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Inverse effects of lipopolysaccharides on anxiety in pregnant mice and their offspring

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ABSTRACT

This study aimed to evaluate the effects of the bacterial lipopolysaccharide (LPS) exposure during early pregnancy on anxiety-related behaviour of both pregnant female mice and their male offspring. Pregnant NMRI mice were treated with subcutaneous injections of LPS (30, 60, 120, 240 and 480 μg/kg) on the tenth gestational day of pregnancy. Pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6 and corticosterone levels, were measured in maternal serum 1.5 h following the LPS injections. Baseline anxiety levels of pregnant mice (1.5 h after LPS administration) and their male offspring (at postnatal days 60–70) were investigated with the elevated plus maze (EPM) test. In addition, anxiety levels in the offspring were measured after 2 h restraint stress or TNF-α (10 μg/kg) administration. Our results demonstrate that LPS administration induces anxiety-like behaviour and a significant increase in cytokines and corticosterone levels in maternal serum. However, in male offspring, prenatal LPS administration has no significant effects on serum cytokines and corticosterone secretion with an exception of the lowest LPS dose that slightly reduced corticosterone levels. Interestingly, prenatal LPS treatment seemed to decrease the baseline anxiety levels, while pretreatment with restraint stress or TNF-α abolished this anxiolytic effects. In summary, our results suggest that prenatal exposure to LPS during early pregnancy may result in reduced baseline anxiety in adult male offspring.

1. Introduction

Fear and anxiety states are normal human emotional experiences; however, excessive or inappropriate anxiety can become a life-impairing illness. In fact, anxiety disorders are at present among the most common mental illnesses affecting around 20% of the world population [1,2]. Studies have shown that anxiety can be shaped by early environmental experiences, the impacts of which are modulated by genetic susceptibility factors [1,3]. Such interactions can induce persistent structural and functional changes in the brain that underlie susceptibility to anxiety states. Studying prenatal factors that influence brain development as well as the plastic changes they trigger may help us to understand neurobiological mechanisms of anxiety disorders [1,4,5].

Increasing amount of data support the notion that many psychiatric disorders, including excessive anxiety, are neurodevelopmental in origin [6]. Maternal exposure to stress during pregnancy presents a parsimonious mechanism that is able to alter foetal brain development. Several studies demonstrate the effects of maternal bereavement, viral infections, malnutrition or an exposure to natural disaster during certain gestational windows on the later emotional and cognitive behaviour of the offspring [7–9]. Epidemiological studies have shown that maternal bacterial and viral infections during pregnancy represent a significant risk factor for several neuropsychiatric disorders. Animal studies have illustrated that bacterial and viral infections in pregnant females cause a spectrum of neuropathological and behavioural abnormalities in offspring resembling the symptoms of schizophrenia and autistic spectrum disorders [10–12].

The wide variety of infectious agents associated with an increased risk for mental disorders, like schizophrenia, suggests that mechanisms common to various prenatal infections may affect foetal development [13,14]. Although the mechanisms underlying this epidemiological relationship remain unclear, the maternal cytokine-associated inflammatory response to infection may be the crucial link [10,15,16].

In the present study, we investigate the effects of prenatal exposure to bacterial endotoxin on the development of HPA axis, anxiety-related behaviour, serum pro-inflammatory cytokine levels and serum corticosterone levels in pregnant mice and their adult male offspring.

2. Materials and methods

2.1. Animals

Female and male NMRI mice (Pasteur institute, Iran and Charles River, Germany) aged 8 to 10 weeks at the time of testing, were housed in groups of 4 animals per cage in a room with a 12:12 h light / dark

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cycle (lights on 07:00 a.m.) and controlled temperature (23 ± 1 °C). Animals had access to food and water ad libitum. Breeding began after 2 weeks of acclimatization period in the new animal facility; breeding procedure and the verification of pregnancy were performed as previously described [10,17]. The number of animals in each litter was standardized (3 male and 3 female pups/dam) in order to standardize the milk availability. Pups were weaned on the postnatal day 21 (PD 21) and housed with 4 animals from the same treatment in a cage. Pregnant dams and their male offspring were randomly divided into control and experimental groups (pregnant dams: $n = 8$–9; male offspring: $n = 8$–12, with max. two pups from one litter per group) [18,19].

All experimental procedures were approved by the Committee on Animal Health and Care of the State of Middle Frankonia (Regierung Mittelfranken, Germany; Az 54-2532.1-2/12-2) and performed in strict compliance with the European Union Directive for the care and use of laboratory animals.

2.2. LPS treatment

In order to model a physiological maternal infection, pregnant mice were administered with subcutaneous injections of LPS (from *Salmonella enterica* serotype *abortus equi*, Sigma-Aldrich, Germany) on the tenth gestational day. This procedure was previously shown to produce optimal fever and cytokine induction in mice while having limited impact on maternal and pup survival (i.e., 30, 60, 120, 240 and 480 μg/kg of LPS, solved in saline, 0.05 ml/animal). LPS-injections were performed between 9:00 and 11:00 a.m. [13,20–23]. Control groups were treated with intradermal injections of saline (0.05 ml/animal).

2.3. Experimental design

All experiments were performed during the activity phase of the animals between 10:00 a.m. and 02:00 p.m. Pregnant mice were divided into 3 groups. Group one was sacrificed 1.5 h after LPS or saline administration, and their blood samples were used for evaluating corticosterone and cytokines levels. Group two mice gave birth and nurtured their offspring naturally, whereas the third group was used for behavioural tests. Adult male offspring (PD 60–70) born and nurtured by the second maternal group either underwent behavioural testing or were used for the evaluation of serum corticosterone and cytokines levels.

2.4. Quantification of serum corticosterone and cytokines

Blood samples of pregnant dams (1.5 h after LPS-injections) and adult offspring (after PD 60) were collected from the retro-orbital vein plexus of the animal's eyes using hematocrit tubes. Serum was prepared by centrifugation at 15000 rpm for 5 min, aliquoted and then stored at −80 °C until the cytokine assays were performed. Concentrations of corticosterone (Kamiya Biomedical Company, KT-510, Seattle, WA, USA), interleukin 1 beta (IL-1β) (Immuno-Biological Laboratories, IB49700, USA), interleukin 6 (IL-6) (BioSource International, CA 93012, USA) and tumour necrosis factor (TNF-α) (Ucytech, CT 302 Netherlands) were determined using commercial ELISA kits in accordance with the manufacturer's instructions. All samples and standards were assayed in duplicate.

2.5. Anxiety test

Elevated plus maze (EPM) test was applied to access anxiety levels of the animals. EPM is a wooden, plus-shaped apparatus, 50 cm elevated above the ground. The maze is composed of two open arms (30 cm × 5 cm) and two enclosed arms (30 cm × 5 cm × 15 cm), with each arm having an open roof. The maze was placed in the centre of a quiet and dimly lit (60 lx) room. Following their respective treatment, mice were placed individually in the centre of the plus-maze, facing one of the open arms. Behavioural data were accessed online by a "blind" observer who was positioned 1 m behind one of the closed arms of the maze. The observer measured: (1) time spent in the open arms, (2) time spent in the closed arms, (3) number of entries into the open arms and (4) number of entries into the closed arms during the 5-min test period. An entry was defined as "all four paws in the arm." The maze was cleaned with distilled water after each mouse was tested. Open-arm activity was quantified as the amount of time that the mouse spent in the open arms relative to the total amount of time spent in any arm (open/total × 100); the number of entries into the open arms was quantified relative to the total number of entries into any arm (open/total × 100) [24–26]. The total number of arms entered as well as the total number of closed arms entered were used as indexes of general locomotor activity [27,28]. EPM-test in dams was carried out 1.5 h after injection of LPS and anxiety test of offspring was carried out at postnatal days 60–70 [7,13,29].

2.6. Evaluating the effects short-term restraint stress on anxiety levels of the male offspring

Separate groups of male offspring were subjected to a short-term restrained stress procedure prior to the EPM-testing in order to evaluate anxiety levels of mice prenatally exposed to LPS (120, 240 and 480 μg/kg) or vehicle under stress-conditions. Restraining was performed for 2 h in a well-ventilated plastic tube, ensuring that the mouse was not compressed. After 2 h, restraint stress mice were returned into their home cages for 30 min before exposing to the EPM [30,31].

2.7. Evaluating the effects TNF-α on the anxiety levels of prenatally LPS-treated mice

Prenatally LPS (120, 240 and 480 μg/kg) or vehicle-treated offspring were intraperitoneally (i.p.) injected with TNF-α (Bioscience, USA) at the dose of 10 μg/kg. After TNF-α injections, mice were returned to their home cages for 30 min before behavioural evaluation on the elevated plus maze was performed [32].

2.8. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) using SPSS 17.0 (Chicago, IL, USA). For post hoc testing, least significant difference (LSD) test was applied, if appropriate, to assess specific group differences. Data are presented as mean ± SEM. Differences with $p < 0.05$ between experimental groups at each point were considered statistically significant.

3. Results

3.1. Corticosterone and cytokine levels increase following LPS administration in pregnant dams

LPS injections at 60, 120, 240 and 480 μg/kg are associated with a significant increase of corticosterone levels in serum of pregnant dams ($F_{5,\ 42\ LPS} = 10.05$, $p < 0.0001$, Fig. 1A).

Furthermore, an administration of bacterial LPS significantly increases serum levels of pro-inflammatory cytokines, IL-1β ($F_{5,\ 42\ LPS} = 18.73$, $p = 0.0001$, Fig. 1B), IL-6 ($F_{5,\ 42\ LPS} = 3.167$, $p = 0.0163$, Fig. 1C) and TNF-α ($F_{5,\ 48\ LPS} = 19.69$, $p = 0.0001$, Fig. 1D) in pregnant dams.

3.2. Increased anxiety in pregnant dams following LPS administration

Fig. 2 exhibits the effect of LPS treatment on the EPM-behaviour of pregnant dams. One-way ANOVA reveals a significant difference in the percentage of open arm time ($F_{5,\ 42\ LPS} = 4.059$, $p = 0.0043$, Fig. 2A) and entries ($F_{5,\ 42\ LPS} = 3.756$, $p = 0.0067$, Fig. 2B) between LPS-treated dams (120, 240 and 480 μg/kg) and the vehicle group. No