Psychoneuroendocrinology (2010) 35, 1525—1535

available at www.sciencedirect.com

ScienceDirect

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

Gender differences in the long-term effects of chronic prenatal stress on the HPA axis and hypothalamic structure in rats

Cristina García-Cáceres [a], Natalia Lagunas [b], Isabel Calmarza-Font [b], Iñigo Azcoitia [c], Yolanda Diz-Chaves [b], Luis M. García-Segura [b], Eva Baquedano [a], Laura M. Frago [a], Jesús Argente [a], Julie A. Chowen [a,*]

[a] *Hospital Infantil Universitario Niño Jesús, Universidad Autónoma de Madrid, CIBER Fisiopatología de Obesidad y Nutrición (CIBERobn), Instituto de Salud Carlos III, Madrid 28009, Spain*
[b] *Instituto Cajal, CSIC. Avenida Dr. Arce 37, Madrid 28002, Spain*
[c] *Departamento de Biología Celular, Facultad de Biología, Universidad Complutense de Madrid, Madrid 28040, Spain*

Received 22 December 2009; received in revised form 19 April 2010; accepted 20 May 2010

KEYWORDS
Immobilization stress;
HPA axis;
Hypothalamus;
Cell turnover;
Gender;
Synaptic proteins;
Astrocytes

Summary Stress during pregnancy can impair biological and behavioral responses in the adult offspring and some of these effects are associated with structural changes in specific brain regions. Furthermore, these outcomes can vary according to strain, gender, and type and duration of the maternal stress. Indeed, early stress can induce sexually dimorphic long-term effects on diverse endocrine axes, including subsequent responses to stress. However, whether hypothalamic structural modifications are associated with these endocrine disruptions has not been reported. Thus, we examined the gender differences in the long-term effects of prenatal and adult immobilization stress on the hypothalamic—pituitary—adrenocortical (HPA) axis and the associated changes in hypothalamic structural proteins. Pregnant Wistar rats were subjected to immobilization stress three times daily (45 min each) during the last week of gestation. One half of the offspring were subjected to the same regimen of stress on 10 consecutive days starting at postnatal day (PND) 90. At sacrifice (PND 180), serum corticosterone levels were significantly higher in females compared to males and increased significantly in females subjected to both stresses with no change in males. Prenatal stress increased pituitary ACTH content in males, with no effect in females. Hypothalamic CRH mRNA levels were significantly increased by prenatal stress in females, but decreased in male rats. In females neither stress affected hypothalamic cell death, as determined by cytoplasmic histone-associated DNA fragment levels or proliferation, determined by proliferating cell nuclear antigen levels (PCNA); however, in males there was a significant decrease in cell death in response to prenatal stress and a decrease in PCNA levels with

* Corresponding author at: Servicio de Endocrinología, Hospital Infantil Universitario Niño Jesús, Avenida Menéndez Pelayo 65, Madrid 28009, Spain. Tel.: +34 91 503 5939; fax: +34 91 503 5939.
E-mail address: jachowen@telefonica.net (J.A. Chowen).

0306-4530/$ — see front matter
doi:10.1016/j.psyneuen.2010.05.006

both prenatal and adult stress. In all groups BrdU immunoreactivity colocalized in glial fibrillary acidic protein (GFAP) positive cells, with few BrdU/NeuN labelled cells found. Furthermore, in males the astrocyte marker S100β increased with prenatal stress and decreased with adult stress, suggesting affectation of astrocytes. Synapsin-1 levels were increased by adult stress in females and by prenatal stress in males, while, PSD95 levels were increased in females and decreased in males by both prenatal and adult stress. In conclusion, hypothalamic structural rearrangement appears to be involved in the long-term endocrine outcomes observed after both chronic prenatal and adult stresses. Furthermore, many of these changes are not only different between males and females, but opposite, which could underlie the gender differences in the long-term sequale of chronic stress, including subsequent responses to stress.

1. Introduction

Exposure of pregnant humans or animals to chronic stress during critical periods of fetal brain development may raise the risk of depression, attention and learning deficits, hormonal imbalances and metabolic disorders in the offspring, some of which have been associated with structural alterations in the brain (Schneider, 1992; Van Os and Selten, 1998; Geddes, 1999; Rhees et al., 1999a; Weinstock, 2001; Wadhwa et al., 2001; Linnet et al., 2003; Maccari et al., 2003; Walker, 2005; Abe et al., 2007; Yaka et al., 2007; Bogoch et al., 2007). Indeed, behavioral disturbances produced by prenatal stress are associated with cellular and synaptic protein alterations in the hippocampus, dentate gyrus and prefrontal cortex of adult offspring (Lemaire et al., 2000; Koo et al., 2003; Van den Hove et al., 2006; Michelsen et al., 2007). Furthermore, many of the long-term behavioral alterations caused by prenatal stress, as well as the structural alterations in brain areas controlling behavior, are gender specific (Reznikov et al., 1999; Rhees et al., 1999a; Bowman et al., 2004; Tobe et al., 2005; Weinstock, 2007; Mueller and Bale, 2008; Zuena et al., 2008).

Prenatal stress also has long-term effects on distinct endocrine axes (Kofman, 2002; Mairesse et al., 2008; Mueller and Bale, 2008), but whether modifications in hypothalamic structures are involved in this process remains largely unknown. Moreover, some of these endocrine alterations in response to early stress are sexually dimorphic (Horst et al., 2009; García-Cáceres et al., 2010). As normal hypothalamic development differs between males and females, resulting in sexually dimorphic hypothalamic structures and functions (Ward, 1972; Rhees et al., 1999a,b), it follows that environmental challenges or changes during this critical period may differentially affect each gender. Hence, it is conceivable that maternal and early stresses not only induce structural changes in the hypothalamus, but that these effects are sexually dimorphic and this could contribute to the gender differences in endocrine outcomes.

As little information is available in the literature regarding the possible structural alterations in the hypothalamus in response to chronic stress or whether these changes are sexually dimorphic, our aims in this study were to: (1) analyze the effect of maternal stress on hypothalamic cell turnover and synaptic density in the adult offspring, (2) determine whether these structural changes are different between males and females, (3) examine whether exposure to a second chronic stress during adulthood results in long-lasting hormonal and hypothalamic alterations and if exposure to chronic prenatal stress modulates this response and (4) determine whether the long-term response of the hypothalamic–pituitary–adrenal axis to maternal stress, which is different between males and females, can be correlated with structural changes in the hypothalamus.

2. Materials and methods

2.1. Materials

All chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO) or Merck (Barcelona, Spain) unless otherwise indicated.

2.2. Animals

All experiments were designed according to the European Union laws for animal care and the study was approved by the local institutional Ethical Committee. Young adult pregnant Wistar rats were housed individually under alternate light (12 h)–dark (12 h) periods and allowed free access to rat chow and tap water.

2.3. Experimental design

Prenatal restraint stress was performed daily in pregnant rats during the last week of gestation (gestational days 14–21) by placing them in transparent plastic cylinders (7 cm inner diameter, 19 cm long) along with bright light exposition, for 45 min, three times a day, as previously described (Ward and Weisz, 1980). Female rats from the control group remained undisturbed in their home cage.

At birth pups were housed with their mother with no handling of either the pups or the mothers until postnatal day 21 (P21) at which time they were weaned. Only litters of 9–14 pups were employed in the study. At P21, pups were distributed (four/cage) according to origin from control or stressed dams, with males and females being housed separately. At approximately P90, 9–10 animals from each of the two groups (control; C or prenatally stressed; PnS) for both sexes were subjected to adult stress (AS). Female rats were subjected to stress starting on day 2 of diestrus (as determined by daily vaginal swab). Adult stress was performed by using a similar protocol to that described for prenatal stress, but over 10 days. Either 2 or 14 days before sacrifice rats received i.p. injections of 5-bromo-2′-deoxyuridine (BrdU; Sigma–Aldrich, St. Louis, MO) for 2 consecutive days (50 mg/kg of body weight at a concentration of 10 mg/ml in sterile saline).