, modulate *via* sympathetic autonomic nervous system the energy expenditure [20].

In rodents, several studies suggest that hypothalamic "malprogramming" begins *in utero* but continues in early postnatal life during the suckling period. It thus may lead to long-lasting disturbed body weight set-point in adulthood. Indeed, maternal reduced nutrition affects the cell proliferation [22], the organization of the feeding circuitry hardwiring [11,13,15] and the cytoarchitectonic organization [47] of the offspring hypothalamus. Maternal nutrient restriction also alters hypothalamic appetite-regulating neuropeptide mRNA levels in the offspring, favoring the orexigenic pathways (*i.e.*, increased NPY mRNA expression) [14,29]. This is often associated with central leptin/insulin resistance [17,22]. Thus, it may predispose them to hyperphagia and an increased risk of developing obesity later in life, particularly when nourished with hypercaloric diet [55]. Finally, maternal reduced nutrition modifies hypothalamic circadian feeding rhythms in the offspring [11,43,53].

Using a model of prenatal maternal 70% food restriction diet in rat pregnant females (FR30) throughout gestation, we have previously shown that FR30 procedure induces IUGR and programs some metabolic syndrome features. Thus, under chow diet, adult FR30 offspring display mild hypertension, hyperleptinemia, hypercorticosteronemia, impaired glucose tolerance and hyperphagia [11,50]. We also reported that FR30 procedure differently disturbs the long-term offspring hypothalamic appetite programming system. In particular, we showed that the response of POMC neurons to energy status variation and light/dark-phase food intake rhythm is modified in adult FR30 offspring. They also exhibit subtle alterations of POMC hypothalamic neurons projections [11]. Although showing a lean phenotype, adult FR30 offspring exhibit white adipose tissue (WAT) gene expression profile programming that may predispose for adiposity [35]. Based on the DOHaD hypothesis, we therefore decided to assess whether postweaning moderate high-fat (HF) diet would amplify the phenotype focusing on the hypothalamus gene expression profile.

This study shows that the POMC gene expression whose fine-tuning regulation is critically involved in the lifelong energy homeostasis, is a key target of developmental programming in prenatally undernourished male rat offspring under HF diet.

2. Materials and methods

2.1. Animals

Wistar rats were purchased from Janvier Laboratories (Saint Berthevin, France) and housed six per cage. After mating with a male, pregnant females were transferred into individual cages with free access to water and to chow diet (SAFE 04, 2900 cal/g, containing 16% protein, 3% fat, 60% carbohydrates; UAR, Augy, France). Control pregnant dams were fed *ad libitum*, while pregnant dams from the food-restricted group fed 30% (FR30) of the daily intake of control pregnant dams throughout the gestation. At parturition, litter size was adjusted to 8 pups per dam. Feed-restricted pups were nursed by FR30 dams fed *ad libitum* during lactation. To obviate any litter effects, animals used for each experiment were randomly chosen in different litters and only a limited number of animals ($n = 1–2$) was used from each litter. After weaning, male offspring from the control (C) or FR30 dams were housed individually and divided into four groups (CN, CHF, FR30N, FR30HF; $n = 16$ per group) to be fed either chow (N) or moderate high-fat (HF) (SAFE, D12451, 4720 cal/g, containing 23% protein, 23% lipid, 40% carbohydrates; UAR, Augy, France). Body weight and food intake of the offspring were measured weekly until adulthood. All parameters of adult male offspring were studied at 4 months of age. Animal use accreditation by the French Ministry of Agriculture (No. 04860) has been granted to our laboratory for experimentation with rats. Experiments were conducted in accordance with the principles of laboratory animal care (European Communities Council Directive of 1986, 86/609/EEC).

2.2. Plasma and tissues collections

At 4 months of age, male rats were rapidly weighed and killed by decapitation between 9 and 10 a.m. Trunk blood samples were collected into prechilled tubes containing EDTA (20 μL of a 5% solution), gently shaken and centrifuged at 4000 × *g* for 10 min at 4 °C. Aliquots of the supernatants were stored at −20 °C until assayed. Hypothalami, liver as well as deposit pads of WAT and interscapular brown adipose tissue (BAT) were rapidly removed, weighed, frozen in liquid nitrogen and stored at −80 °C until use.

2.3. Food intake and metabolic parameters

Food intake of rats was recorded weekly from weaning (postnatal day 22, PND22) to adulthood (postnatal day 127, PND127) in the four groups. This parameter was measured at the beginning of the light phase (9 a.m.). All animals were presented with the same amount of food and their intake was measured by subtracting the uneaten food.

For oral glucose tolerance tests (OGTT), rats (PND113) were fasted overnight (16 h). Basal blood glucose level defined as T0 was determined using an automatic glucometer (Glucotrend 2, Roche Diagnostics, France) before the glucose administration (2 g/kg of body weight) ($n = 12$ per group). Tail vein blood glucose was then measured at 0, 30, 60, 90 and 120 min after administration. Plasma insulin concentrations were measured by enzyme-linked immunosorbent assay (DRG, International, Inc., USA).

2.4. Endocrine parameters

All plasma endocrine parameters levels were investigated with commercially available kits. Blood glucose and plasma insulin levels were determined as described above. Plasma leptin concentration was measured with murine enzyme-linked immunosorbent assay kits (Diagnostic Systems Laboratories, Inc., USA; Adipogen Inc., Korea). Plasma corticosterone levels were determined by a competitive enzyme immunoassay (Immunodiagnostic Systems Ltd., Boldon, UK). Assay kits were applied to determine the contents of plasma triglycerides and total cholesterol (61238 Triglyceride Enzymatique PAP100, 61218 Cholesterol Liquide, BioMérieux, France) as well as free cholesterol and free fatty acid (FFA) (references 279-47106 and 999-75406, Wako Chemicals, Neuss, Germany). Each point has been measured in duplicate.