Prenatal Stress Up-Regulated Hippocampal Glucocorticoid Receptor Expression in Female Adult Rat Offspring

Estrés Prenatal Expresión delReceptor de Glucocorticoides del Hipocampo Regulado por Aumento en Crías de Ratas Hembras Adultas

Libin Liao; Xueping Yao; Jufang Huang & Shengbin Bai

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**SUMMARY:** Accumulating evidence from preclinical and clinical studies indicates prenatal exposure to stress or excess glucocorticoids can affect offspring brain. Glucocorticoid receptor (GR) is an important target of glucocorticoid. Therefore the aim of the present study was to investigate the expression of GR in prenatally stressed adult offspring and the relationship between GR expression and behavior in offspring. Pregnant rats received restraint stress during the last week of pregnancy. Hippocampal glucocorticoid receptor expression levels in the offspring were detected on postnatal 60 (P60). Cognition function was also detected. It shows significantly lower hippocampal GR expression was observed in female prenatally stressed offspring compared with their controls at P60. Corresponding to the expression of GR, female prenatally stressed offspring exhibited poorer spatial learning and memory abilities in the Barnes maze than control. This suggests that cognitive impairment in prenatally stressed rat offspring attribute lower hippocampal GR expression.

**KEY WORDS:** Prenatal stress; Cognitive impairment; Glucocorticoid receptor; Hippocampus.

**INTRODUCTION**

Early life events have long lasting impacts on tissue structure and function. Pregnancy is a specific time in a woman’s life. During pregnancy, any external environmental influences such as maternal psychological stress not only affect the mother’s health but also have lifelong consequences for her developing unborn (Rakers et al., 2017). Prenatal stress is a public health problem that has been reported in 10 %-35 % of children worldwide (Markham & Koenig, 2011). It is now widely recognized that exposure to an adverse environment during pregnancy is associated with an adverse pregnancy outcome and increases the individual’s risk to develop neurobehavioral disorders, including depression, attention deficit hyperactivity disorder and schizophrenia (Markham & Koenig; Walker et al., 2008; Ronald et al., 2011; McEwen et al., 2011) cardiovascular and metabolic disease in later life.

A major mechanism to explain the association between adverse prenatal stress and postnatal health is overexposure to glucocorticoids. During the process of prenatal stress, over-activation of a pregnant mother’s hypothalamo-pituitary adrenal (HPA) axis, produces excess maternal glucocorticoids and affects the structure and the function of the brains of the offspring. However, currently, little is known about the underlying molecular mechanisms by which excess maternal glucocorticoid levels affect the brains of offspring (Pallarés et al., 2017).

The glucocorticoid receptor is the target of glucocorticoids. It is an important modulators in HPA axis activation response to stress. Importantly, GR is expressed at high levels in the fetal brain of many species, including humans (Moisidis et al., 2014). Liu et al. (1997) showed that in rats, exposure to high levels of postnatal care leads to
increase GR mRNA expression in the hippocampus and reduced HPA axis response to restraint stress as adults compared with offspring raised by low licking-grooming dams. Human studies also indicated hippocampal GR targets might be subjected to programming by early life events (Cottrell & Seck, 2009). These results indicated that glucocorticoid receptor may has associated with behavioural outcomes influenced by prenatal stress. Therefore, the objective of the present study is to analyze the effects of prenatal stress in the expression of glucocorticoid receptor in hippocampus of adult offspring.

MATERIAL AND METHOD

Animals. Adult female Sprague-Dawley rats (250–300 g) were used in this study. They were housed in an animal care facility within the Department of Laboratory Animals in Central South University, Hunan, P.R. China, under stable temperature (22 ± 1°C) and lighting conditions (12-h light/12-h dark cycle). Food and water were provided, along with constant care and clean conditions.

All animal experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23), revised in 1996, and were approved by the Animal Ethics Committee of the Third Xiangya Hospital (Changsha, China). All efforts were taken to minimize the number, suffering, and discomfort of all laboratory animals used in this study.

Prenatal stress procedure. Pregnant females were housed individually and were randomly assigned to a stressed (n = 6) or control (n = 6) group. The prenatal stress protocol was adapted from previous studies and was performed at the last week of pregnancy. Pregnant dams were placed into transparent plastic restrainers (8.6 cm in diameter × 21.6 cm in length) at 09:00 h, 13:00 h, and 17:00 h for 45 min daily. Control dams were left undisturbed in their home cages. After birth, the pups were raised with their mothers. The offspring were weaned on postnatal day 21 and group-housed with littermates of the same sex.

Behavioral analysis. The Barnes maze was used to test the spatial learning and memory abilities of the subjects. This test was performed as previously described (Barnes, 1979). The maze used in the present study was a white, circular disk (122 cm in diameter) with 18 holes (9.5 cm in diameter, placed every 20°, and close to the edge of the disk) and a high stand (140 cm height) supporting the disk. The first trial was the habituation phase. Each rat was placed in a cylindrical black start chamber in the middle of the maze. After 15 s had elapsed, the chamber was lifted. Then, the rat was exposed to a bright light and gently guided to the escape box. The rat was allowed 3 min to search for the escape box following stimulation with the same light. If the rat failed to locate the escape box, it was gently guided to the box. Once the rat was inside the box, the light was turned off, and the rat was permitted to stay in the escape box for 2 min. The entry hole in the box was covered with a black sheet. The spatial location of the target box and the corresponding hole were relatively fixed over the 4-day test (3 trials/day), as were the spatial cues in the room. The rat was then returned to its home cage for a 15-min interval before the next trial. Between each trial, the surface of the disk was cleaned with 75 % ethanol to remove any potential odor cues. The escape latency for reaching the target hole and the escape errors were recorded and compared.

Tissue collection. Rats were sacrificed via decapitation after anesthetization with 5 % sevoflurane, and whole brains (including the entire hippocampus) were quickly removed. The brain tissues used for immunostaining were post-fixed with 4 % paraformaldehyde for 24 h at 4°C. The tissues were then sequentially placed in 15 % and 30 % sucrose in 0.1 M PBS at 4°C. Next, the brain tissues were embedded in Tissue-Tek optimal cutting temperature medium (Sakura Finetek Japan Co. Ltd. Tokyo) frozen in liquid nitrogen, and stored at -80°C. For Western blot analysis, samples of the entire hippocampus were immediately isolated, frozen in liquid nitrogen and stored at -80°C.

Western blot analysis. Proteins were extracted from the hippocampal regions. Protein concentrations were determined using a bicinchoninic acid (BCA) protein assay kit (CWBio, China). Proteins (40 µg) were separated on 8 % denaturing acrylamide gels (SDS-PAGE) and transferred to membranes. The membranes were incubated with rabbit monoclonal rabbit monoclonal anti-GR (1:1000; Proteintech Group) antibodies. After rinsing with Tris-buffered saline (Sigma, USA) containing 0.3 % Tween-20 (Beijing solarbio science technology Co. Ltd. Beijing, China), the membranes were incubated with an IRDye® 800CW goat anti-rabbit secondary antibody (1:8000; 926-32211, Li-COR®, USA) for 1 h at room temperature.

After washing, the immunoblotted bands were visualized using an Odyssey-CLX infrared imaging system (Li-COR®). GAPDH was used to normalize protein levels as an internal reference control. The integrated density values of specific proteins were quantified using Image J software (National Institutes of Health, MD, USA), and the relative expression levels of the proteins were normalized by calculating the ratio of the target proteins (GR) to GAPDH.
**Immunohistochemistry.** The hippocampal tissues were serially cut into 20 mm thick sections on a freezing sliding microtome (Leica CM1950, Leica, Germany). Free-floating sections of hippocampal tissues were washed with PBS (Sinopharm Group, Co. Ltd, Beijing, China) and then sequentially treated with 3 % hydrogen peroxide (Sinopharm Group Co. Ltd) in 0.01 M PBS for 10 min and 5 % bovine serum albumin (BSA, Sigma, MO, USA) in 0.01 M PBS containing 0.3 % Triton X-100 (Beijing solarbio science technology Co. Ltd) for 1 h. Next, the tissue sections were incubated with a polyclonal rabbit anti-GR antibody (1:200; Proteintech Group, Wuhan, China) overnight at 4°C. Then, the sections were washed with PBS and subsequently exposed to the corresponding biotinylated secondary antibody (1:200; Vector Laboratories, USA). After a 1 h incubation with avidin-biotin complex reagents (ABC Elite Kit, Vector Laboratories, USA), the immunoreaction products were visualized using DAB kits (Beijing Zhongshan Jinqiao Biological Technology Co., Ltd., China). Finally, the floating sections were mounted, dehydrated, cleared, and coverslipped in Permount™ mounting medium (Sinopharm Group, Co. Ltd). Photographs were captured under a microscope (Nikon, Tokyo, Japan) and analyzed.

**Statistical analysis.** The data are presented as means ± standard errors (means ± SEM), and statistical graphs were processed using GraphPad Prism 5.0 software (GraphPad Software Inc., La Jolla, CA, USA) and Adobe Photoshop CS4 software (Adobe Systems Incorporated, CA, USA). The results of the Barnes maze test were analyzed with repeated-measures ANOVA using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). Other results were statistically analyzed using unpaired Student’s t-tests. P < 0.05 was considered statistically significant.

**RESULTS**

Prenatal stress reduced birth weight. We detected the weight of the females offspring on postnatal 0 day, compared to control offspring group, the birth weight significantly decreased in the prenatally stressed adult offspring (t=7.035, P < 0.001).

Prenatal stress impaired the spatial learning and memory abilities of adult offspring. Regarding the Barnes maze test, data were analyzed using repeated-measures ANOVA. Compared to the control offspring, the latency to

![Graph 1. Weight of the offspring on postnatal 0 day. Data are expressed as means ± SEM. ** P < 0.005 compared with the control, as measured by t-tests (n = 28 per group). control: normal offspring , stress: prenatally stressed offspring.](image)

![Graph 2. Prenatal stress causes abnormal behavior in adult female offspring. A The latency to reach the target hole is increased in the female prenatally stressed offspring (F(1,14) = 3.640, P = 0.008 as measured by repeated-measures ANOVA). B Female prenatally stressed offspring committed a significantly greater number of errors than control female offspring (F(1,14) = 17.015, P = 0.001, as measured by repeated-measures ANOVA). control: normal offspring , stress: prenatally stressed offspring.](image)
Pregnant rats subjected to prenatal restraint stress or control conditions. On P60, hippocampal GRs expression in the female prenatally stressed offspring was significantly increased in DG and CA3 area, but no difference in CA1 area compared to that in control offspring (DG: T = 3.386, p = 0.0012; CA1: T = 1.233, p = 0.057; CA3: T = 3.556, p = 0.0119, Fig. 3). Western blot analysis also showed on P60, significantly higher hippocampal GR expression was observed in female prenatally stressed offspring than in control female offspring (T = 7.400, P = 0.0100 Fig. 4).

DISCUSSION

Prenatal stress transmits its affect on developing fetus and on pregnancy outcomes in adult offspring (Weinstock, 2017). Many studies have shown that administered with

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In conclusion, our findings provide evidence that maternal prenatal stress upregulated hippocampal GR expression in female rat offspring, GR mediates offspring behavioral.

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Corresponding author:
Dr. Jufang Huang
Department of Anatomy and Neurobiology
Central South University School of Basic Medical Sciences
Changsha, Hunan
CHINA

Email: huangjufang@csu.edu.cn

Corresponding author:
Dr. Shengbin Bai
Department of Histology and Embryology
Basic Medical College of Xinjiang Medical University
Ürümqi, Xinjiang
CHINA

Email: bsbxx@a26.com

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