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Mineralocorticoid receptor antagonist spironolactone prevents chronic corticosterone induced depression-like behavior

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Summary High level of serum corticosteroid is frequently associated with depression, in which a notable HPA (hypothalamus-pituitary-adrenal) axis hyperactivity is often observed. There are two types of corticosteroid receptors expressed in the hippocampus that provide potent negative feedback regulation on the HPA axis but dysfunction during depression, i.e. the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). The balance between hippocampal MR and GR during chronic stress plays an important role in the occurrence of depression. The aim of this study is to explore if chronic corticosterone administration would induce depression-like behavior and affect the expression and function of hippocampal MR and GR, in addition to assess whether manipulation of corticosteroid receptors would modulate depressive behaviors. Hence, mice were treated with corticosterone (40 mg/kg) for 21 days followed by assessment in a battery of depression-like behaviors. The results show that chronic corticosterone-treated animals displayed an increased immobility time in a forced-swimming test, decreased preference to sucrose solution and novel object recognition performance, and enhanced hippocampal serotonin but decreased MR expression in both hippocampus and hypothalamus. On the other hand, co-administration of MR antagonist, spironolactone (25 mg/kg, i.p. $\times$ 7 days) in corticosteroid-treated animals reduced immobility time in a forced-swimming test and improved performance in a novel object recognition test. In conclusion, we demonstrate that chronic corticosterone treatment triggers several depression-like behaviors, and in parallel, down-regulates MR expression in the hippocampus and hypothalamus. Administration of an MR antagonist confers an anti-depressant effect in chronic corticosterone-treated animals.

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1. Introduction

Depression encodes a serious psychological illness with multifactorial causes (Heim and Nermeroff, 2001; Caspi et al., 2003). Core symptoms of major depression include anhedonia, cognitive impairment and despair along with frequent anxiety and comorbid diseases (Nestler et al., 2002; De Kloet et al., 2007). Although meta-analyses from epidemiological studies indicate that depression is largely heritable (Sullivan et al., 2000), chronic intense stress has been recognized as one of the crucial components in the emergence of major depression (McEwen, 2007; Pittenger and Duman, 2008). When a subject is exposed to a stressor, CRF (corticotrophin-releasing factor) from the paraventricular nucleus of the hypothalamus stimulates the release of corticotrophin (ACTH) from the anterior pituitary, which in turn evokes glucocorticord (cortisol in human or corticosterone in rodents) secretion from the adrenal cortex (Vermeer et al., 2003; Berton and Nestler, 2006; Lanfumey et al., 2008). Under extreme stress, excessive corticosteroids would trigger negative feedback regulation in various brain regions, particularly the hippocampus and amygdale. HPA (hypothalamus-pituitary-adrenal) axis activity is inhibited either directly or indirectly, thus reducing the high levels of circulating corticosteroids (De Kloet et al., 1998; Ozawa, 2005). Previous studies reported that the hippocampal feedback was impaired in major depression patients resulting in hyperactivity of the HPA axis and increased levels of humeral corticosteroids (Brown et al., 2004; Wulsin et al., 2010). In this context, patients with Cushing's disease or those undergoing long-term pharmacotherapy with glucocorticoids exhibit an extremely high rate of depression (Sonino et al., 2010).

The two most highly expressed corticosteroid receptors in the limbic brain are the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) (Gass et al., 2001). The MR is a high affinity receptor which binds corticosterone at low concentrations compared to the GR. Thus the MR is speculated to be occupied under homeostatic conditions while the GR becomes substantially occupied during stressful conditions (De Kloet et al., 1998). Accordingly, numerous studies have assessed GR expression or function in response to stress, and concluded that a causal relationship exists between high corticosterone or reduced forebrain GR levels and major depression (Boyle et al., 2005; Gregus et al., 2005). Functional GR is known to associate with heat shock proteins (Hsp70 and Hsp90) for protein folding as well as translocate and interact with glucocorticoid responsive elements (GREs) (Srinivasan et al., 1994; Caamano et al., 2001). It has been shown that acute stress decreases cytosolic GR and Hsp70 in hippocampus (Filipovic et al., 2005), providing further molecular evidence of GR dysfunction under stress conditions.

In addition to the well described changes in GR, morphological examination of post-mortem human brain also found a reduced level of MR in patients with major depression (Lopez et al., 1998; Webster and Carlstedt-Duke, 2002) as well as bipolar disorder (Xing et al., 2004). Consistent with a role for altered MR expression in depression, a recent animal study demonstrated that chronic corticosterone exposure reduced hippocampal MR expression while GR levels remained the same (Xu et al., 2010). Collectively, it appears that decreases in levels of either MR or GR within the hippocampus, or other limbic structures, might disrupt negative feedback regulation on the HPA axis, ultimately leading to depression. Of importance, there is evidence that the expression and function of GR and MR in the hippocampus are tightly regulated by serotonin (5-HT) in the median raphe nucleus (Azmitia and Segal, 1978; Lai et al., 2003). It was previously shown that stress increases 5-HT release in the hippocampus (Keeney et al., 2006) and alters the expression of hippocampal GR and MR in a 5-HT dependent manner (Robertson et al., 2005). Stress-induced 5-HT hyperactivity that results from the down-regulation of pre-synaptic 5-HT1A autoreceptor in the dorsal and median raphe (Fairchild et al., 2003) may occur through MR- or GR-mediated cellular and/or transcriptional regulation (Hesen and Joels, 1996; Ou et al., 2001; Wissink et al., 2000). Additionally, lesioning 5-HT terminals in rodents is known to potentiate stress-evoked blood corticosterone (Richardson, 1984).

To understand the causal relationship between chronic corticosteroid and depression and the underlying mechanism, which gained less attention in previous literatures, we treated animals (ICR mice) with repetitive corticosteroid (CORT) (40 mg/kg) for 21 days. Animals went through a series of behavioral measurements including forced swim test (FST), tail suspension test (TST), novel object recognition test (NORT), locomotor activity as well as sucrose consumption. To correlate the behaviors with underlying mechanism, we measured the amount of hippocampal 5-HT, MR and GR expression and GR-associated Hsp90 as well as levels of MR and GR expression in the hypothalamus. The finding that levels of MR decreased after chronic CORT treatment led us to test if administration of MR antagonist, spironolactone could reverse episode(s) of depressive behaviors.

2. Materials and methods

2.1. Animals

Male ICR mice aged 5 $\sim$ 6 weeks (BioLASCO Taiwan Co., Ltd) at the beginning of the study were used. Mice were housed five per cage under a 12-h light—dark cycle (07:00—19:00 h) and at constant temperature (25 °C) and humidity in a controlled room for at least 1 week prior to experimentation. Food and water were available ad libitum. All the experimental procedures were performed during the light cycle. All procedures were performed in accordance with the NIH Guide for Care and Use of Laboratory Animals (**NIH Publication No. 8023) and were approved by the Animal Committee of Chang-Gung University. Numbers were strictly controlled to reduce the unnecessary use of the animal.

2.2. Drug treatment

Mice were injected subcutaneously with 40 mg/kg CORT (corticosterone 21-acetate, Sigma, MO) dissolved in sesame oil (Sigma) continuously for 21 days. Control mice received the same volume (1 ml/kg) of sesame oil. In a separate experiment, spironolactone (25 mg/kg; Sigma) was suspended in dH_2O containing one drop of Tween® 20 (Sigma) and mice were dosed by intraperitoneal injection. The dosage and route of administration were chosen based on previous reports that can effectively blocked MR-mediated effects but did not affect spontaneous behaviors (Koenig and