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Research Report

Prenatal stress may increase vulnerability to life events: Comparison with the effects of prenatal dexamethasone

Karin S. Hougaard[a,*], Maibritt B. Andersen[b], Sanna L. Kjær[a], Åse M. Hansen[a], Thomas Werge[b], Søren P. Lund[a]

[a]National Institute of Occupational Health, Lersø Parkallé 105, DK-2100 Copenhagen, Denmark
[b]Sct. Hans Hospital, DK-4000 Roskilde, Denmark

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Abstract

Prenatal stress has been associated with a variety of alterations in the offspring. The presented observations suggest that rather than causing changes in the offspring per se, prenatal stress may increase the organism's vulnerability to aversive life events. Offspring of rat dams stressed gestationally by chronic mild stress (CMS, a variable schedule of different stressors) or dexamethasone (DEX, a synthetic glucocorticoid, i.e., a pharmacological stressor) was tested for reactivity by testing their acoustic startle response (ASR). Two subsets of offspring were tested. One was experimentally naïve at the time of ASR testing, whereas the other had been through blood sampling for assessment of the hormonal stress response to restraint, 3 months previously. Both prenatal CMS and dexamethasone increased ASR in the offspring compared to controls, but only in prenatally stressed offspring that had been blood sampled 3 months previously. In conclusion, similarity of the effects of maternal gestational exposure to a regular stress schedule and of exposure to a synthetic glucocorticoid suggests that maternal glucocorticoids may be a determining factor for changes in the regulatory mechanisms of the acoustic startle response. Further, a single aversive life event showed capable of changing the reactivity of prenatally stressed offspring, whereas offspring of dams going through a less stressful gestation was largely unaffected by this event. This suggests that circumstances dating back to the very beginning of life affect the individual's sensitivity towards experiences in life after birth. The prenatal environment may thus form part of the explanation of the considerable individual variation in the development of psychopathology.

Theme: Development and regeneration
Topic: Nutritional and prenatal factors, hormones and development

Keywords: Acoustic startle response; Chronic mild stress; Corticosterone; Dexamethasone; Fear potentiation; Prenatal stress

1. Introduction

Maternal stress during gestation may lead to a variety of behavioral, neuroendocrine, and neuroanatomical alterations in the offspring. Hence, cognitive function, social behavior, and levels and distribution of regulatory neurotransmitters have shown susceptible to maternal stress during fetal life [37]. In the absence of a direct neural connection between the developing fetus and the dam, maternal hormones have been hypothesized to mediate the effects of prenatal stress, particularly through alterations in the maternal hypothalamic–pituitary–adrenal (HPA) axis. The trophic hormones of the HPA axis, i.e., adrenocorticotropic hormone and corticotropin releasing factor (CRF), do not traverse the placenta in the rat [5,42]. In contrast, the adrenal glucocorticoids passes from the maternal to the fetal compartment [48]. The role of glucocorticoids as mediators of prenatal stress effects has been investigated by administering

Abbreviations: ASR, acoustic startle reaction; CMS, chronic mild stress; DEX, dexamethasone; PPI, prepulse inhibition

* Corresponding author. Fax: +45 39 16 52 01.
E-mail address: ksh@ami.dk (K.S. Hougaard).
URL: www.ami.dk. (K.S. Hougaard).

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corticosterone (or synthetic analogues, e.g., dexamethasone) to pregnant dams and observing the offspring. Results from these studies suggest that fetal glucocorticoid exposure poses part of the mechanism by which prenatal stress programs the offspring (reviewed in [22,38]).

Only few studies have investigated the consequences of prenatal stress for postnatal development in humans, and most are limited by the use of retrospective designs, small sample sizes, or lack of control for confounders. Further, the results from the few well-designed human studies are concordant, i.e., decreased adaptation to novelty, altered attention, and increased emotionality [15]. Although the range of behavioral abnormalities is much more limited in animals than in humans, animal models allow for control of environmental factors and hypothesis testing based on manipulation of not only the prenatal but also the postnatal environment [37]. Animal models are therefore of great value in order to identify which behavioral domains or physiological systems that are particularly vulnerable to prenatal stress, to investigate whether individual sensitivity plays a role and which physiological mechanisms that mediate the effects of prenatal stress [15]. Findings in prenatally stressed animals and humans show many similarities. Thus, changes associated with the 'prenatal stress syndrome' point towards increased fearfulness in novel situations as well as reduced abilities to cope with stress [37,38]. The adult offspring of rat dams subjected to stressors during gestation displays an increase in anxiety-related behaviors, e.g., suppressed exploration of the open areas of the elevated plus maze [27,49] and increased defensive withdrawal [35]. An important physiological characteristic of prenatally stressed offspring is a hyper-active HPA axis, most often observed as an enhanced and/or prolonged plasma corticosterone response to stressors [36]. Also neural systems related to CRF are susceptible to stress in fetal life. In the amygdala, there are reports of increased CRF concentration, release, and receptor density after prenatal stress [2,35,40]. Gestational stress has been observed to facilitate the development of CRF-containing neurons [6,29], and in prenatally stressed animals, CRF receptor antagonists normalize fearful behavior [35].

In a previous study, we found that prenatally stressed offspring displayed a persistently enhanced acoustic startle response (ASR, a characteristic sequential contraction of the skeletal musculature evoked by a sudden and intense acoustic stimulus) [14]. Specifically CRF has been shown to intensify the acoustic startle response [20,28,32]. The apparent up regulation of CRFergic systems in prenatally stressed animals therefore poses an attractive means of explaining our observation of enhanced ASR in prenatally stressed animals [14,41].

The ASR may also be enhanced by aversive expectation about potential dangers. In context aversive conditioning a repulsive event is linked to the experimental context of this event. Subsequent exposure of the animal to this or resembling contexts will trigger anxiety. In the case of ASR, this will be displayed as an enhanced startle response, i.e., fear potentiated startle [9]. Prenatal stress has been shown to enhance conditioned fear and increase the sensitivity to environmental perturbations throughout life [8,30]. In our previous study, the increased ASR was observed in prenatally stressed animals that had been blood sampled for investigation of their hormonal stress response. This procedure involved 20 min of restraint and repeated blood sampling (described in [14]). Restraint is aversive to rats [7], and part of the experimental procedure of ASR measurement bears resemblance to aversive elements of the blood sampling procedure, i.e., confinement in a test tube. We therefore hypothesized that the aversiveness associated with blood sampling lead to the pronounced ASR in prenatally stressed animals, due to enhanced fear potentiated startle. To evaluate whether prenatal stress effects on ASR occurred as a consequence of fetal glucocorticoid exposure, the ASR was investigated in prenatally stressed offspring as well as offspring of dams injected gestationally with a potent synthetic glucocorticoid, dexamethasone.

2. Materials and methods

2.1. Animals

66 time-mated young adult nulliparous female Wistar rats (HanTac:WH, Taconic M&B, Denmark) were randomly distributed to white plastic cages (27 × 43 × 15 cm) with pine-bedding (Lignocel S8) in pairs, upon arrival at gestation day (GD) 3. Environmental conditions were automatically controlled with a 12-h light–dark cycle. Food (Altromin Standard Diet 1324) and tap water were provided ad libitum. Clean cages and new bedding were provided twice weekly.

The animal welfare committee, appointed by the Danish Ministry of Justice, granted ethical permission for the studies. All procedures were carried out in compliance with the EC Directive 86/609/EEC and with the Danish law regulating experiments on animals.

2.2. Exposure

The day after arrival (GD 4), the animals were weighed and assigned to three groups, control, dexamethasone (DEX), and chronic mild stress (CMS). Body weights were recorded at GD 4, 7, 10, 13, 15, 17, 19, and 21. Dexamethasone (100 μg/kg per day, Sigma-Aldrich, Denmark) dissolved in 4% ethanol–0.9% saline (100 μg/mL) was injected s.c. in the skin of the neck during the last week of gestation (GD 14–21). Control and CMS animals received vehicle injections during the same period. The CMS model is a schedule of chronic stress, where various relatively mild stressors are presented in a random schedule, e.g., change of partner and isolation housing. The model was developed as an alternative to conventional animal models of chronic stress that often include physical and potentially painful stressors [31]. For