Available online at www.sciencedirect.com
ScienceDirect
Neuroscience Letters 419 (2007) 99–103
Neuroscience Letters
www.elsevier.com/locate/neulet

Prenatal undernutrition decreases the sensitivity of the hypothalamo-pituitary-adrenal axis in rat, as revealed by subcutaneous and intra-paraventricular dexamethasone challenges

Mario Navarrete [a], Héctor Núñez [a], Samuel Ruiz [b], Rubén Soto-Moyano [a], Luis Valladares [a], Allan White [c], Hernán Pérez [a,*]

[a] *Laboratory of Hormones and Receptors, Institute of Nutrition and Food Technology (INTA), University of Chile, Santiago, Chile*
[b] *Faculty of Biomedical Sciences, Diego Portales University, Santiago, Chile*
[c] *Program of Physiology and Biophysics, ICBM, Faculty of Medicine, University of Chile, Santiago, Chile*

Received 12 March 2007; accepted 10 April 2007

Abstract

Prenatal undernutrition is known to disturb the hypothalamo-pituitary-adrenal (HPA) axis, possibly through the programming of decreased expression of hypothalamic and pituitary glucocorticoid receptors. To test this hypothesis, we examined the corticosterone response to moderate subcutaneous (100 μg/kg) and intra-paraventricular (50 pmol, bilaterally) dexamethasone (DEX) challenges in normal eutrophic and prenatally undernourished young rats. Undernutrition was induced during fetal life by restricting the diet of pregnant mothers to 10 g daily, while mothers of eutrophic rats received the same diet ad libitum. At day 40 of postnatal life (i) undernourished rats showed increased plasma corticosterone concentration compared to normals; and (ii) subcutaneous and intra-paraventricular administrations of DEX led to reduced corticosterone levels in normal and undernourished animals, the effect of DEX (administered either peripherally or centrally) being significantly lower in the latter group. Results suggest that the low sensitivity of the HPA axis to DEX as well as the increased plasma corticosterone observed in prenatally undernourished rats could be due to the already reported glucocorticoid receptor underexpression found in the hypothalamus and pituitary of in utero undernourished animals, but alternative explanations involving central noradrenergic adaptive changes could also be possible.

Keywords: Prenatal undernutrition; Hypothalamo-pituitary-adrenal axis; Corticosterone; Dexamethasone; Paraventricular nucleus

Epidemiological studies point out an association between low birth weight and arterial hypertension, coronary disease and type 2 diabetes during adult life, suggesting that these dysfunctions are programmed during fetal life [1]. One of the main factors determining fetal growth is the nutritional status of the mother, in such a way that periods of malnutrition during pregnancy will lead to growth retardation during prenatal life as well as to elevated blood pressure at later postnatal ages [1]. This notion is supported by studies in animals showing that pregnant rats submitted to different forms of protein or protein–calorie restriction gave birth pups that developed hypertension during postnatal life in spite of they were nutritionally rehabilitated during lactation [11,24,39].

Such effect of maternal undernutrition in offspring could be related to disturbances in the fetal hormonal environment, particularly in the activity of the hypothalamo-pituitary-adrenal (HPA) axis. In fact, there is evidence that both maternal protein [12] and maternal food [13] restrictions to pregnant rats result in lower placental 11β-hydroxy-steroid dehydrogenase type 2 (11βHSD2) activity, the enzyme that converts physiological glucocorticoids to inactive 11-keto products, thus resulting in overexposure of the fetus to maternal glucocorticoids. Deficiency in placental 11βHSD2 has also been reported in babies with reduced body weight at birth [37]. Overexposure to maternal glucocorticoids caused by prenatal undernutrition has been found to reduce glucocorticoid receptor expression in

* Corresponding author. Tel.: +56 2 9781488; fax: +56 2 2214030.
E-mail address: hperez@inta.cl (H. Pérez).

0304-3940/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2007.04.019

the hippocampus [13], hypothalamus [2] and pituitary [8] of the offspring, which are important sites of feed-back control upon the HPA axis. Hypothetically, reduced glucocorticoid receptor expression in these sites would result in decreased negative feed-back control by glucocorticoids and thereby in increased HPA activity. This possibility is supported by studies reporting higher expression of hypothalamic corticotropin-releasing hormone (CRH) as well as greater plasma levels of adrenocorticotropin hormone (ACTH) and corticosterone/cortisol in rats and lambs that underwent different forms of prenatal undernutrition [7,19,22,26,34]. Epidemiologic studies have also associated low birth weight of babies, an index of intrauterine undernutrition, with increased basal plasma cortisol levels when adults [25].

In spite of the synthetic glucocorticoid dexamethasone (DEX) has been widely used for in vitro and in vivo studies of the glucocorticoid effects on a number of different cellular and physiological responses, the DEX suppression test has not still been employed for determining the status of the negative feed-back control of the HPA axis in subjects that experienced undernutrition during fetal life. In this regard, several studies in rat have demonstrated a pituitary rather than a central site of action in the suppression of HPA axis if moderate amounts of DEX are administered [4,5], since the expression of the efflux transporter P-glycoprotein hampers the penetration of DEX into the brain [16] and, on the other hand a moderate dose of DEX results in suppression of endogenous corticosterone secretion [10]. Therefore, central administration of DEX into the paraventricular nucleus (PVN) would be necessary to study DEX-induced suppression of HPA axis at the hypothalamic level. In the present investigation we examined the corticosterone response to moderate subcutaneous and intra-PVN DEX challenges in normal eutrophic and prenatally undernourished young rats in order to test at both, the pituitary and hypothalamic levels respectively, changes in sensitivity of the negative feed-back control of the HPA axis that would be programmed by undernutrition during fetal life.

The experimental protocol and animal management were in accordance with the NIH Guide for the Care and Use of Laboratory Animals [18]. The experiments were carried out on male and female Wistar rats, born from mothers subjected during pregnancy to one of the following nutritional conditions: (i) normal pregnant rats, with free access to a 21% protein standard laboratory diet (Champion, Santiago, Chile); (ii) undernourished pregnant rats, with restricted access (10 g daily) to the standard laboratory diet throughout pregnancy; this amount of food is about 40% of that consumed by normal pregnant rats [34], and was given two times daily (5 g at 09:00 h and 5 g at 19:00 h) in order to minimize anxiety for feeding in food restricted pregnant dams. To prevent undernutrition of pups during postnatal life, prenatally undernourished pups were at birth fostered to well-nourished dams giving birth on that day, according to rearing procedures already described [17]; pups born from well-nourished mothers were also fostered to well-nourished dams, in order to equalize among groups other factors that may depend on the rearing conditions (i.e. stress due to cross-fostering). During the lactation period all litters were adjusted to eight pups per dam, and all dams continued to receive the standard laboratory diet ad libitum. After weaning at 22 days of age, all pups were housed eight per cage and fed on the standard laboratory diet, under controlled laboratory conditions (a 12-h light/dark cycle with lights on at 09:00 h). During the light cycle, light intensity was maintained at 300 lx as measured at the level of the cage floor. The body weight of pregnant mothers and the body weight of pups were measured daily.

All experimental groups were composed by six rats (three males and three females) arising from different litters. On day 40 of postnatal life, at 19:00–20:00 h, six normal and six prenatally undernourished rats were s.c. injected with a single low dose of 100 μg/kg DEX-21-phosphate (Sigma–Aldrich, St. Louis, MO) dissolved in 0.9% (w/v) NaCl to induce a decrease in plasmatic corticosterone. Another six normal and six prenatally undernourished rats receiving the same volume of s.c. saline served as controls. Other groups of six normal and six prenatally undernourished rats were used to study the effect of intra-PVN microinjection of DEX on plasma corticosterone. Six additional normal and six additional prenatally undernourished rats microinjected with saline into the PVN served as controls. In order to microinjecting DEX or saline, animals were anesthetized with sodium pentobarbital (45 mg/kg body weight) and placed in a stereotaxic apparatus for rats (Narishige ST-7, Narishige Scientific Instrument Lab., Tokyo, Japan). The horizontal zero plane of the stereotaxic apparatus was tangent to the upper incisor bar and 5 mm above the interaural line. The skull was exposed and two 2.0-mm diameter holes, centered at 0.5 mm lateral to the midline and 0.6 mm rostral to the bregma point, were drilled in the right and left parietal bones for approaching both PVNs at coordinates A, 6.4; L, 0.5; V, −1.6 (in mm) [21]. DEX-21-phosphate (Sigma–Aldrich, St. Louis, MO) was bilaterally injected (50 pmol DEX/0.2 μl saline) using a 10-μl Hamilton syringe directed at each PVN. This dose was taken from studies using centrally microinjected DEX in other brain nuclei, such as the nucleus tractus solitarius, to produce modification in cardiovascular parameters [20,38]. Similar saline volumes were microinjected into the PVNs of control animals. The injections were performed gradually over a period of 2 min. Afterwards, rats were kept one animal per cage until the next morning.

Fourteen hours after the DEX challenge (s.c. or intra-PVN), rats were killed rapidly by decapitation between 09:00 and 10:00 h in a room separate from that in which the other animals are kept. Trunk blood was collected in heparinized Eppendorf tubes and immediately centrifuged (1700 × *g*, 10 min, 4 °C) and plasma stored at −20 °C. Plasma corticosterone was measured using a RIA based on ^{125}I-labelled rat corticosterone that was performed according to the manufacturer's instructions (Diagnostic Products Corporation, Los Angeles, CA, USA). This Coat-a-Count rat corticosterone kit is a solid-phase RIA, in which rat ^{125}I-corticosterone competes for antibody sites with corticosterone in the sample during a fixed time (120 min). The antibody is coated on the wall of a polypropylene tube. Decanting the supernatant is sufficient to terminate the competition and to isolate the antibody-bound fraction of the radiolabelled corticosterone. Counting the tube in a gamma counter (Riastar