Preventive effect of estrogen on depression-like behavior induced by chronic restraint stress

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Abstract: Objective To investigate the roles of estrogen and kalirin-7 in chronic restraint stress (CRS)-induced depression and the pathophysiological mechanism of depression. **Methods** Healthy female mice from Institute of Cancer Research (ICR) were randomly divided into 3 groups: control group, CRS group, and estrogen + CRS group. CRS was used to establish the animal model of depression. Forced swimming test and immunohistochemistry method were utilized to investigate the animal behavior and kalirin-7 expression in the hippocampus, respectively. **Results** Compared with the control group, the CRS mice displayed depression-like behaviors, including a significant reduction in body weight, a significant increase in immobility time in forced swimming test, and a dramatic decrease in kalirin-7 expression in the hippocampus. However, administration of estrogen attenuated the CRS-induced negative behaviors, and simultaneously increased kalirin-7 expression. **Conclusion** Estrogen replacement therapy (ERT) could prevent CRS-induced depression-like behaviors in female ICR mice. Besides, kalirin-7 also plays a role in preventing CRS-induced depression-like behaviors.

Keywords: chronic restraint stress; depression; estrogen; neural plasticity; kalirin-7

1 Introduction

Depression is a devastating disorder with a high prevalence and mortality, resulting in massive socioeconomic burdens (World Health Organization 2001)^[1]. Many studies have revealed that stress, especially chronic stress, is correlated with the emergence and the exacerbation of depression^[2,3]. Stress can activate the hypothalamo-pituitary-adrenal (HPA) axis and stimulate the secretion of glucocorticoids (GCs) from the adrenal cortex^[4,5]. Repeated activation of the HPA axis during chronic stress induces production of high levels of

GCs, which leads to the down-regulation of hippocampal corticoid receptors and impairment in brain structure and functions^[6]. Chronic stress can also deprive the hippocampus of its regulatory capacity of providing a negative feedback to HPA axis activation, leading to the further hypersecretion of GCs^[7-10]. Actually, previous studies have found that the stress-induced activation of HPA axis can greatly inhibit the female reproductive axis^[11].

As the most common psychiatric disorder, depression attracts attention from all over the world. However, the underlying pathophysiological mechanism is complex and has been poorly understood. In fact, depression incidence in the female, particularly during childbearing period, is twice as frequent as that in the male^[12]. One reason for this discrepancy may be the estrogen. Estrogen production is derived from ovary changes periodically in women since the puberty,

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and the prevalence of depression increases accordingly. Premenstrual dysphoric disorder, postpartum mood disorders and perimenopausal depressive symptoms have been reported to correlate with a decline in plasma estrogen concentration^[13-15]. Clinical studies have reported that estrogen replacement therapy (ERT) is an effective treatment for those mood disorders^[16]. Similarly, studies using animal models have revealed the positive effects of estrogen in female rodents. For example, the proestrous rats that typically have a higher level of estrogen than diestrous or male rats, show improvement in depressive behaviors in the forced swimming test^[17,18]. Removal of the primary source of E2 through ovariectomy (OVX) can induce the depressive behaviors in female rats and mice, and E2 replacement can reverse this effect^[18,19].

One of the most fascinating features of the brain is the plastic changes in brain during adult life. Some regions of the adult brain, especially the limbic areas such as the hippocampus, the amygdale and the piriform cortex, retain particularly high degrees of structural plasticity^[20]. Studies have shown that neural plasticity is involved not only in the function of learning and memory, but also in the pathophysiological process of feeling, emotion and cardiovascular function. Evidence concerning the relationship between stressful life events and depression, and findings from neuroimaging and postmortem studies indicate that mood disorders may be associated with impairment in structural plasticity within specific neural networks^[21].

Rearrangement of the actin cytoskeleton has been implicated in the generation of structural plasticity^[22,23]. Kalirin, a multi-domain protein, was originally identified through its interaction with peptidylglycine α -amidating monooxygenase (PAM)^[24]. Northern blot analysis of adult rat tissues has revealed multiple isoforms of kalirin^[25]. Kalirin-7 is the most prevalent isoform of kalirin in adult rat brain, and is concentrated in the postsynaptic density fraction^[25-27]. It is one of the few proteins whose primary effect is to increase the number of spines^[28]. Over-expression of kalirin-7 can cause the formation of spine-like structures in cortical neurons^[27].

However, it is still unclear whether there is a certain relationship between stress-induced depression and

estrogen, or whether estrogen takes part in the pathophysiological process. Besides, whether kalirin-7 is involved in stress-induced depression through influencing the changes of dendrites has not been revealed until recently. Here, the female mice were used and chronic restraint stress (CRS) was adopted to establish the animal model of depression. Assays were conducted to solve the above-mentioned problems and provide some references for the therapy of affective disorders.

2 Materials and methods

2.1 Animals and materials A total number of 36 female mice from Institute of Cancer Research (ICR) (weighing approximately 20 g) were individually housed in standard (27×21×14 cm³) polypropylene cages at (21±1) °C, under a 12 h:12 h light-dark cycle, with free access to food and water except when food and water deprivation was needed by the experiment. The mice were allowed 2 weeks to adapt to the laboratory setting. They were randomly divided into 3 groups ($n=12$ in each group): the control group, in which the mice were deprived of food and water only during the corresponding 6-h stress session, to exclude the interference of food and water deprivation; the CRS group, in which the mice were subject to a 6-h CRS session each day for 21 d, and during this session, mice did not receive any food or water; and the CRS+estrogen group, in which the mice were subject to the 6-h CRS session each day for 21 d, and received intramuscular injection of estrogen on the day before CRS, and on days 6, 12, and 18 during CRS. During the 6-h session, mice either received no food or water.

After 21-d CRS, forced swimming test was carried out (on day 22). On day 23, all the mice were transcardially perfused, and brains were removed and frozen.

β -Estradiol 3-benzoate (EB) was purchased from Sigma and was dissolved in sesame oil (8 μ g EB/50 μ L). The injection dosage for EB solution was 2 mL/kg.

2.2 Mice treatment The CRS paradigm was utilized to establish the animal model of depression, based on the well-established methods from literatures. During the 21-d stress period, mouse was placed in a plexiglas restrainer tube (2.9 cm in diameter, 14 cm in length) for 6 h (between 09:00-15:00) without access to food or water. The restrainers had ventilation

air holes and limited room for movement. Animals were not physically compressed and did not experience pain. After the stress session, mice were placed back to their home cages with free access to water and food. During the 6-h stress session, mice in the control group were deprived of food and water, but without stress treatment. All the animals were weekly weighed during the 21-d period.

2.3 Forced swimming test The experiment was conducted according to the modified version of Porsolt Forced Swim Test (FST)^[29]. Briefly, mice were placed in a glass cylinder (12 cm in diameter) filled with 10-cm deep water (23-25 °C). Animal behaviors were observed for 6 min, and the immobile time during the last 4 min was measured. Immobility was determined when mice exhibited no obvious movement except some small movement necessary to keep the head above the water.

2.4 Immunohistochemistry method One day after FST, animals were deeply anesthetized with sodium pentobarbital (200 mg/kg, i.p.) and transcardially perfused with 0.1 mol/L phosphate buffered solution (PBS, pH 7.4) followed by 4% paraformaldehyde in 0.1 mol/L PBS (pH 7.4). Brains were then removed and postfixed at 4 °C overnight in 4% paraformaldehyde (prepared in 0.1 mol/L PBS), then in 30% sucrose (prepared in 0.1 mol/L PBS, pH 7.4). Coronal sections (40 µm) were cut on freezing microtome (Leica, CM1900, Germany) and collected in 0.01 mol/L PBS. Sections were rinsed with 0.01 mol/L PBS for 5 min for 3 times, and then blocked with 0.5% bovine serum albumin for 2 h at 37 °C. After that, the sections were incubated in rabbit anti-rat kalirin-7 antibody (1:250; a gift from Department of Neuroscience, University of Connecticut Health Center) for 72 h at 4 °C, and rinsed 3×5 min. Then the sections were incubated in biotinylated sheep anti-rabbit immunoglobulin for 1.5 h at 37 °C and rinsed 3×5 min. After that, sections were incubated in avidin-biotin-horse-radish peroxidase complex (ABC) for 1.5 h at 37 °C. After being rinsed, the sections were stained in diaminobenzidine (DAB, Boster) in PBS for 5 min. Cells that were stained as brown particles, or had brown contours that could be distinguished from background, were considered to be kalirin-7-positive cells. The anatomical localization of positive cells was conducted according to the stereotaxic atlas^[30]. The number of positive cells was quantified bilaterally in 3-4 sec-

tions in the hippocampus.

2.5 Statistical analysis Data were expressed as mean±SEM and analyzed with SPSS 12.0 by one-way analysis of variance (ANOVA). $P < 0.05$ was considered as statistically significant.

3 Results

3.1 Body weight change As shown in Table 1, the animals exposed to CRS displayed lower body weight on days 7, 14 and 21 ($P < 0.01$), compared to the control group. However, mice with estrogen treatment showed higher body weight than CRS mice ($P < 0.01$).

Table 1. The body weight changes in 3 groups during the 21-d experimental period (mean±SEM)

Groups	Day 1	Day 7	Day 14	Day 21
Control	25.15±0.37	26.96±0.44	28.71±0.30	29.76±0.51
CRS	23.59±0.33	22.14±0.26 ^a	23.58±0.45 ^a	24.17±0.41 ^a
CRS+estrogen	23.08±0.61	25.62±0.44 ^b	25.48±0.54 ^b	25.81±0.53 ^b

^a $P < 0.01$ vs control group; ^b $P < 0.01$ vs CRS group.

3.2 Behavioral measures The immobile time was quantified during the last 4 min. The mice exposed to 21-d CRS exhibited an increase in immobility time, compared with the control group ($P < 0.01$). However, estrogen treatment induced a significant decrease in immobility time, compared with that in CRS group ($P < 0.01$) (Table 2).

Table 2. The immobility time of mice in 3 groups as detected by FST (mean±SEM)

Groups	Immobility time (s)
Control	53.90±12.72
CRS	153.00±12.01 ^a
CRS+estrogen	83.38±12.29 ^b

^a $P < 0.01$ vs control group; ^b $P < 0.01$ vs CRS group.

3.3 Immunohistochemistry As shown in Fig. 1, the expression level of kalirin-7 in dentate gyrus in CRS group was lower than that in control group. In addition, administration of estrogen notably increased the number of kalirin-7-positive cells, compared with that in CRS group. The quantification of kalirin-7-positive cells was listed in Table 3.

Table 3. Kalirin-7 expression in dentate gyrus of mice in 3 groups (mean±SEM)

Groups	The average number of kalirin-7-positive cells in dentate gyrus
Control	66.76±2.84
CRS	32.56±1.17 ^a
CRS+estrogen	48.20±2.26 ^b

^a $P < 0.01$ vs control group; ^b $P < 0.01$ vs CRS group.

Fig.1 Hippocampal expression of kalirin-7 (arrows) in the control group (A), the CRS group (B) and the CRS+estrogen group (C), as revealed by immunohistochemistry method ($n=12$ in each group). Scale bar: 50 μ m.

4 Discussion

4.1 There is a relationship between CRS and estrogen Depression is one of the most complex human diseases. Individuals suffering from depression are more vulnerable to physical illness, and have decreased social functioning and a high mortality^[31]. Although epidemiological investigation has shown that women are more vulnerable to mood disorders, whether estrogen is involved in stress-induced depression in female rodents has not been known until recently. In this study, CRS was used to establish an animal model of stress-induced depression, aiming to explore the function of female hormone in affective disorders.

Our results show that CRS could lead to a decrease in body weight, which is consistent with that reported by Homerira Zardooz *et al.*^[32]. The restraint of body weight growth is an obvious phenomenon during the process of depression. Matthew *et al.* believe that the stress-induced change of weight growth is more predominant than anhedonia^[33].

The forced swimming test was first proposed as a simpler variation of learned helplessness test, and is probably the most widely used screening test for selecting potential antidepressant compounds^[34-36]. The immobility time here was used as an indicator for the severity of depression. Our results show that mice exposed to the 21-d restraint stress exhibited an increase in immobility time, compared with that in the control group, suggesting that CRS could significantly influence the physiological and psychological processes, and produce a negative effect on behaviors. Some researchers believe that the effects of restraint stress are greatly influenced by procedural differences among the experiments, such as duration, types of restraint, and different strains of rodents employed^[37]. We find that it is feasible to choose the ICR female mice for model establishment of stress-induced depression, which provides a foundation for our further research.

Our data also show that estrogen treatment could attenuate CRS-induced behavioral deficits. Plasma estrogen level in women with depression is often significantly lower^[11]. Estrogen-treated mice showed an increase in affective state,

suggesting that ERT may also be potentially effective in treating stress-induced depression. This result suggests that there may be a relationship between the development of CRS-induced depression and estrogen level.

4.2 Kalirin-7 is involved in CRS-induced depression Current theories have proposed that the major depressive disorders may be associated with impairment of neural plasticity^[38,39], and the recurrences of depressive episodes may enhance the deleterious effect of depression on structural and functional plasticity^[21].

Kalirin-7 is just one of the components of the cascades of neural plasticity-involving processes. By *in situ* hybridization, the highest level of kalirin-7 mRNA is detected in the cerebral cortex, hippocampus, and Purkinje cells^[40]. Studies have revealed that kalirin-7 is necessary for the maintenance of dendritic spines. Reduced expression level of kalirin-7 results in decreases in dendritic length and complexity, and retraction of apical dendrites and basal dendrites^[41]. Thus, it is of great interest whether kalirin-7 is involved in the pathology of stress-induced depression through influencing the changes of dendrites.

Our results show that the expression level of kalirin-7 in the hippocampus was down-regulated following 21-d restraint stress, while estrogen administration could largely reverse this effect. These data suggest that the change in kalirin-7 expression would induce dendrite remodeling in the hippocampus, which might be associated with the pathophysiology of depression. It has also been shown that estrogen plays a key role in the neuroprotective function of antidepressant, by enhancing expression of kalirin-7.

In conclusion, our studies further demonstrate the involvement of estrogen in affective disorders. We propose that the decline of estrogen level may be responsible for the CRS-induced depression in female mice. ERT could offer an approach to prevent this disorder. Furthermore, we have observed a link between chronic stress-induced depression and the expression level of kalirin-7 in hippocampus regulated by estrogen, providing new insights into the etiology and treatment strategy of such psychiatric diseases.

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雌激素在慢性束缚应激性抑郁发生中的作用

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摘要: **目的** 探讨雌激素和 kalirin-7 在慢性束缚应激性抑郁发生中的作用。**方法** 采用慢性束缚应激性抑郁动物模型, 运用强迫游泳测试和免疫组织化学方法, 分别检测动物行为学表现及海马中 kalirin-7 蛋白的表达。**结果** 慢性束缚应激能显著降低动物体重、延长游泳不动时间、抑制海马中 kalirin-7 蛋白的表达。注射雌激素能明显改善动物抑郁样行为, 并且海马 kalirin-7 表达显著增加。**结论** 慢性束缚应激能诱发产生抑郁样行为, 而雌激素替代疗法则能预防慢性束缚应激性抑郁的发生。此外, kalirin-7 在防止慢性束缚应激性抑郁的发生中起到重要作用。

关键词: 慢性束缚应激; 抑郁; 雌激素; 神经可塑性; kalirin-7