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FKBP5 and specific microRNAs via glucocorticoid receptor in the basolateral amygdala involved in the susceptibility to depressive disorder in early adolescent stressed rats

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ABSTRACT

Exposure to stressful events induces depressive-like symptoms and increases susceptibility to depression. However, the molecular mechanisms are not fully understood. Studies reported that FK506 binding protein51 (FKBP5), the co-chaperone protein of glucocorticoid receptors (GR), plays a crucial role. Further, miR-124a and miR-18a are involved in the regulation of FKBP5/GR function. However, few studies have referred to effects of early life stress on depressive-like behaviours, GR and FKBP5, as well as miR-124a and miR-18a in the basolateral amygdala (BLA) from adolescence to adulthood. This study aimed to examine the dynamic alternations of depressive-like behaviours, GR and FKBP5, as well as miR-124a and miR-18a expressions in the BLA of chronic unpredictable mild stress (CUMS) rats and dexamethasone administration rats during the adolescent period. Meanwhile, the GR antagonist, RU486, was used as a means of intervention. We found that CUMS and dexamethasone administration in the adolescent period induced permanent depressive-like behaviours and memory impairment, decreased GR expression, and increased FKBP5 and miR-124a expression in the BLA of both adolescent and adult rats. However, increased miR-18a expression in the BLA was found only in adolescent rats. Depressive-like behaviours were positively correlated with the level of miR-124a, whereas GR levels were negatively correlated with those in both adolescent and adult rats. Our results suggested FKBP5/GR and miR-124a in the BLA were associated with susceptibility to depressive disorder in the presence of stressful experiences in early life.

1. Introduction

Depression is a common psychiatric disorder characterized by significant and lasting low mood, loss of interest, cognitive impairment and some physical symptoms (Willner et al., 2013; Yang et al., 2016). The dysregulation of the hypothalamus-pituitary-adrenal (HPA) axis is closely related to the aetiology and pathogenesis of depression in which glucocorticoid receptor (GR) plays a crucial rule in regulation of feedback of the HPA axis (de Kloet, 1998; Keller et al., 2016; Min et al., 2012). Exposure to prolonged stress causes an increase in glucocorticoid (GC) levels and results in the disruption of GR and hypoactive negative feedback of the HPA axis. Repeated administration of dexamethasone (DEX) in animals can disrupt HPA axis function and induce depressive-like behaviours, which supports the conclusion that GC/GR is involved in the pathogenesis of depression (Sigwalt et al., 2011; Skupio et al., 2015). More importantly, studies confirm that the developing brain has more sensitivity to higher levels of GC because it induces neuron apoptosis, inhibits neuritis outgrowth and decreases synapse formation (Brown and Spencer, 2013; McEwen, 1987), then results in more susceptibility to psychosis in adulthood (Andersen, 2003). The decreased GR and neurogenesis was reported in patients with depression as well as depressive animal models, whereas the alterations were relieved with antidepressant treatment, which suggested that GR might be the pivot of depression (Anacker et al., 2011; Ruksee et al., 2014).

FK506 binding protein51 (FKBP5), as the chaperone protein of heat-shock protein 90 (HSP90) and the co-chaperone protein of GR, plays a key role in regulation of GR sensitivity. Briefly, in the absence of glucocorticoids, FKBP5 is integrated into GR via HSP90

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consisting of the mature GR heterocomplex, which is maintained inactive in the cytoplasm and has lower affinity for its ligand (Binder, 2009; Wochnik et al., 2005). Under the induction of glucocorticoids, FKBP5 is replaced by FKBP4 and the steroid receptor is activated, thus allowing the nuclear translocation of GR-mature and further genetic transcription (Davies et al., 2002). In addition, GC can induce the expression of FKBP5, completing an intracellular ultra-short negative feedback loop to regulate GR activity (Hubler and Scammell, 2004; Vermeer et al., 2003). Clinical studies reported that the T allele of the *FKBP5* gene was associated with structural changes of emotional processing brain areas in depressed patients who suffered childhood adversity (Tozzi et al., 2016). In addition, chronic mild stress increased expression of FKBP5 and decreased nuclear GR levels in hippocampus and prefrontal cortex of rats and the alterations were modulated by chronic antidepressant treatment (Guidotti et al., 2013; Xing et al., 2015). Therefore, FKBP5 is thought to be an important element associated with pathogenesis of depression as an existing mutual adjustment between FKBP5 and GR.

Micro-ribonucleic acids (miRNAs) are a class of small noncoding RNAs (~22 nucleotide) characterized by highly conserved Rip miRNAs loaded into Argonaute (Ago) proteins in the cells to form the RNA-induced silencing complex. The miRNAs regulate gene expression level posttranscriptionally by either inhibiting translation or inducing degradation of messenger RNA (Huntzinger and Izaurralde, 2011). Some miRNAs are involved in neuronal differentiation, synapse formation, and stress responsiveness (Bian and Sun, 2011; Hollins and Cairns, 2016). A previous study has suggested that the overexpression of miR-124a in the hippocampus contributed to depressive-like behaviour induced by social defeat stress of rats, and inhibited miR124a expression by injecting miR124a-silencers into the hippocampus and displayed antidepressants-like effects in rats (Bahi et al., 2014). Upregulation of miR-124a decreased GR protein levels and interfered with GR translational activity by binding to 3′ untranslated regions of GR (Vreugdenhil et al., 2009) and miR-18a inhibited translation of GR mRNA by acting on its putative target site of GR-3′-untranslated region in vitro experiments (Uchida et al., 2008). Further, acute stress induced decreased GR expression and increased miR-124a level in the corpus callosum of mice, whereas it led to lower GR and higher miR-18 levels in the hypothalamus of mice. However, yokukansan (YKS), a Kampo medicine, increased GR expression and downregulated miR124a and miR-18 levels (Shimizu et al., 2015a, 2015b). However, long-term alterations of GR, miR-124a, and miR-18a expression in adolescent and adult rats undergoing stress during the adolescent period have not yet been clarified.

The amygdala, as part of the limbic system, plays an important role in emotion regulation and fear conditioning (Phelps and LeDoux, 2005). Patients with depression showed abnormal amygdala volumes, functional connectivity, and alterations in molecular aspects in comparison with individuals without depression (Hu et al., 2015; Schuhmacher et al., 2012). In particular, the basolateral amygdala (BLA) has a projection to the prefrontal cortex, the hippocampus, and the nucleus accumbens, which are composed of crucial subregions of brain in affective regulation (Orsini et al., 2011; Price and Drevets, 2010). However, few studies have investigated the long-term effects of early life stress on the BLA.

Because adolescence is the crucial period of mental development, stress specifically affects the brain during this stage and results in an increased risk of mental disorders in later life (Heim and Nemeroff, 2001; Holmes and Robins, 1987). The current study aimed to examine the long-term effects of stress on depressive-like behaviours, GR and FKBP5 expressions, as well as miR-124a and miR-18a expressions in BLA from adolescence to adulthood using depressive animal models and animals undergoing dexamethasone administration. Meanwhile, the antagonist of GR, RU486, was used to block the binding of GR agonists in the study and the reverse effect of blocking GR on the aforementioned variables of stressed rats was explored.

2. Methods and materials

2.1. Animals and grouping

A total of 100 male Wistar rats (21 days old) were supplied by the Experimental Animal Center of Shandong University. Animals were housed (five per cage) under standardized laboratory conditions (temperature 23 ± 2 °C, 12-h light/dark cycle), with free access to food and water for 7 days of acclimatization before the follow-up experiments. Procedures and care of animals of the study were approved by the Animal Ethics Committee of Shandong University. The study was designed to minimize the number and suffering of the laboratory animals.

Rats were first randomly divided into 5 groups (20 in each group): control group (C), chronic unpredictable mild stress (CUMS) group (S), CUMS and RU486 group (S + R), dexamethasone group (D), dexamethasone and RU486 group (D + R). Following 3 weeks of animal modeling and drug administration, 10 rats randomly selected from each group were sacrificed at the age of 55 days after conducting behavioural tests. The rest of the rats were raised to adulthood and were sacrificed after conducting behavioural tests at the age of 90 days (see Fig. 1). The current study had in total 10 groups including adolescent and adult control group (AdoC and AduC, together referred to as C group), adolescent and adult CUMS group (AdoS and AduS, together referred to as S group), adolescent and adult CUMS and RU486 group (AdoS + R and AduS + R, together referred to as S + R group), adolescent and adult dexamethasone group (AdoD and AduD, together referred to as D group), and adolescent and adult dexamethasone and RU486 group (AdoD + R and AduD + R, together referred to as D + R group).

2.2. Chronic unpredictable mild stress

Rats from S group and S + R group were subjected to CUMS for 21 days according to previous studies described (Filho et al., 2015; Li et al., 2016). The stressors included: (1) food deprivation for 24 h; (2) water deprivation for 24 h; (3) noise (92 dB, 1500 Hz) for 2 h; (4) pinching tail for 1 min; (5) hot stress in oven at 45 °C for 5 min; (6) day and night reversal for 24 h; (7) unpredictable foot shocks (1 mA, one shock/5 s, 10-s duration for a total of 10 min). The time and form of stimulation daily were at random and each type of stressor was guaranteed to be performed three times during the CUMS period.

2.3. Drug administration

Rats of S + R group were treated with RU486 (subcutaneously, dissolved in saline) at the dose of 10 mg/kg/d 30 min before CUMS for 21days (Aisa et al., 2008). Rats of D group were treated with repeated administration of dexamethasone (DEX, subcutaneously, dissolved in saline) at the dose of 1.5 mg/kg/d for 21 days (Sigwalt et al., 2011). Rats of D + R group were treated with RU486 30 min before the application of dexamethasone for 21days. Rats of non-drug groups and one-time drug administration were treated with saline to balance the systematic error. All drugs were purchased from Qilu Hospital, China.

2.4. Behaviour tests

Behaviour tests were conducted on the following day after the