Int. J. Devl Neuroscience 38 (2014) 23–29

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

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

Perinatal undernutrition increases meal size and neuronal activation of the nucleus of the solitary tract in response to feeding stimulation in adult rats

Lívia A. Lira[b], Larissa C.A. Almeida[b], Amanda A.M. da Silva[b], Taisy C.F. Cavalcante[c], Diogo D.C.B. de Melo[b], Julliet A. de Souza[c], Renata C.F. Campina[a,b], Sandra L. de Souza[a,b,c,*]

[a] Department of Anatomy, Universidade Federal de Pernambuco – UFPE, Recife, PE, Brazil
[b] Postgraduate Neuropsychiatry and Behavioral Sciences, Universidade Federal Pernambuco – UFPE, Recife, PE, Brazil
[c] Department of Nutrition, Universidade Federal de Pernambuco – UFPE, Recife, PE, Brazil

ARTICLE INFO

Article history:
Received 26 April 2014
Received in revised form 27 July 2014
Accepted 27 July 2014
Available online 4 August 2014

Keywords:
Perinatal undernutrition
Feeding behaviour
Nucleus of the solitary tract
Rats
Fos protein

ABSTRACT

During the early periods of development, i.e., gestation and lactation, the influences of stimulus such as undernutrition can lead to several behavioural and morphofunctional damages to organs and systems in general, including pathways and structures that control energy balance and feeding behaviour. Although a large body of evidences have shown the effects of this stimulus on structures such as hypothalamus, only few studies have directed their attention to the long-term effects of undernutrition on the nucleus of the solitary tract (NTS). The aim of this study was to investigate the effects of early undernutrition on the NTS and control of food intake in adulthood. Male Wistar rats were divided into two groups according to the diet offered to the dams during gestation and lactation: control group (C, diet containing 17% casein) or isocaloric low-protein group (LP, diet containing 8% casein). On 35 or 180 days, we evaluated the rats' body weight, food intake, behavioural satiety sequence and c-Fos protein expression in the NTS in response to food stimulus. Based on these assessments, it was found that perinatal undernutrition promoted an increase in food intake and the number of activated cells in rostral and, mainly, medial NTS in response to food stimulation in adulthood. These results indicated that the NTS is a structure particularly vulnerable to the influences of nutritional manipulation in the early stages of development with effects on food control in adulthood.

1. Introduction

Nutritional deficiencies during perinatal period, such as undernutrition, may promote the development of chronic diseases in adulthood (Horton, 2008). Diet consumption is one of the major environmental factors that affects some aspects of health and is involved in the risks of several diseases (Jimenez-Chillaron et al., 2012). The association between undernutrition and late disease has been well described in the Developmental Origins of Health and Disease Hypothesis (DOHaD). This theory suggests that environmental stimuli, such as nutrition, act during critical period of development and can produce permanent changes in the cell structures, tissues and functions through long-lasting modifications of the expression of target genes (Gluckman and Hanson, 2004).

Modifications in the regulatory systems of feeding behaviour may be the key points for understanding the actions of neonatal events that generate metabolic diseases in adulthood. The intake of low-protein diets during perinatal period could produce hyperphagia in the later life (Orozco-Solis et al., 2009). There are several changes in the gene expression of hypothalamic orexigenic and anorexigenic peptides (Bouret and Simerly, 2006). It has been observed in animal models of undernutrition that expression of orexigenic peptide neuropeptide Y (NPY) is increased in

* Corresponding author at: Universidade Federal de Pernambuco, Departamento de Anatomia, Centro de Ciências Biológicas – CCB/UFPE Av. Prof Moraes Rêgo, 1235 Recife, PE 50670-420, Brazil. Tel.: +55 81 21268555; fax: +55 08121268567.
E-mail addresses: ila_lira@yahoo.com.br (L.A. Lira), ftlarissaalmeida@gmail.com (L.C.A. Almeida), amandabiomedica10@gmail.com (A.A.M. da Silva), taisyferro@yahoo.com.br (T.C.F. Cavalcante), diogo.brasileiro.melo@hotmail.com (D.D.C.B. de Melo), jully_jesus@hotmail.com (J.A. de Souza), renatacampina82@gmail.com (R.C.F. Campina), sanlopesufpe@gmail.com, ilalira@hotmail.com (S.L. de Souza).

http://dx.doi.org/10.1016/j.ijdevneu.2014.07.007
0736-5748/

contrast to the expression of anorexigenic peptide proopiomelanocortin (POMC) that decreased. Besides, the rhythmicity of feeding behaviour also appears to be modified (Stevens et al., 2011).

The nucleus of the solitary tract (NTS) in the dorsal medulla are important mediators with integrative and interoceptive functions, and are involved in the modulation of some aspects of feeding behaviour, such as initiation, termination, duration as well as meal size (Grill and Kaplan, 2001; Grill, 2006, 2010; Grill and Hayes, 2009). This nucleus has an array of neurons and circuits directly involved in the ingestion, digestion and absorption of food (Zheng et al., 2005).

Besides, NTS integrates peripheral information from the gastrointestinal tract, pancreas and adipose tissue, and is also responsive to gustatory stimuli and information related to motivation and food reward (Grill, 2006; Appleyard et al., 2007; Grill and Hayes, 2009). Moreover, the NTS maintains direct and reciprocal connexions with other brain structures involved in food intake control and energy balance, such as hypothalamus, nucleus accumbens, amygdala, ventral tegmental area, dorsal raphe nucleus and area postrema (Rinaman, 2010).

Although NTS plays a fundamental role in the control of energy balance, the available information about the long-term consequences of inadequate nutritional intake in the early stages of development on the ability of this nucleus in regulating feeding behaviour is scarce. Rubio et al. (2004), using the model of perinatal food restriction by reducing the food intake during pregnancy and lactating period, found changes in morphology of the NTS neurons, which become hypotrophic compared to the controls. Furthermore, interneurons showed fewer and shorter dendritic prolongations. In a rehabilitated group with restricted food before birth but normal food intake during the lactating period, the neuronal morphology was similar to that of controls. In spite of the fact that this work did not show the direct relationship between undernutrition and its consequences on ability of NTS in regulating food intake, it shows that NTS is a structure vunerable to perinatal influences. Therefore, this study evaluated the effects of perinatal undernutrition on neuronal activation in response to food stimulation in the NTS.

2. Materials and methods

2.1. Animals

All the experiments were performed in accordance with the recommendations of the Ethic Committee for use of animals (CEUA, Comissão de ética no uso de animais/Ethics number 23076.024837/2009-11). Virgin female Wistar rats weighing 200–250 g were obtained from facilities of Department of Nutrition, Federal University of Pernambuco and placed under a 12-h light/dark cycle with food and water ad libitum.

The rats were mated at a ratio of one male to two females. After confirmation of mating by the visualisation of spermatozoa through vaginal smear and body weight, the rats were housed individually and fed with either a control diet (17% of casein) or an isocaloric low-protein (LP) diet (8% of casein) during the entire gestation and lactation periods (Table 1). Birth was recorded as postnatal day zero (P0) for the pups. Sexing was performed at 24 h after birth, and the numbers of pups were adjusted to give 8 pups per mother with an equal ratio (4:4) of males and females. In this paper, female pups were used only to complete the litters maintaining the same male: female ratio. The experimental groups consisted of two male rats from each litter and, at the end, a total of 10 animals of each control and low-protein restrict groups were used. After weaning (21 days), all the male pups from the control and LP groups were fed with standard laboratory chow diet.

The analyses were performed by using 35- and 180-day-old rat. It is known that full maturation of the central nervous system occurs in rodents after 35 days (Rice and Barone, 2000; Semple et al., 2013). Accordingly, the present study aimed to analyse the effect of short- and long-term perinatal undernutrition on neuronal activation of the NTS in response to food intake.

2.2. Measurement of body weight and food intake

The body weight was recorded on days 1, 6, 11, 16, 21, 26 and 31 of life. On days 35 and 180, each animal of the C or LP groups was housed in individual cages, and its body weight and food intake were determined. To measure the food intake, each animal was deprived of food for 4 h. The food intake was measured during 90 min and determined by the difference between the food provided and the amount of food rejected. Body and food weights were recorded to 0.01 g.

Table 1
Composition of the experimental diets offered during perinatal period (g/100 g diet).

Macronutrient	Control (17% protein)	Hypoproteic (8% protein)
Protein		
Casein[a]	21.3	10.0
Carbohydrate		
Cornstarch	51.0	63.0
Sucrose	10.0	10.0
Fat		
Vegetable oil	7.0	7.0
Cellulose	5.0	5.0
Vitamin mix	1.0	1.0
Mineral mix	0.3	0.3
Methionine	0.3	0.3
Energy density (Kcal/g)	3.58	3.59
% Macronutinent (Kcal/g)		
Protein	19.7	9.3
Carbohydrate	61.6	72.0
Fat	17.9	17.5

The diets were formulated according to the guidelines of the American Society for Nutritional Sciences (AIN 93).

[a] Casein with 80% of biodisponibility.

2.3. Behavioural sequence satiety

Behavioural sequence satiety (BSS) is considered as a reflex of physiological operational process, which occurs during post-ingestive period. BSS was analysed in accordance with adaptation of Halford's protocol (Halford et al., 1998). The 150-day-old animals of each experimental group were fasted for 4 h. After this period, it was offered a diet, and during a 60 min, feeding and non-feeding behaviours were continuously scored using a video system coupled to a computer in a nearby room by a highly trained experimenter blind to the nutritional status of the animals. Behaviours were categorised as: eating (ingesting food, gnawing, chewing or holding food in paws), drinking, active (exploring movements around the cage, rearing), grooming (body care movements with the mouth or forelimbs), and resting (sitting or lying in a resting position, sleeping animal). Other measures scored from the behavioural observation of feeding were: latency to eat (time to begin eating after the presentation of chow), meal duration (time over the entire monitoring period the animal is actually eating food), and feeding rate (amount of food consumed/meal duration). Food was weighed at the beginning and at the end of each session.

2.4. c-Fos immunohistochemistry

On days 35 and 180, the animals were fasted for 4 h and were given standard diet 90 min before transcardiac perfusion to stimulate c-Fos expression in both C ($n = 5$) and LP ($n = 5$) groups. After 90 min, the animals were deeply anesthetised with a combination of ketamine andxylazine (50 mg/kg), followed by transcardiac perfusion, first with saline (0.9% NaCl) and then with 4% paraformaldehyde (PFA). The brains were removed and post-fixed in 4% PFA plus 20% sucrose for 4 h, and then transferred to 0.1 mol/l phosphate buffer plus 20% sucrose for 48 h at 4 °C. Subsequently, coronal sections of 40-μm thickness were cut on a cryostat. Five series of sections were collected for each animal in 25-well acrylic plates containing ethylene glycol and sucrose in phosphate-buffered saline (PBS) and stored at −20 °C. One group of the sections was used for c-Fos immunohistochemistry. The sections were removed from the storage solution and washed thrice with PBS (10 min for each wash). Subsequently, the sections were washed for 5 min with 0.6% hydrogen peroxide to block endogenous peroxidase activity and some nonspecific antibody binding sites, and were washed again for three times with PBS (10 min each) to remove any fixative residue. Then, the sections were incubated in PBS containing 0.3% Triton X-100, 5% normal goat serum and Fos protein antibody (1:10,000 dilution; Calbiochem, CAT#PC38, Bad Soden, Germany) for 48 h. After that, the sections were washed thrice with PBS (10 min each) and incubated in PBS containing 0.3% Triton X-100 and a 1:200 dilution of the biotinylated secondary antibody (Catalogue No. B8895; Sigma–Aldrich, St. Louis, MO, USA) for 90 min and then incubated with 1% avidin–biotin peroxidase (Vec-tastain; Camon, Wiesbaden, Germany) for 90 min. The reaction was visualised with 3,3-diaminobenzidine (D5637; Sigma–Aldrich) diluted in distilled water with nickel ammonium sulfate, 0.2 mol/l sodium acetate ammonium chloride and β-D-glucose. Subsequently, the sections were mounted on gelatinised slides, dehydrated in 100% ethanol, cleared using xylene, covered with a cover slip and viewed under a light microscope. Photomicrographs were taken by using a digital camera connected to a microscope and a computer, and identification and quantification of the neurons located in the rostral NTS (bregma −12.72 to −12.96) and medial NTS (bregma −13.96 to −14.16) were performed by using these