Hormones and Behavior 62 (2012) 345–355

Contents lists available at SciVerse ScienceDirect

Hormones and Behavior

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

Neonatal immune challenge alters reproductive development in the female rat

Luba Sominsky [a,*], Crystal L. Meehan [a], Adam K. Walker [b], Larisa Bobrovskaya [c], Eileen A. McLaughlin [d], Deborah M. Hodgson [a]

[a] Laboratory of Neuroimmunology, School of Psychology, Faculty of Science and IT, The University of Newcastle, Australia
[b] Integrative Immunology and Behavior Program, Department of Animal Sciences and Department of Medical Pathology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
[c] School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia
[d] ARC Centre of Excellence in Biotechnology & Development, School of Environmental & Life Sciences, Faculty of Science and IT, The University of Newcastle, Australia

ARTICLE INFO

Available online 14 February 2012

Keywords:
LPS
Postnatal
Immunological challenge
Reproductive development
Ovarian follicular reserve
Puberty onset
HPA axis
HPG axis
TH phosphorylation
Transgenerational

ABSTRACT

This article is part of a Special Issue "Neuroendocrine-Immune Axis in Health and Disease."

Neonatal lipopolysaccharide (LPS) exposure alters neuroendocrine, immune and behavioural responses in adult rats. Recent findings indicate that neonatal LPS treatment may have a more pronounced effect on the mating behaviours of females compared to males. The current study further explored the impact of neonatal inflammation on reproductive development in the female rat. Wistar rats were administered LPS (0.05 mg/kg, i.p.) or saline (equivolume) on postnatal days (PNDs) 3 and 5. The immediate effect of treatment was assessed on plasma corticosterone and tyrosine hydroxylase (TH) phosphorylation in the adrenal medulla. Weight gain and vaginal opening were recorded, and oestrous cyclicity was monitored post-puberty and in late adulthood. Blood and ovaries were collected throughout development to assess HPA and HPG hormones and to examine ovarian morphology. Reproductive success in the first (F1) generation and reproductive development in the second (F2) generation were also assessed. Neonatal LPS exposure resulted in increased TH phosphorylation in the neonatal adrenals. LPS treatment increased the corticosterone concentrations of females as juveniles, adolescents and adults, and reduced FSH in adolescence. Increased catch-up growth was evident in LPS-treated females, prompting earlier onset of puberty. Diminished follicular reserve was observed in neonatally LPS-treated females along with the advanced reproductive senescence. While fertility rates were not compromised, higher mortality and morbidity were observed in litters born to LPS-treated mothers. Female offspring of LPS-treated mothers displayed increased corticosterone on PND 14, increased catch-up growth and delayed emergence of the first oestrous cycle. No differences in any of the parameters assessed were observed in F2 males. These data suggest that neonatal immunological challenge has a profound impact on the female reproductive development, via the alteration of metabolic and neuroendocrine factors which regulate sexual maturation. Evidence of altered development in the female, but not male offspring of LPS-treated dams suggests increased susceptibility of females to the deleterious effects of neonatal immunological stress and its possible transferability to a subsequent generation.

Introduction

Extensive evidence has indicated sexual dimorphism with regard to human disease prevalence and susceptibility. Men are known to be more susceptible to cardiovascular and infectious diseases (Schroder et al., 1998), while women demonstrate higher rates of autoimmune and affective disorders (Bale, 2009; Whitacre, 2001). Several mechanisms have been suggested to underpin these sex-related differences. The primary hypothesis is based on differences in sex hormones, initiated via their organising effects in early life, and continuing into activating effects which influence the development of physiological and behavioural aspects of adult life (Van Goozen et al., 1995). In addition to the role played by gonadotrophin-releasing hormones (GnRH) and gonadal steroids in sexual differentiation and reproduction, sex hormones are also involved in immune system modulation and development (Tanriverdi et al., 2003; Verthelyi, 2001), as well as playing an important role in brain ontogeny (Harris, 1964). Sexual dimorphism in immune and neuroendocrine responses, driven by reproductive hormonal fluctuations, has been investigated in a number of human and animal studies (Grossman, 1985; Naor et al., 2009). Oestrogen has been shown to attenuate, and testosterone to exacerbate, inflammatory responses in rats (Razmara et al., 2005). In humans, oestrogen has been suggested to have an immunoprotective role, while testosterone has been found to be immunosuppressive (Schroder et al., 1998).

* Corresponding author. School of Psychology, The University of Newcastle, Callaghan, NSW 2308, Australia. Fax: +61 2 49216980.
E-mail address: luba.sominsky@newcastle.edu.au (L. Sominsky).

0018-506X/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2012.02.005

Given that major hormonal changes occur in adolescence, it is not surprising that sex differences in disease prevalence typically emerge during this period (Goel and Bale, 2009; McCormick and Mathews, 2007). However, the foundation for such susceptibilities is thought to be laid down earlier in life. The impact of the perinatal environment on development and predisposition to pathology has been gaining increasing attention in the last few decades. The developmental origins of health and disease (DOHaD) hypothesis proposes that exposure to adverse events during the perinatal period alters long term health outcomes (Barker and Osmond, 1987). Epidemiological data, for instance, have demonstrated an association between malnutrition experienced in early life and increased risk of cardiovascular disease, stroke and diabetes in later life (Barker et al., 1989; Barker, 1994, 2006). More recently the DOHaD hypothesis has been extended to explain the impact of a variety of stressors during the perinatal period. Typically stress during the perinatal period is associated with altered stress responsivity in the offspring, leading to an increased risk of health complications, including psychopathologies, later in life. A common occurrence during the perinatal period is exposure to infection, which activates the host immune response. Early life immune activation is known to impact normal development in both animals and humans resulting in long-term metabolic (Dimock et al., 2011; Walker et al., 2006), immune (Galic et al., 2009; Hodgson et al., 2001; Martinez et al., 1998; Walker et al., 2010), neuroendocrine (Matthews, 2002; Shanks et al., 2000) and behavioural (Meyer et al., 2006; Walker et al., 2009b) alterations. Sexual dimorphism in response to a neonatal immune challenge has also been recently shown to predict differences in adult immune and behavioural responses to inflammation (Kentner et al., 2010).

A growing body of evidence suggests that reproductive health and sexual behaviour might be affected by bacterial exposure occurring early in life (Knox et al., 2009; Kentner and Pittman, 2010; Wu et al., 2011). A recent study in our laboratory by Walker et al. (2011) has demonstrated that immune activation during early life compromises reproductive fitness in adulthood. Specifically, the study reported that male and female rats exposed to a bacterial mimetic (lipopolysaccharide: LPS) on days 3 and 5 postpartum exhibited altered puberty onset, and diminished hypothalamic–pituitary–gonadal (HPG) hormonal function. Interestingly, in this study males but not females exhibited increased anxiety-like behaviours in adulthood. When sexual behaviour was examined in adulthood, both sexes had impaired sexual performance, however, a greater number of behaviours were affected in females compared to males treated neonatally with LPS. Walker et al. (2011) also reported neonatal exposure to an immune challenge to induce long-term changes in testicular development and spermatogenesis. To date the impact of early life exposure to an immunological challenge on the development of female reproductive morphology and function has not been examined.

Given the previous findings indicating that neonatal immune activation has a robust effect on female sexual behaviour (Walker et al., 2011), and given the important role of the female rodent in the regulation of mating behaviour (Agmo, 1997), the aim of this study was to further explore the impact of neonatal immune activation on reproductive development in the female rat. The current study examined the effect of exposure to a neonatal immune challenge on the onset of puberty, female gonadal development by examining ovarian morphology at several critical time points in reproductive development and determined the onset of reproductive senescence. Finally, we assessed the impact of neonatal LPS exposure on reproductive success in the first (F1) generation (in terms of mating and offspring outcomes) and on reproductive development in the second (F2) generation. The important question being addressed in this component of the study is whether the reproductive history of the mother is transmitted to her offspring.

In an attempt to understand the mechanisms mediating alterations to reproductive fitness, this study also assessed the role of the sympathetic nervous system (SNS). Sympathetic innervation of the ovary is known to affect reproductive function and can be altered by systemic sympathetic activity (Greiner et al., 2005). Catecholamines, and in particular norepinephrine, play a significant role in the control of ovarian steroidogenesis and follicular development at puberty (Aguado et al., 1982; Aguado and Ojeda, 1984). Moreover, loss of sympathetic innervation in neonatal rats has been shown to result in the delayed onset of puberty and disruption of oestrous cyclicity (Lara et al., 1990). Catecholaminergic assessment is particularly relevant to this study given that we have recently reported that male rats exposed to LPS during the neonatal period exhibit an immediate and sustained activation of the sympatho-adrenomedullary system, by increasing phosphorylation of tyrosine hydroxylase (TH) (Sominsky et al., 2012). TH is a rate-limiting enzyme in catecholamine synthesis (Dunkley et al., 2004). It is unknown at this point whether the same activation occurs in female rats in response to neonatal LPS.

Methods

Experiment 1 — First generation (F1) study

Animals

Naïve female Wistar rats (8–10 weeks of age) obtained from the University of Newcastle animal house were mated in the University of Newcastle Psychology vivarium. 28 litters were randomly and evenly allocated into either LPS or saline control conditions at birth (postnatal day [PND] 1). No significant differences in litter size and male-to-female ratios were observed between LPS and saline-treated litters. On PND 3 and PND 5, pups were briefly removed from their home cages, weighed, and administered intraperitoneally (i.p.) with either 0.05 mg/kg LPS (Salmonella enterica, serotype enteritidis; Sigma-Aldrich Chemical Co., USA, dissolved in sterile pyrogen-free saline) or an equivolume of non-pyrogenic saline (Livingstone International, Australia). Drug administration procedures and housing conditions were identical to those previously described in A.K. Walker et al. (2009, 2010). A subgroup of animals (26 females: 12 LPS, derived from 3 litters; 13 Saline, derived from 4 litters) was sacrificed at 4 h and 24 h following neonatal drug administration on PND 5 when trunk blood and adrenal glands were collected to assess the immediate effect of treatment on HPA axis and SNS function. All male rats were treated identically to females and remained within the litters until weaning. The remaining female rats were randomly allocated for assessment at five developmental groups: (1) PND 14 (9 LPS, derived from 6 litters; 8 Saline, derived from 5 litters); (2) puberty onset (~PND 33) (40 LPS, derived from 8 litters; 33 Saline, derived from 8 litters); (3) adolescence (~PNDs 45–50) (20 LPS, derived from 6 litters; 22 Saline, derived from 7 litters); (4) adulthood (~PND 85) (7 LPS, derived from 5 litters; 7 Saline, derived from 5 litters); and (5) oestrous cyclicity decline in late adulthood (9–12 months) (11 LPS, derived from 3 litters; 8 Saline, derived from 2 litters). A final group of animals was used for mating purposes (4 LPS, derived from 3 litters; 4 Saline, derived from 3 litters) in order to assess the potential for transgenerational changes in sexual development in the second (F2) generation. Figs. 1(a and b) provides a schematic timeline of the protocols. Apart from those sacrificed on PND 5 and PND 14, animals were left undisturbed until weaning (PND 22) when they were separated into same-sex pair housing (41.5 cm × 28.0 cm × 22.0 cm cages; Mascot Wire Works, Sydney). Following weaning, female rats underwent daily assessment of pubertal markers. Animals were maintained under a normal 12 h light/dark schedule (lights on 06:00 h); temperature (21 ± 1 °C), with food and water available ad libitum. All experimentation occurred in accordance with the 2004 NH&MRC Australian Code of Practice for the care and use of animals for scientific practice.

Neonatal blood and adrenal gland collection

Four hours following injection on PND 5 blood and tissues were obtained for assessment of plasma corticosterone responses to