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Corticosterone exposure augments sensitivity to the behavioral and neuroplastic effects of fluoxetine in C57BL/6 mice

Shivon A. Robinson [a], Bethany R. Brookshire [a], Irwin Lucki [a, b, *]

[a] Department of Psychiatry, University of Pennsylvania, Philadelphia, PA 19104, USA
[b] Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104, USA

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ABSTRACT

Both genetic background and pre-existing stress play critical roles in the effects of antidepressant drugs. The current studies showed this principal by demonstrating that exposure to the stress hormone corticosterone (CORT) allowed behavioral and neurogenic effects to emerge following chronic treatment with fluoxetine of C57BL/6 mice, a strain ordinarily resistant to these effects. Adult male mice were implanted subcutaneously with 21-day slow-release CORT pellets (10 mg) or placebo and then co-treated with 5 mg/kg fluoxetine (b.i.d., i.p.) or saline for 14 days. Animals were then assessed for approach behavior in the novelty-induced hypophagia (NIH) test, hippocampal cell proliferation, corticosteroid receptor expression, and CORT plasma levels. Co-treatment of CORT with fluoxetine significantly reduced approach behavior in the novel environment of the NIH test and increased hippocampal cell proliferation whereas fluoxetine given alone was ineffective. CORT given alone did not alter approach behavior in the novel environment and caused a smaller increase of cell proliferation. The CORT effect was blocked by adrenalectomy and was likely due to increased adrenal feedback. Cell proliferation in CORT-treated animals was associated with reduced mineralocorticoid, but not glucocorticoid, receptor mRNA expression. Although the pellets were advertised to release CORT for 21 days, plasma CORT levels were increased at 1 day after implantation but were not sustained when measured at 7 days or longer intervals. Nevertheless, the transient CORT increase was sufficient to induce long-lasting behavioral and molecular changes when followed by fluoxetine treatment. These studies warrant further investigation into the role of glucocorticoids and environmental stress as adjunctive facilitators of the response to antidepressants, especially for treatment-resistant patients.

1. Introduction

Major Depressive Disorder (MDD) is one of the most common psychiatric disorders, with a lifetime prevalence of 17% in the United States and 4% worldwide (Eaton et al., 2008; Kessler et al., 2005). In terms of years lost to disability, MDD is considered one of the most disabling medical conditions and is predicted to become a leading contributor to the worldwide burden of disease (Mathers and Loncar, 2006). The majority of pharmacotherapies developed for the treatment of MDD target brain monoamine systems, primarily serotonin (5-HT), norepinephrine, and dopamine. The most common of these, the selective serotonin reuptake inhibitors (SSRIs) and selective norepinephrine reuptake inhibitors (SNRIs), comprise a large proportion of pharmaceutical sales and are considered first line treatments for MDD. Unfortunately, an estimated 40% of patients fail to respond to these therapies (Cipriani et al., 2009; Culpepper, 2010). Further insight into the neurobiological mechanisms underlying antidepressant response is needed for the development of more efficacious antidepressant regimens.

The combination of genetic vulnerabilities and environmental factors, such as stress, are thought to be significant contributors to the onset of depression in humans (Charney and Manji, 2004). The likelihood of experiencing a depressive episode is greatly increased following a stressful life event or after accumulation of chronic minor stresses (Caspi et al., 2003; Harkness and Monroe, 2006). Moreover, many patients suffering from depression exhibit signs of dysfunctional hypothalamic-pituitary-adrenal (HPA) axis activity,

* Corresponding author. University of Pennsylvania, Department of Psychiatry, Translational Research Building, 125 South 31st Street, Room 2204, Philadelphia, PA 19104, USA.
E-mail address: lucki@mail.med.upenn.edu (I. Lucki).

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as demonstrated by elevated basal cortisol levels and resistance to dexamethasone, an exogenous steroid that suppresses cortisol in healthy individuals (Gillespie and Nemeroff, 2005; Pariante and Miller, 2001). Interestingly, successful antidepressant treatment is often associated with restored suppression of HPA axis response (Schule, 2007). Together, these findings suggest a potential role of stress hormones, such as cortisol (corticosterone (CORT) in rodents), in the pathology and treatment of depression.

CORT produces its effects in the central nervous system *via* activation of glucocorticoid (GR) and mineralocorticoid (MR) receptors. Though these receptors are ubiquitous throughout the brain, they are highly abundant in the hippocampus, where they provide crucial inhibitory feedback signals to the HPA axis (Jacobson and Sapolsky, 1991; Sapolsky et al., 1984). A reduction or absence of these inhibitory signals can promote hyperactivation of the axis and augmented secretion of glucocorticoids (Anacker et al., 2011; McEwen et al., 2012). In a healthy individual, elevated corticosteroid activity helps facilitate the physiological and behavioral adaptations required to appropriately respond to stressors and reinstate homeostasis. However, prolonged exposure to CORT can inhibit the proliferation and survival of adult-born hippocampal neurons, which have been shown to play an important role in the behavioral and neuroendocrine components of stress responses in rodents (Gould and Tanapat, 1999; Snyder et al., 2011). Conversely, chronic treatment of normal rodents with SSRIs, such as fluoxetine, increases hippocampal neurogenesis and neurotrophins such as brain derived neurotrophic factor (BDNF) (Duman and Monteggia, 2006; Krishnan and Nestler, 2008; Schmidt and Duman, 2006). Increased hippocampal neurogenesis is associated with behavioral indications of antidepressant efficacy in rodents, such as reduced hyponeophagia in the novelty-induced hypophagia (NIH) test and performance in the forced swim test (Dranovsky and Hen, 2006).

Not all strains of mice respond to the behavioral and neurogenic effects of antidepressant treatments. For example, normal C57BL/6 mice are unresponsive to the behavioral effects of chronic fluoxetine treatment, measured in the NIH test, and do not exhibit increased hippocampal cell proliferation (Balu et al., 2009a). Rodent strains that are unresponsive to antidepressants could provide information about treatment resistance. However, the effects of antidepressants may be altered after exposure to stress. CORT is a vital component of the central nervous system's stress response circuitry. Although corticosteroids alone do not encompass all aspects of stress exposure (Belzung, 2014), previous studies have shown that chronic CORT exposure can induce a depressive-like motivational state in rodents that is similar to that produced by a chronic mild stress paradigm (Gourley et al., 2008). Moreover, CORT treatment alone is sufficient to alter molecular targets that are implicated in depression and antidepressant efficacy, such as hippocampal neurogenesis (Bilsland et al., 2006; Gourley and Taylor, 2009). In a small clinical study Dinan et al. (1997), found that 4-day dexamethasone therapy significantly enhanced antidepressant response to SSRIs in treatment-resistant patients. Therefore, we hypothesized that activation of stress circuitry might be important to reveal the behavioral and neurogenic effects of the SSRI fluoxetine in C57BL/6 mice, a non-responsive mouse strain.

In the current study we investigated the effects of exposure to commercial CORT pellets for 21 days in augmenting fluoxetine's behavioral and proliferative effects in C57BL/6 mice. The results of this study showed that chronic fluoxetine produced behavioral effects in the NIH test only in mice exposed to CORT. Furthermore, CORT administration with fluoxetine co-treatment augmented hippocampal cell proliferation, an effect potentially mediated by alterations in hippocampal corticosteroid receptor expression. Interestingly, analysis of plasma at the end of treatment revealed a paradoxical decrease in CORT levels in animals treated with the pellets, suggesting that the CORT pellets did not work as advertised. Adrenalectomized animals implanted with CORT pellets revealed a sharp drop in CORT plasma levels by day 7 of treatment, indicating that this method of CORT exposure produced transiently elevated, but not sustained, CORT levels. Nevertheless, these experiments revealed the important finding that CORT exposure potentiates the behavioral and neurogenic effects of chronic fluoxetine administration in a mouse strain that is otherwise non-responsive to this antidepressant treatment.

2. Materials and methods

2.1. Animals

Intact and adrenalectomized male C57BL/6J, 7–8 weeks old upon arrival, were purchased from Jackson Laboratories (Bar Harbor, ME). Mice were housed in groups of 4 (except for those used in the NIH test whom were housed in pairs) in polycarbonate cages and maintained on a 12 h light–dark cycle (lights on at 0700 h) in a temperature (22 °C)- and humidity-controlled environment. Food and water were available ad libitum. All experiments were approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania.

2.2. Experimental design

2.2.1. Experiment 1

Intact animals were implanted with CORT pellets (10 mg) or placebo pellets. Beginning on day 7 of CORT treatment, animals were dosed with either fluoxetine (5 mg/kg b.i.d., i.p.) or saline daily for the remaining 14 days of the experiment. Cohort 1: Animals were tested in the NIH and home cage test on the last two days of drug treatment ($n = 8–10$ per group). Cohort 2: Animals received a single injection of BrdU on the last day of drug treatment and were sacrificed 24 h later. In these animals hippocampal tissue was dissected and analyzed for BrdU positive cells and corticosteroid mRNA expression. Trunk blood was collected at time of sacrifice and analyzed for plasma CORT levels ($n = 15–19$ per group).

2.2.2. Experiment 2

Adrenalectomized animals were implanted with CORT pellets (10 mg) or placebo pellets and received chronic fluoxetine treatment as described in Experiment 1. All mice received additional CORT replacement through the drinking water (25 μg/ml in 0.9% saline) to prevent the loss of electrolyte homeostasis (Funder, 2006) and eliminate the confounding effects of adrenalectomy alone on neurogenesis (Cameron and Gould, 1994). Animals received a single injection of BrdU on the last day of drug treatment and were sacrificed 24 h later. Hippocampal tissue was dissected and analyzed for BrdU positive cells ($n = 7–10$ per group).

2.2.3. Experiment 3

Intact animals were implanted with CORT pellets (2.5 mg) or placebo pellets and received chronic fluoxetine treatment as described in Experiment 1. Animals received a single injection of BrdU on the last day of drug treatment and were sacrificed 24 h later. Hippocampal tissue was dissected and analyzed for BrdU positive cells ($n = 9–10$ per group).

2.2.4. Experiment 4

Adrenalectomized animals were implanted with CORT pellets (10 mg) or placebo pellets and then sacrificed 1, 7, 14, or 21 days after implantation. Trunk blood was collected at time of sacrifice and analyzed for plasma CORT levels ($n = 5–6$ per group).