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EFFECTIVENESS OF DIFFERENT CORTICOSTERONE ADMINISTRATION METHODS TO ELEVATE CORTICOSTERONE SERUM LEVELS, INDUCE DEPRESSIVE-LIKE BEHAVIOR, AND AFFECT NEUROGENESIS LEVELS IN FEMALE RATS

J. M. KOTT, S. M. MOONEY-LEBER, F. A. SHOUBAH AND S. BRUMMELTE *

Department of Psychology, Wayne State University, 5057 Woodward Avenue, Detroit, MI 48202, USA

Abstract—High levels of chronic stress or stress hormones are associated with depressive-like behavior in animal models. However, slight elevations in corticosterone (CORT) – the major stress hormone in rodents – have also been associated with improved performances, albeit in a sex-dependent manner. Some of the discrepancies in the literature regarding the effects of high CORT levels may be due to different administrations methods. The current study aims to compare the effects of ~40 mg/kg given either via subcutaneous injection, through an implanted pellet, or in the drinking water, for ~21 days on CORT serum levels, depressive-like behavior in the forced swim test (FST), and neurogenesis levels in the dentate gyrus (DG) in adult female rats. We found that animals exposed to the daily injections showed elevated CORT levels throughout the administration period, while the pellet animals showed only a transient increase, and drinking water animals revealed no elevation in CORT in serum. In addition, only the injection group exhibited higher levels of immobility in the FST. Interestingly, animals receiving CORT via injection or drinking water had lower numbers of doublecortin-positive cells in the ventral DG one week after the last CORT administration compared to animals implanted with a CORT pellet. These results will contribute to the growing literature on the effects of chronic CORT exposure and may help to clarify some of the discrepancies among previous studies, particularly in females.

Key words: females, corticosterone, neurogenesis, depression, stress, administration methods.

INTRODUCTION

Chronic stress has well-known consequences for overall health and well-being (Juster et al., 2010) and glucocorticoids (cortisol (the major glucocorticoid in humans) and corticosterone (CORT; major glucocorticoid in rodents)) have been identified as the major culprits in the pathway from stressful experience to negative health outcomes (Keller et al., 1983; Rabin et al., 1990). Studies have investigated the effects of direct administration of CORT to male rodents instead of exposing the animals to chronic stress (Kalynchuk et al., 2004; Gregus et al., 2005; Murray et al., 2008) However, less is known about the effects of chronic CORT administration in females and whether different administration methods may have different impacts on body weight, depressive-like behavior, or CORT serum levels.

Chronic stress studies in animals are often used to induce depressive-like behavior or other adverse physiological and behavioral outcomes, however, there is a lot of variability in the reported effects and consequences. This may be due to the type of stressor used, or due to individual, or sex-dependent differences in stress sensitivity. For instance, rats easily adjust to predictable stressors and can fail to exhibit increased CORT levels over time, so paradigms using predictable stress may be less effective compared to unpredictable stressors (De Boer et al., 1989; Herman, 2013). Another interesting concern is that some stress models seem to be less effective in females compared to males (Dalla et al., 2010). For example, females seem to be more sensitive to the stressful effects of the forced swim test (FST) than males, but display a decrease in helplessness behavior in response to footshock than do males (Dalla et al., 2008). Further, female rats have higher basal CORT levels in serum (Jezova et al., 1996) than males and their unique physiology may allow them to adapt differently to stress than males. For instance, females undergo a unique down-regulation of their hypothala mus–pituitary–adrenal (HPA) axis sensitivity during pregnancy and the postpartum (Neumann, 2001; Brunton et al., 2008). Though males undergo endocrine changes in response to fatherhood such as reduced testosterone levels (Gettler et al., 2011a) and testosterone and glucocorticoids are known to interact with each other (Viau and Meaney, 1996; Gettler et al., 2011b), recent animal studies suggest that these parenthood-induced changes may

*Corresponding author. Tel: +1-313-577-2834.
E-mail address: sbrummelte@wayne.edu (S. Brummelte).
Abbreviations: ANOVA, analysis of variance; CBG, corticosteroid-binding globulin; CORT, corticosterone; DAB, 3,3′-daminobenzidine; DCX, doublecortin; DG, dentate gyrus; FST, forced swim test; HPA, hypothalamus–pituitary–adrenal; NIH, National Institutes of Health; OFT, open field test; PBS, Phosphate-buffered saline; s.c., subcutaneous.

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not have pronounced effects on stress-responsiveness in fathers, in contrast to the well-documented effects of pregnancy and lactation in mothers (Saltzman and Ziegler, 2014). It is conceivable that the unique female ability to downregulate HPA axis responses during reproductive times may also help them with stress adaptation in regular adulthood compared to males. However, despite these findings, animal models test female rats far less frequently than males (Martin and Brown, 2010), though new National Institutes of Health (NIH) regulations may change this for pre-clinical studies in the future.

An alternative method to using chronic stress paradigms is the direct administration of CORT, the end product of HPA activation, to mimic the effects of stress. One advantage of this method is that it reduces the individual and sex-dependent variability in response to stressful experience, as well as the variability in effectiveness of the various chronic stress models currently in use. Administering CORT has successfully been used in adrenalectomized animals to provide glucocorticoids, as well as a means to transiently elevate glucocorticoid levels similar to stress-induced HPA axis activation in intact animals. For instance, high levels of CORT (40 mg/kg s.c. for 21 days) induces depressive-like behavior in both males and females (Gregus et al., 2005), reduces body weight and hippocampal neurogenesis, and maintains high levels of CORT throughout a three-week period (Brummelte and Galea, 2010). Further, high levels of CORT (40 mg/kg) given to dams during the postpartum period has led to permanent alteration in the offspring (Brummelte et al., 2006). Notably, there were sex-specific effects in the offspring, with male offspring showing more impulsivity-like and anxiety-like behavior and reduced hippocampal cell proliferation, while females seemed somewhat less affected (Brummelte et al., 2006, 2012). Further, other studies using CORT during the postpartum period also found adaptations in the offspring that suggest a negative impact of CORT during development (Pavlovska-Teglia et al., 1995; Taylor et al., 2000; Brummelte et al., 2006; Brummelte and Galea, 2010). Despite the fact that using exogenous CORT instead of stress should reduce variability between studies, other studies have reported quite the opposite outcomes in regard to the effects of exogenous CORT administration. For instance, Cataloni and colleagues administered CORT through the drinking water to postpartum dams and found protective effects of maternal CORT elevation in the offspring (Catalani et al., 1993, 2000, 2002; Casolini et al., 2007) compared to the rather negative effects described above (Pavlovska-Teglia et al., 1995; Taylor et al., 2000; Brummelte et al., 2006; Brummelte and Galea, 2010). Another study showed that CORT-treated dams produced pups less vulnerable to the damaging effects of global brain ischemia in adulthood (Casolini et al., 2007). Further, CORT in the drinking water resulted in improved learning in the water maze task, a reduction in fearful behavior in the light–dark task, and reduced anxiety-like behavior in the plus maze in the adult offspring (Catalani et al., 2002). The studies described above emphasize that maternal exogenous CORT administration can have a profound impact on the offspring, but that there is still a great deal of variability between studies which may be due to different doses or administration protocols.

There are currently three major routes that are commonly used for CORT administration: subcutaneous (s.c.) injection of CORT, CORT solution in drinking water, and s.c. placement of a time-release CORT pellet. Each has certain advantages and disadvantages. For instance, the pellet implantation requires a brief surgery and the dose cannot be adjusted during the experiment (i.e. for weight gain or loss). Further, the pellets provide a continuous release of the drug that is uncontrollable once the pellet is implanted. Drinking water is a non-invasive way to administer CORT and requires no handling or manipulation of the animals, which is a major advantage. However, the dose cannot be controlled or adjusted, as the animals control the amount of water consumed *ad libidum*. Daily s.c. injections require daily handling and invasive injections, but the dose can be adjusted by weight daily if necessary. Further, injections deliver the full dose at once rather than over the course of the day, which is an important difference between these routes. CORT and cortisol serum levels vary significantly throughout the day, with levels being high at the beginning of the active period (mornings in humans, evenings in rats) and drop throughout the course of the day/night. This diurnal rhythm and HPA-axis regulation may play a crucial role in depressive pathology. Patients who improved their HPA regulation after one week of treatment had lower depression scores and shorter admission times compared to patients that continued to display HPA axis dysregulation (Schule et al., 2009). Thus, some of the variance between studies may be explained by continuous versus all-at-once release of the chosen CORT dose, as this will have an impact on the diurnal CORT profile and HPA regulation. Though all routes have been proven effective in at least some studies to increase CORT levels in males, no study to date has looked at the effectiveness of the different administration routes in female rats, who naturally have higher circulating levels of CORT than males (Tinnikov, 1999).

The aim of the current study was to determine the effects of chronic CORT administration via three different routes in female rats: an implanted pellet, through the drinking water, or via s.c. injection. We were interested in serum levels of CORT during and after a three-week administration paradigm, as well as the effects on body weight, depressive-like behavior, anxiety-like behavior, and neurogenesis in female rats. To measure depressive-like behavior, we use the Porsolt FST (Porsolt et al., 1978). In addition, we test the rats in the open field test (OFT) that serves as a test for anxiety-like behavior as well as for overall locomotor activity. Route-dependent variations in CORT-induced effects in female rats may also help to better understand different possible outcomes of offspring that have been exposed to pre- or postnatal elevation of maternal CORT. While these methods of exogenous CORT administration have been effective in males, but not consistently so in