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A mouse model of depression induced by repeated corticosterone injections

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Abstract

A rat model of depression has been recently developed by exogenous corticosterone administration. In this study, we further determined whether corticosterone administration also increased depression-like behavior in mice and explored the brain biochemical consequences of exposure to this administration paradigm. Mice received repeated injections of vehicle and 20 mg/kg of corticosterone for 1, 3 and 5 weeks, and then were subjected to the forced-swim and tail suspension tests. The results showed that repeated corticosterone injections increased immobility behavior in the forced-swim and tail suspension tests in a time-dependent manner. Meanwhile, this injection paradigm produced a time-related effect on tyrosine hydroxylase (TH) levels in the hippocampus of mice. These results are consistent with correlations in stress-induced depression models, and suggest that the repeated corticosterone injection paradigm provides a useful and reliable mouse model within which to further study the role of stress and glucocorticoids in depressive illness, as well as screen for antidepressants or preventive drugs.

Keywords: Corticosterone; Depression; Animal model; Tyrosine hydroxylase; Stress; (Mouse)

1. Introduction

According to large studies, depression is a severe illness with a lifetime prevalence of between 10 and 20%. It is estimated, by the World Health Organization, that depression will be the most important cause of disability in the world by the year 2020 (Murray and Lopez, 1997). In terms of unipolar depression, it appears to have both a genetic and environmental basis. Twin studies suggest that about 25–30% of the variance is genetic and that environmental factors account for about 75% of the variance (Henn et al., 2004). The most important environmental factor is stress (Charney and Manji, 2004; Paykel, 2003).

The human stress experience contributes to the pathogenesis of depression, and may also play a role in the severity and recurrence of this debilitating illness. The connection between stress and depression was initially drawn from observations of over activity of the hypothalamic–pituitary–adrenal (HPA) axis, elevated cortisol levels and disrupted cortisol rhythmicity in depressed patients (Dinan, 1994; Reus and Miner, 1985). Cortisol is further linked to depressive symptomatology by observations that patients experiencing elevated glucocorticoid levels as a result of Cushing's disease or synthetic glucocorticoid therapy develop psychiatric and cognitive symptoms consistent with those observed in major depression (Antonijevic and Steiger, 2003; Brown et al., 2004; Brown and Suppes, 1998).

Based on such clinical findings, animal models were developed to further study the molecular effects of stress and the underlying neurobiological mechanisms of depression. For pharmacologists, these animal models of depression can also be used to screen for antidepressants or preventive drugs which target the HPA axis or cortisol receptors. Of the animal models that currently exist, those involving repeated exposure to stress hold promise for modeling depression, as they simulate the presumed etiology of the disorder. Animal models of repeated stress have utilized a wide range of stimuli to invoke HPA axis activation, ranging from chronic mild stress exposure to repeated restraint stress (Gamaro et al., 2003; Grønli et al., 2004). The advantage of these repeated stress models is that they provide an excellent means to study the neurobiological changes produced

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by repeated stress exposure (Beck and Luine, 2002; Gamaro et al., 2003; Nestler et al., 2002a; Pharm et al., 2003). However, the effects of restraint stress on emotional behavior are variable. Some studies have reported that repeated restraint stress increases emotional behavior (Beck and Luine, 2002; Faraday, 2002), and other studies have found no increases in emotional behavior (Dinan, 2001; Gregus et al., 2005; Platt and Stone, 1982). One possibility is that the effects of restraint stress are quite sensitive to procedural differences between experiments, such as different durations and types of restraint. A second possibility is that there is a lack of control over individual differences in HPA axis activation and subsequent corticosterone levels in terms of experimenter-applied stress models. Stressful stimuli can differ in their physical qualities, and in terms of their psychological qualities (Jesberger and Richardson, 1985). This may result in differing corticosterone levels between different animals exposed to the same stressor, which in turn could lead to increased experimental variability (Nestler et al., 2002b). Finally, the variable behavioral results produced by restraint stress may be due to habituation. That is, animals may have habituated to the adverse effects of repeated restraint (Gregus et al., 2005; Luine et al., 1996). For example, it has been shown that by day 14 of repeated restraint stress, corticosterone levels in male rats are significantly lower than on days 1 and 7 of the restraint procedures (Galea et al., 1997).

One way to avoid these problems is by using exogenous corticosterone administration as a means to study the effects of elevated corticosterone levels, which would occur as a consequence of stress exposure. There are a number of ways to administer corticosterone to animals, but a repeated corticosterone-injection model has some advantages compared to other methods of administration. For example, a repeated corticosterone-injection model provides control over increases in circulating glucocorticoids (Johnson et al., 2006; Sousa et al., 1998a), which may not be achieved with other corticosterone administration methods (e.g., corticosterone pellet implantation or corticosterone in drinking water). Presently, accumulating evidence suggests that repeated corticosterone injections administered to male rats may produce changes in emotional behavior that correspond to symptoms of clinical depression (Gregus et al., 2005; Johnson et al., 2006; Stone et al., 1988).

However, few investigations were conducted to evaluate the effects of repeated corticosterone injections on depression-like behavior in mice. There were also few studies concerning the time-related effects of repeated corticosterone injections on depression-like behavior (e.g., one-week, three-week or five-week). Therefore, the primary objective of the present research is to determine whether repeated corticosterone injections also produce effects on depression-like behavior in mice. This question is addressed by observing the effects of one-week, three-week or five-week daily corticosterone injections on forced-swim and tail suspension behavior at the dose of 20 mg/kg.

A second objective for this experiment is to determine the effects of daily corticosterone injections on levels of tyrosine hydroxylase (TH) in hippocampal tissues of mice. TH is a rate-limiting enzyme in the dopamine and noradrenergic system (Glavin, 1985). This system is believed to play a very important role in the pathogenesis of human depression (Brown et al, 1993; Kapur and Mann, 1992; Ressler and Nemeroff, 1999; Tsao et al, 2006). In several previous animal studies, gene expression of TH was found to change in different brain regions of rodent animals after repeated stress exposure or corticosterone administration. However, different groups have ended with different results. For example, a rise in TH mRNA levels was observed after a 12-day forced walking stress (Wang et al., 1998) or 16 days of corticosterone subcutaneous implantation (Ortiz et al., 1995), while no change was noticed after two-week variable stress (Prieto et al., 2003). Additionally, three-week mild stress induced a decrease of TH mRNA in the rat locus coeruleus (Dunčko et al., 2001). In view of these facts, we hypothesized that the duration might play a very important role in gene expression of TH in response to stress or corticosterone administration. In order to test this hypothesis, we assessed hippocampal TH protein and mRNA levels after performing forced-swim and tail suspension test at weeks 1, 3 and 5, respectively. The aim is to determine whether daily corticosterone injections produce a time-related effect on gene expression of TH. Additionally, we also want to determine whether TH contributes to the depression-like behavior induced by this injection paradigm.

2. Methods and materials

2.1. Animals and corticosterone administration

Male C57BL/6N mice (16–20 g, Vital River Laboratory Animal Technology, Beijing, China) were housed in a 12-h light/dark cycle, with lights off at 18:00 h, at a constant temperature of 25 ± 1 °C and free access to food and tap water. Animals were treated according to the Guidelines of Accommodation and Care for Animals Formulated by the Chinese Convention for the protection of vertebrate animals used for experimental and other scientific purposes. Mice were randomly assigned to six experimental groups (n = 10/group). Three groups were administrated subcutaneously with corticosterone (20 mg/kg, suspended in physiological saline containing 0.1% dimethyl sulfoxide (DMSO) and 0.1% Tween-80) once a day in a volume of 5 ml/kg at random times during the light phase, while the other three groups were administrated only with vehicle. A similar corticosterone injection paradigm has been reported to cause a persistent elevation of plasma corticosterone levels (i.e., lasting for 24 h), with peak levels occurring within 4 h of the injection (Sousa et al., 1998a). After being administered for 1, 3 and 5 weeks, mice depression-like behavior was observed in forced swimming test and tail suspension test. Then, mice were weighted and sacrificed for neurochemical measurement. Five animals per group were randomly chosen for western blot analysis, and the rest were used for RT-PCR analysis.

2.2. Forced swimming test and tail suspension test

Forced swimming test was similar to that described by Porsolt et al. (1977). Briefly, mice were individually placed in 10 cm of ambient temperature water (25 ± 1 °C) in 2000 ml glass

beakers and were allowed to swim for 5 min, and the durations of immobility were recorded during the last 4 min of the test. Duration of immobility is defined as the absence of active, escape-oriented behaviors, such as swimming, jumping, rearing, sniffing or diving.

Tail suspension test was similar to that described by Steru et al. (1985). After forced swimming test, mice were allowed to have a rest for 24 h, and then suspended on the edge of a shelf 58 cm above a tabletop by adhesive tape, placed approximately 1 cm from the tip of the tail. They were allowed to hang for 6 min, and the duration of immobility was recorded during the last 4 min of the test. Mice were considered immobile only when they hung passively and completely motionless.

2.3. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated by trizol (Bio Basic Inc., CA) extraction according to the manufacturer's instructions. Total RNA (2 μg per same) was reverse-transcribed into first-strand cDNA using the MMLV First-Strand Synthesis System kit (Bio Basic Inc., CA) and Oligo-dT_{12-18}. PCR reactions were carried out by using the Ready-to-Use PCR kit (Bio Basic Inc., CA). The oligonucleotide primers were designed based on GeneBank® sequence using PCR primer designing software Primer 5.0 to ensure specific and efficient amplification of target sequences. The primers synthesized by Sangon Biotechnology (Sangon, Beijing, China) and parameters for PCR amplification of TH and β-actin were listed in Table 1. PCR products were separated on 1.5% agarose gels, visualized by ethidium bromide (Sigma) staining, and analyzed using a FR-200A Electrophoresis Image Analysis System (Furi, Shanghai, China). Semi-quantity of PCR product was measured by the factor optical density multiplied by the area of the band. The values of TH PCR product were normalized against the amount of PCR product for β-actin obtained for the same RT sample.

2.4. Western blot analysis

Mice were sacrificed by decapitation while under ether anesthesia. After sacrifice, the brains were rapidly removed from the skull and hippocampal tissues were dissected on an ice-cold plate. Then, dissected hippocampal tissues were immediately homogenized at 4 °C with 0.5 ml of lysis buffer [50 mM Tris–HCl, 0.1% sodium dodecyl sulfate (SDS), 1% nonidet-P40 (NP-40, Sigma), 1 mM ethylenediaminetetraacetic acid (EDTA), 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride (PMSF, Sigma), 1 mM NaF, 1 mM Na_3VO_4, 1 μg/ml aprotinin (Sigma), 1 μg/ml leupeptin (Sigma), pH ≈ 7.5]. Aliquots of the clarified homogenized liquid, containing 75 μg of protein, were denatured in a sample buffer [1% SDS, 1% dithiothreitol (DTT, Sigma), 10 mM Tris–HCl, 10% glycerol, 1 mM EDTA, pH ≈ 8.0] at 95 °C for 5 min. The samples were then analyzed by 12% SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride (PVDF) membranes (Bio-Rad). The membrane was blocked in a buffer (PBS containing 5% non-fat dried milk and 0.2% Tween-20) for 1 h at 37 °C. Then, the membrane was in turn incubated with primary antibody, biotin-tagged secondary antibody and peroxidase-tagged streptavidin. Finally, the membrane was developed in 10 ml of freshly prepared substrate solution [0.03% H_2O_2, 6 mg/ml 4-chloro-1-naphthol (Sigma) and 2 mg/ml 3, 3′-diaminobenzidine tetrahydro-chloride (Sigma) in PBS]. The protein bands were quantified using a FR-200A Electrophoresis Image Analysis System. The values of TH levels were normalized against the amount of β-actin obtained for the same sample. The specific anti-TH and anti-β-actin polyclonal antibodies were purchased from Bioss Biotechnology (Bioss, Beijing, China).

2.5. Statistical analysis

Data was expressed as mean ± S.E.M. for the indicated number of experiments and analyzed using the statistical package for social sciences (SPSS) computer program version 10.1. Statistical significance was determined by two-way analysis of variance (ANOVA) with treatment and time as independent, between-subjects factors. In case of significant interactions, Fisher least-significant differences test of multiple comparisons was performed. The significance level was set at $P \leq 0.05$ for all statistical comparisons.

3. Results

3.1. Body weight

The effects of repeated corticosterone injections on body weight are shown in Fig. 1. The corticosterone injections decreased body weight over the injection period. The body weight differences between normal and corticosterone-treated groups arrived at 2.5 g, 3.2 g and 3.4 g at weeks 1, 3 and 5, respectively. The statistical details of these observations are given below.

At week 0, average body weight (g) of six groups of mice was 18.4 ± 0.4 vs. 18.6 ± 0.6, 18.5 ± 0.6 vs. 18.6 ± 0.4 and 18.6 ± 0.4 vs. 18.8 ± 0.5, respectively. All normal mice gained weight over time, as shown by a significant effect of time ($F_{2,\ 54} = 8.135$; $P < 0.001$). Body weight of normal mice increased 0.6 g (3.3%), 1.5 g (8.1%) and 2.4 g (12.9%) at weeks 1, 3 and 5, respectively.

Table 1
The primers and parameters for PCR amplification of TH and β-actin

cDNA	Primer	Size of product (base pairs)	PCR amplification
TH (mouse)	Up: 5′-CGG CRG GRA GGT TTG ATC TTG-3′ Down: 5′-GGT CTA CTG TCT GCC CGT GAT-3′	414 bp	95 °C (45 s), 57 °C (30 s), 72 °C (1 min) 30 cycles, Mg^{2+} (2 mM)
β-actin (mouse)	Up: 5′-GCC CAT CTA CGA GGG CTA T-3′ Down: 5′-GCT GGA AGG TGG ACA GTG AG-3′	570 bp	95 °C (45 s), 56 °C (30 s), 72 °C (1 min) 25 cycles, Mg^{2+} (1.5 mM)

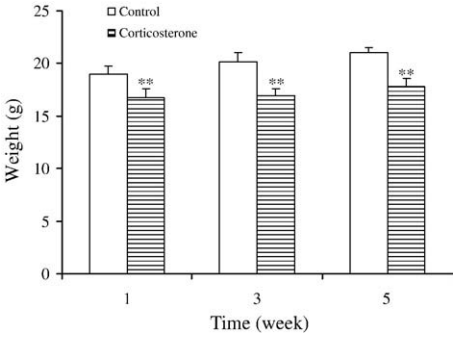

Fig. 1. Effects of repeated corticosterone injection on body weight of mice. Data is expressed as mean±S.E.M. (n=10). At week 0, body weight (g) of six groups of mice was 18.4±0.4 vs. 18.6±0.6, 18.5±0.6 vs. 18.6±0.4 and 18.6±0.4 vs. 18.8 ±0.5, respectively. Data was analyzed by using two-way analysis of variance (ANOVA) with treatment and time as factors. **P<0.01, for the corticosterone vs. control mice, Fisher least-significant differences test.

However, repeated corticosterone injections inhibited weight gain, as shown by a significant effect of treatment ($F_{1,\ 54}$= 35.897; P<0.001) and a significant interaction between time and treatment ($F_{2,\ 54}$=6.754; P<0.01). In fact, repeated corticosterone injections even caused mice to lose body weight.

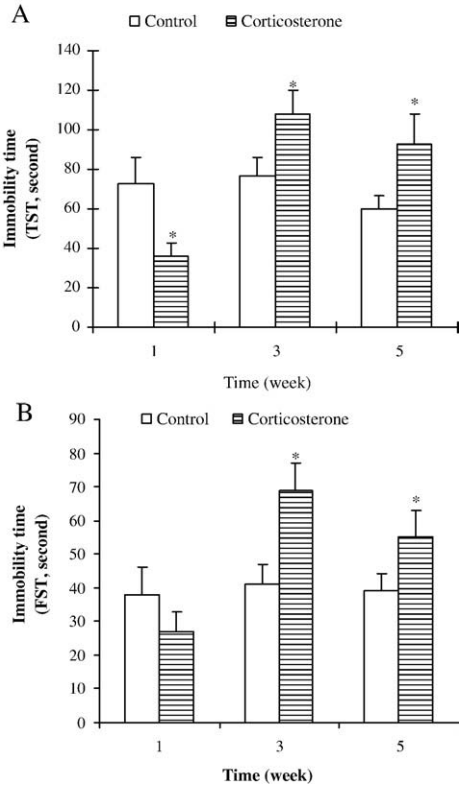

Fig. 2. Effects of repeated corticosterone injection on depression-like behavior of male mice in the forced-swim test (FST) and tail suspension test (TST) (n=10). The mean (±S.E.M.) of time spent immobile during the last 4 min of the tail suspension test is shown in panel (A), and the mean (±S.E.M.) of time spent immobile during the last 4 min of the forced swimming test is shown in panel (B). Data was analyzed by using two-way analysis of variance (ANOVA) with treatment and time as factors. *P<0.05, for the corticosterone vs. control mice, Fisher least-significant differences test.

Body weight of corticosterone-treated mice lost 1.9 g (10.2%), 1.7 g (9.1%) and 1.0 g (5.3%) at weeks 1, 3 and 5, respectively.

3.2. Forced swimming test and tail suspension test

After repeated corticosterone injections, depression-like behavior of mice was assessed by using forced swimming test and tail suspension test at weeks 1, 3 and 5, respectively. In general, repeated corticosterone injections significantly increased depression-like behavior in both tests. The statistical details of these observations are given below.

In the forced swimming test, there was a significant effect of time ($F_{2,\ 54}$=5.135; P<0.01) and treatment on immobile time ($F_{1,\ 54}$=3.897; P<0.05), as well as a significant interaction between treatment and time ($F_{2,\ 54}$=8.754; P<0.001). Multiple comparisons test showed that these corticosterone injections significantly increase immobile time of mice at week 3 (P<0.05) and week 5 (P<0.05), but not at week 1 (Fig. 2A).

In the tail suspension test, two-way ANOVA also showed significant main effects of treatment ($F_{1,\ 54}$=5.274; P<0.05) and time ($F_{2,\ 54}$=3.911; P<0.05) with a significant interaction between these factors ($F_{2,\ 54}$=5.013; P<0.05). Multiple comparisons test revealed that the immobile time of mice injected daily with corticosterone decreased significantly at week 1 (P<0.05), but increased significantly at week 3 (P<0.05) and week 5 (P<0.05) (Fig. 2B).

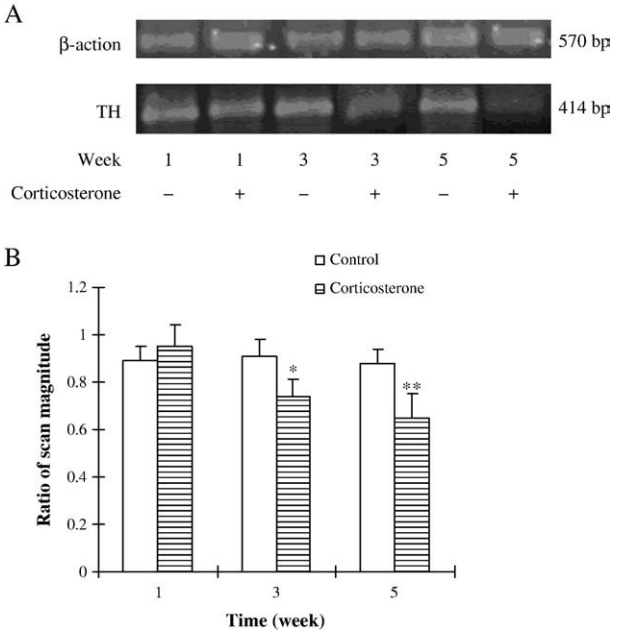

Fig. 3. Effects of repeated corticosterone injection on TH expression in the hippocampus tissues of mice. Data is expressed as mean±S.E.M. (n=5). TH transcription was studied by using RT-PCR (A). PCR products of β-actin and TH were separated on 1.5% agarose gels, visualized by ethidium bromide staining, and analyzed using a FR-200A Electrophoresis Image Analysis System. The values of TH (B) PCR products were normalized against the amount of PCR product for β-actin obtained from the same RT sample. Data was analyzed by using two-way analysis of variance (ANOVA) with treatment and time as factors. *P<0.05; **P<0.01, for the corticosterone vs. control mice, Fisher least-significant differences test.

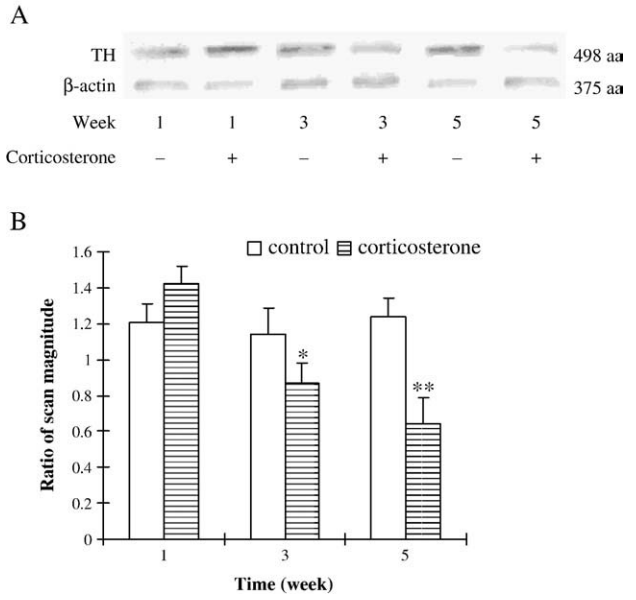

Fig. 4. Effects of repeated corticosterone injection on TH protein levels in the hippocampus tissues of mice. Data is expressed as mean±S.E.M. (n=5). TH protein levels were determined by western blot analysis using anti-TH and anti-β-actin antibodies (A). Protein samples were analyzed by 12% SDS-polyacrylamide gel electrophoresis. Then, the gel was transferred to Immuno-Blot PVDF membranes (0.22 μm) at a different voltage, respectively. The protein bands were quantified using a FR-200A Electrophoresis Image Analysis System. The values of TH levels (B) were normalized against the amount of β-actin obtained for the same sample, respectively. Data was analyzed by using two-way analysis of variance (ANOVA) with treatment and time as factors. *P<0.05; **P<0.01, for the corticosterone vs. control mice, Fisher least-significant differences test.

3.3. TH mRNA expression

The effects of repeated corticosterone injections on hippocampal TH transcription are shown in Fig. 3. In general, repeated corticosterone injections significantly decrease TH mRNA levels in hippocampal tissues. The statistical details of these observations are given below.

Two-way ANOVA revealed a significant effect of treatment ($F_{1,\ 24}$=12.720; P<0.001) and time ($F_{2,\ 24}$=4.924; P<0.05) on TH expression with a significant interaction between two factors ($F_{2,\ 24}$=3.999; P<0.05). Multiple comparisons test showed that there was no obvious difference for TH mRNA levels between two groups at week 1. A significant decrease for TH mRNA levels appeared at weeks 3 and 5, with a decrease of 31.9% (P<0.05) and 47.6% (P<0.01), respectively (Fig. 3B).

3.4. TH protein levels

The effects of repeated corticosterone injections on hippocampal TH protein levels are shown in Fig. 4. In general, repeated corticosterone injections significantly decreased TH protein levels in hippocampal tissues. The statistical details of these observations are given below.

Two-way ANOVA revealed a significant effect of treatment ($F_{1,\ 24}$=15.340; P<0.001) and time ($F_{2,\ 24}$=4.424; P<0.05) on TH protein levels, with a significant interaction between two factors ($F_{2,\ 24}$=4.112; P<0.05). Multiple comparisons test showed that there was a tendency to increase TH protein levels in hippocampal tissues of mice treated with 1-week daily corticosterone (P=0.132). However, we found that 3-week and 5-week daily corticosterone injections significantly decreased TH protein levels by 19.9% (P<0.05) and 37.1% (P<0.01), respectively (Fig. 4B).

4. Discussion

The forced-swim and tail suspension tests are commonly employed as a behavioral screen for antidepressant treatments (Cryan et al., 2002). Because antidepressants generally delay and decrease immobility, a depressive phenotype is inferred from prolonged immobility and reduced active behavior. Results from several previous studies have indicated that repeated corticosterone treatments can influence rat behavior in the forced-swim test. For example, two previous papers reported that 21 daily injections of 40 mg/kg corticosterone increased rat forced-swim test immobility (Gregus et al, 2005; Kalynchuk et al., 2004). In addition, 20 consecutive daily injections of 20 mg/kg corticosterone were also found to increase rat forced-swim test immobility (Hill et al., 2003). Recent results from Dr. Kalynchuk's lab further demonstrated that the effects of repeated corticosterone injections on depression-like behavior were dose-dependent (Johnson et al, 2006). These results suggest that repeated corticosterone injections can reliably prolong immobility and increase depression-like behavior in a dose-dependent manner in male rats.

In the present study, we extended this finding in several important ways. We first found that mice, another rodent animal, could also be induced by repeated corticosterone injections to display increased depression-like behavior. The direct evidence was that 3-week and 5-week corticosterone injections significantly prolonged immobility time of mice in both the forced-swim and tail suspension tests. However, our conclusion that exposure to repeated corticosterone injections increases depression-like behavior in mice should be considered in light of the effects of the corticosterone injections on body weight. In this experiment, repeated corticosterone injections obviously inhibited body weight gain and even caused mice to lose body weight, which is consistent with previous findings (Sousa et al., 1998b). This leads to the question of whether the behavioral changes we observed could simply be a non-specific effect of weight loss (and the accompanying decrease in muscle mass), rather than a depressogenic effect per se. For example, it may be that the corticosterone-injected rats show more immobility behavior because they have less muscle mass.

In previous studies, investigators ever tried to demonstrate that it is not likely that the behavioral changes we observed in the forced-swim test can be accounted for simply by weight loss. For example, corticosterone-injected rats did not always engage in less locomotive activity than control rats. Corticosterone-injected rats displayed a similar amount of open-field exploration as vehicle-injected controls (Brotto et al., 2001; Gregus et al., 2005; Kalynchuk et al., 2004). In the Morris Water maze, rats subjected to repeated high doses of corticosterone show

similar swim distances to control rats (Sousa et al., 2000). In our studies, we found that 1-week corticosterone injections, which can significantly decrease body weight gain, did not influence immobility time in the forced-swim test and even decreased immobility time in the tail suspension test. Thus, the behavioral changes we observed could be a depressogenic effect per se, rather than a simple non-specific effect of weight loss.

Meanwhile, TH level changes in hippocampal tissues also provide the indirect evidence for increased depression-like behavior of mice. TH is a rate-limiting enzyme in the biosynthesis of dopamine and norepinephrine, and is routinely regarded as a marker of the dopamine or noradrenergic system that was responsible for depression and also related to the effects of antidepressants (Brown et al., 1993; Kapur and Mann, 1992; Petty and Sherman, 1979; Ressler and Nemeroff, 1999). In our studies, we found that 3-week and 5-week corticosterone injections produced a graded decrease in TH mRNA and protein levels in hippocampal tissues, which was consistent with increased depression-like behavior of mice. Taken together, such direct and indirect evidence we observed in our studies suggests that repeated corticosterone treatment can induce mice to exhibit increased depression-like behavior.

In the present study, we also uncovered another important and interesting finding, that short-term and long-term corticosterone injections produce a different effect on mouse depression-like behavior and hippocampal TH levels. Our results showed that 3-week and 5-week corticosterone injections significantly prolonged immobility time of mice in both the forced-swim and tail suspension test, while 1-week corticosterone injections tended to decrease immobility time of mice in the forced-swim test. Furthermore, in the tail suspension test, 1-week corticosterone injections obviously decreased immobility time of mice. In terms of hippocampal TH levels, we also found that 3-week and 5-week corticosterone injections induced a graded decrease, but 1-week corticosterone injections tended to induce an increase. This data suggested that daily injection of 20 mg/kg of corticosterone could produce a time-related effect on mouse depression-like behavior and hippocampal TH levels.

Although ours is the first study to clearly demonstrate a time-related effect of corticosterone on mouse depression-like behavior and hippocampal TH levels, results from previous studies have ever hinted that repeated corticosterone treatment can cause a biphasic effect on animal memory. It shows that short-term elevations of corticosterone appear to facilitate memory consolidation (Sandi and Rose, 1997), whereas prolonged exposure to elevated corticosterone levels has been associated with deficits in learning, memory and retrieval (Bodnoff et al., 1995; Dachir et al., 1993). On TH mRNA and protein levels, previous studies also show that repeated corticosterone treatment or stress exposure induces a dual effect. For example, a rise in TH mRNA levels was observed after repeated stress exposure or corticosterone administration, such as 12-day forced walking stress (Wang et al., 1998), 13-day social stress (Watanabe et al., 1995), two-week cold stress (Miner et al., 2006) or 16 days of corticosterone subcutaneous implantation (Ortiz et al., 1995), while no change was noticed after repeated foot-shock and restraint (Smith et al., 1991), 2 weeks of corticosterone subcutaneous implantation (Makino et al., 2002) or two-week variable stress (Prieto et al., 2003). On the other hand, three-week mild stress induced a decrease of TH mRNA in the rat locus coeruleus (Dunčko et al., 2001). It seems that less than 3 weeks of corticosterone injections or stress exposure induces an increase or no change in TH levels, while more than 3 weeks of corticosterone injections or stress exposure causes a decrease in TH levels. Taken together, both our and previous findings support the fact that short-term and long-term repeated corticosterone injections produce positive and negative effects on animal behavior, respectively.

It is well known that glucocorticoid hormones can readily enter the brain and bind directly to mineralocorticoid receptors and glucocorticoid receptors (de Kloet, 1991; Reul and de Kloet, 1985). These two receptor types differ in their affinity for corticosterone. Glucocorticoid receptors have a low affinity for corticosterone and become occupied only during stress and at the circadian peak, when circulating levels of glucocorticoids are high. In contrast, mineralocorticoid receptors have a 10-fold higher affinity for corticosterone and are almost saturated under basal conditions (Reul and de Kloet, 1985). Thus, it is likely that positive effects of corticosterone on animal behavior are due to activation of glucocorticoid receptors, which has been supported by several findings. For example, a study examining the effects of systemic administration of different doses of corticosterone to adrenalectomized rats showed that the level of glucocorticoid receptor occupancy was significantly correlated with spatial memory performance (Conrad et al., 1999). Additionally, post-training infusions of a glucocorticoid receptor antagonist were found to impair memory for an avoidance task in chicks and block the enhancing effects of post-training corticosterone (Roozendaal and McGaugh, 1997; Sandi and Rose, 1994).

Interestingly, the activation of glucocorticoid receptors is also believed to be involved in the negative effects of corticosterone on animal behavior (Radeley and Morrison, 2005). A popular structural hypothesis shows that activation of glucocorticoid receptors can suppress neurogenesis through reducing the synthesis of neurotrophins, such as brain-derived neurotrophic factor (BDNF) (Henn et al., 2004). Supporting evidence also includes that glucocorticoid receptor knockout animals clearly have alterations in sensitivity to repeated stress exposure (Gass et al., 2001; Urani and Gass, 2003), and that mifepristone, an antagonist of glucocorticoid receptors, produces a reduction of the symptoms in patients with psychotic depression (Belanoff et al., 2001). Thus, it seems that glucocorticoid receptors are responsible for both positive and negative effects produced by short-term and long-term corticosterone, respectively. If this is true, the numerous studies on roles of glucocorticoid receptors in positive and negative effects of corticosterone only further support the fact that the effects of corticosterone seem to be dual. This, however, is still unknown, and why short-term and long-term corticosterone treatment will produce an opposite effect on animal behavior is most certainly a question worth further investigation.

In conclusion, the present study has served to further explore the behavioral and biochemical consequences of exposure to a repeated corticosterone injection paradigm. We have shown that

mice, another rodent animal, can also be induced by repeated corticosterone injections to appear increased depression-like behavior, suggesting that we can save money and energy through adopting cheaper animals and more convenient model-making materials. Furthermore, the effects of repeated corticosterone injections on depression-like behavior are time-dependent, indicating that we can use this model to explore the potential mechanisms of corticosterone dual effects, and that we need to take the duration into consideration while making this mouse model of depression. These observations suggest that the repeated corticosterone injection paradigm provides a useful and reliable mouse model within which to further study the role of stress and glucocorticoids in depressive illness, as well as screen for antidepressants or preventive drugs.

Acknowledgements

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