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Protective effect of early prenatal stress on the induction of asthma in adult mice: Sex-specific differences

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HIGHLIGHTS

- Prenatal stress early in pregnancy programmed behavioral responses in adult mice.
- Prenatally stressed females presented attenuated allergic response in adulthood.
- The effects of prenatal stress on asthma development depend on the stage of pregnancy.

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ABSTRACT

Adversities faced during the prenatal period can be related to the onset of diseases in adulthood. However, little is known about the effects on the respiratory system. This study aimed to evaluate the effects of prenatal stress in two different time-points during pregnancy on pulmonary function and on the inflammatory profile of mice exposed to an asthma model. Male and female BALB/c mice were divided into 3 groups: control (CON), prenatal stress from the second week of pregnancy (PNS1) and prenatal stress on the last week of pregnancy (PNS2). Both PNS1 and PNS2 pregnant females were submitted to restraint stress. As adults, fear/anxiety behaviors were assessed, and animals were subjected to an asthma model induced by ovalbumin. Pulmonary function, inflammatory parameters in bronchoalveolar lavage (BAL) and histology were evaluated. There was a significant decrease in the number of entries and time spent in the central quadrant on the open field test for the PNS1 animals. Females (PNS1) showed improved pulmonary function (airway resistance, tissue damping and pulmonary elastance), significant increase in the percentage of neutrophils and lymphocytes and a decrease in eosinophils when compared to controls. There was a significant decrease in inflammatory cytokines in BAL of both males (IL-5 and IL-13) and females (IL-4, IL-5 and IL-13) from PNS1 and PNS2 when compared to the CON group. Prenatal stress starting from the beginning of pregnancy reduces the impact of asthma development in adult female mice, showing an improved pulmonary function and a lower inflammatory response in the lungs.

1. Introduction

Adversities faced during the prenatal period might be implicated in fetal programming and in the development of diseases in adulthood, as explained by The Developmental Origins Theory [1,2]. Stress during the prenatal period can cause alterations in different organs and systems [3], such as changes in fear/anxiety behaviors [4], dysfunctions related to diabetes mellitus type 2 [5], predisposition to cardiovascular diseases [6] and impairment in the development and functioning of structures of the Central Nervous System (CNS) [7], among others.

The development of the respiratory system in rodents starts early in gestation (around day 9) [8], which allows changes in the prenatal period to program the development of this system [9]. This seems to be related to epigenetic modifications of genes encoding proteins that are involved in the regulation/activation of the hypothalamic-pituitary-adrenal (HPA) axis, leading to long-lasting effects [10]. Regardless of the precise mechanism, besides behavioral changes such as increased anxiety, prenatal stress can induce an enhancement in airway hyperreactivity, with augmented production of mucus in adult animals. Also, exposure to prenatal stress could lead to an increment in the recruitment of eosinophils to the lungs, which is a key feature in asthma [9]. On the other hand, the stimulatory role of glucocorticoids on prenatal lung development is well-known [11,12]. Hence, it is possible that exposure to continuous stress in this period could produce an increase in glucocorticoid secretion and result in protective effects on

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the development of the respiratory system. However, the complexity of long-term effects of prenatal stress on asthma is still poorly studied.

Asthma is a highly prevalent chronic disease [13] that presents scarcely known multifactorial (genetic and environmental) origins, which are related to atopy [14,15]. Thus, considering the impact of asthma in worldwide populations, it is important to study the mechanisms and effects that premature variations early in life, such as the ones caused by stress, can have on aspects related to the disease physiopathology.

Moreover, several studies have shown that the effects of prenatal stress may be different in males and females [16–19]. Also, it is known that asthma is more prevalent among males [20] and that early life stress effects can affect the response to asthma in a sex-dependent manner [21], which highlights the importance of studying the influence of sex on the interaction between prenatal programming and respiratory diseases.

Thus, considering the importance of the prenatal period in the development of numerous systems and the lack of studies on long-term effects of stress during this period on pulmonary development and on the expression of respiratory diseases, we evaluated the consequences of stress in different time-points of the gestational period and their connection to asthma development in adulthood, including the analysis of potential sex differences. Therefore, the goal of this study was to evaluate outcomes of prenatal stress induced by restraint in two different time-points of pregnancy on pulmonary function and on inflammatory profile of male and female mice exposed to a model of asthma induced by ovalbumin. Also, as a control of long-term programming effects induced by alterations in the prenatal environment, anxiety-like behavior (open field test) and HPA axis response (corticosterone measurement) were evaluated.

2. Material and methods

2.1. Animals

BALB/c mice were acquired from the Center of Biological and Experimental Models - CeMBE from PUCRS and maintained in a vivarium under light/dark cycles of 12 h with free access to food and water. All animal procedures followed the guidelines described in the *Guide for the Care and Use of Laboratory Animals* [22], and the study was previously approved under registry number 11/00270 by the University ethics committee in animal use (CEUA).

2.2. Experimental design

Adult BALB/c primiparous females (6–8 weeks) were monitored for their estrous cycle and then were mated to a male (6–8 weeks). Pregnancy was identified by the presence of a vaginal plug, and females were then separated individually in boxes and divided in three groups: control, prenatal stress 1 (PNS1) and prenatal stress 2 (PNS2). After birth, the litter was culled to 5–7 animals and pups were weighed on days 1, 10 and 21, when they were weaned. Males and females were separated in boxes with a maximum of 5 animals. Mice were kept until adulthood for experiments without any type of intervention except for routine cleaning. Fear/anxiety behavior analyses were performed between days 50–54 through the open field test. On the 56th day of life, males and females were sensitized by two subcutaneous (s.c) injections with ovalbumin (OVA) on days 1 and 7. Then, an intranasal (i.n) challenge was performed on days 14, 15 and 16. On the 17th day, animals were anesthetized and a pulmonary function test was performed for the evaluation of airway resistance, tissue damping and elastance. Bronchoalveolar lavage (BAL) was collected and used for total and differential cell count and for the evaluation of inflammatory cytokines. After animals were euthanized, the left lung was removed and processed for histological analysis. The experimental design as described is shown on Fig. 1.

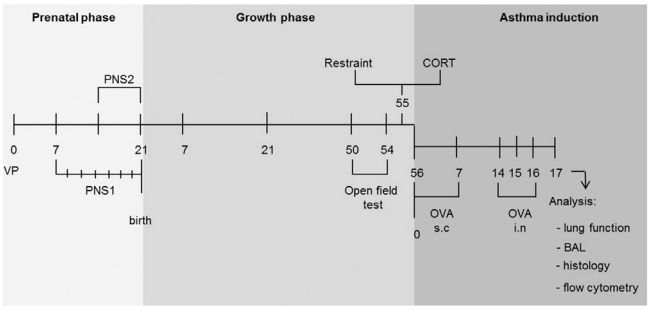

Fig. 1. Experimental design used in the study. VP: vaginal plug; PNS1: prenatal stress from the second week of pregnancy; PNS2: prenatal stress at the last week; Restraint: 30 min restraint stress; CORT: corticosterone measurement; OVA: ovalbumin; s.c: subcutaneous; i.n: intranasal; BAL: bronchoalveolar lavage.

2.3. Maternal exposure to stress

Stress induction was performed by restraint in a closed cylinder (Insight, Brazil) for 30 min. Pregnant females from PNS1 group were subjected to stress early in the gestational period, from the 8th day of pregnancy until birth, every other day. Females from PNS2 group were subjected to stress later in the gestational period (from day 14 to 21), everyday during the last week of pregnancy. The control group did not undergo any intervention during the gestational period.

2.4. Behavioral analysis

Behavioral analysis was performed as a control of long-term programming effects induced by alterations in the prenatal environment. For that, an open field test was performed to evaluate fear/anxiety behavioral responses in adulthood (50–54 days), as previously described [23]. Briefly, this test consists in a squared black box/arena (45 cm × 45 cm × 15 cm of height) divided in central quadrant and periphery. The test consists of placing the animal in a corner of the open field (randomly chosen) and recording the activity of the mouse on video for 10 min. Two specific behaviors were determined: number of entries in the central quadrant and time spent in the central quadrant (measured in seconds). Video analyses of the behaviors recorded were performed using ANY-maze software (version 4.7).

2.5. Corticosterone measurement

In order to evaluate the HPA axis response to a second hit stress, the circulating corticosterone level was measured. When animals (males and females) reached adulthood they were subdivided in two groups (i) decapitated under basal conditions or (ii) subjected to a new restraint stress (as previously described) of 30 min and immediately decapitated for blood collection. Blood was then centrifuged at 13,000 rpm and serum was stored at −80 °C for further analysis. Corticosterone concentration was measured by ELISA (Corticosterone EIA Kit, Cayman Chemical Company, USA) according to the manufacturer. Final results are expressed as pg/mL. The lower limit of detection was 30 pg/mL.

2.6. Asthma induction protocol

On the 56th day of life, males and females were again divided into groups: OVA and phosphate-buffered saline (PBS). Animals on the OVA group were sensitized with ovalbumin (grade VI; Sigma-Aldrich). Two subcutaneous injections (s.c) were performed with 20 μg of OVA diluted in total volume of 200 μL (PBS) per animal (days 1 and 7). Animals were sedated with isoflurane in an anesthetic chamber and then an intranasal challenge was performed, with 100 μg of OVA in 50 μL of PBS applied on each animal (days 14, 15 and 16). Animals on