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Effects of corticosterone on contextual fear consolidation in intact and ovariectomized female rats

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ABSTRACT

Previous studies have shown that post-training administration of glucocorticoids enhances memory consolidation in male rats, but theirs effects on female rats are not known. Thus, this study was conducted to examine the effects of corticosterone (CORT) on contextual fear memory consolidation in intact and ovariectomized (OVX) female rats. In Experiment 1, post-training administration of CORT (0.3, 3, and 10 mg/kg) to OVX female rats impaired memory consolidation at a 0.3 mg dose of CORT. In Experiment 2, post-training injection of CORT (0.3 mg/kg) to female rats in proestrus stage (when the levels of estrogens are highest) enhances and in the estrus stage (when the levels of estrogens are lowest) impaired memory retention. In Experiment 3, OVX female rats injected with CORT (0.3 mg/kg) and one of the three doses of 17β-estradiol (1, 10 or 100 μg/kg) following training. 48-h memory retention test indicated that CORT enhanced memory retention in OVX female rats that received concurrent injection of 10 or 100 μg doses of 17β-estradiol. These findings indicate that cognitive effects of CORT in female rats can be modulated with the plasma levels of estrogens: when the levels of estrogens are low, corticosterone has a negative effect, while when the levels of estrogens are high; the corticosterone has a positive enhancing effect.

1. Introduction

Extensive evidence from animal and human studies indicates that glucocorticoid hormones (corticosterone in rats; cortisol in humans) that are released during stress or emotional experiences can influence various phases of cognitive functions (Roozendaal, 2000). Studies of the effects of corticosterone, as well as synthetic glucocorticoid receptor agonists and antagonists, indicate that memory consolidation for a variety of tasks including inhibitory avoidance, Morris water maze, and contextual fear conditioning (CFC) is enhanced by glucocorticoid agonists and impaired by antagonists (McGaugh & Roozendaal, 2002; Roozendaal, 2002). Recent research has revealed that glucocorticoid–induced enhancement of memory consolidation require arousal-induced noradrenergic activation within the basolateral complex of the amygdala (Roozendaal, Okuda, Van der Zee, & McGaugh, 2006).

Female sex hormones can influence cognitive functions. Both endogenous and exogenous estrogen levels influence hippocampus-dependent learning and memory in a variety of tasks (Daniel, 2006). For example, in comparison with rats in estrus (when endogenous estrogen levels are lowest), female rats in proestrus (when endogenous plasma levels of estrogen are highest) exhibited performance impairment on different tasks such as fear conditioning, and Morris water maze that requires hippocampus integrity (Markus & Zecevic, 1997; Warren & Juraska, 1997). Exogenous administration of estrogen to adult ovariectomized (OVX) female rats shows a dose-dependent effect between estradiol and memory and learning. Low but physiological levels of exogenous estradiol improve performance (Fader, Johnson, & Dohanich, 1999; Holmes, Wide, & Galea, 2002), whereas administration of high exogenous pharmacological or physiological levels of estradiol impair performance on the radial arm maze task, as a hippocampus-dependent task (Galea et al., 2001; Holmes et al., 2002). A recent study showed that acquisition of hippocampus-dependent contextual fear conditioning is influenced by 17β-estradiol, estrone, and 17α-estradiol with a low dose of 17β-estradiol and 17α-estradiol enhanced and a middle or high dose of either form of estradiol impaired contextual fear conditioning (Barha, Dalton, & Galea, 2010). Recent studies have shown that estradiol can influence fear extinction and the activity of brain regions involved in fear memory in women and female rats. For example, it was reported that women using hormonal contraceptives (HC) exhibited significantly poorer extinction recall compared with naturally cycling women. A single administration of estradiol to naturally cycling women significantly enhanced their ability to recall

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extinction memories (Graham & Milad, 2013). Similarly, HC-treated female rats showed fear extinction impairment which was associated with reduced serum estradiol levels. The impairment could be rescued either by terminating HC treatment after fear learning or by systemic injection of estrogen-receptor agonists before fear extinction (Graham & Milad, 2013). Likewise, post-extinction training administration of estradiol facilitated extinction memory consolidation and increased c-Fos expression in the medial prefrontal cortex reducing it in the amygdala in intact female rats. In parallel, natural variance in estradiol in premenopausal cycling women modulated medial prefrontal and amygdala reactivity and facilitated extinction recall (Zeidan et al., 2011). These findings indicate that estrogens have complex effects on different aspects of cognitive functions.

Evidence from human and animal studies reveals a vital association between sex differences in stress sensitivity and the predisposition toward mood disorders (Bale, 2006). Animal studies support the hormonal differences contribute to explaining sex differences in stress response notion (Goldstein, Jerram, Abbs, Whitfield-Gabrieli, & Makris, 2010). Activity of the hypothalamic–pituitary–adrenal axis (HPA) is markedly influenced by sex steroids, as illustrated by the pronounced elevations in glucocorticoid levels exhibited in female rodents compared with male counterparts (Handa, Burgess, Kerr, & O'Keefe, 1994; McCormick, Smythe, Sharma, & Meaney, 1995). In adult OVX female rats, estrogen potentiates the corticosterone responses to numerous stress, including restraint stress (Figueiredo, Ulrich-Lai, Choi, & Herman, 2007; Viau & Meaney, 1991), resulting in glucocorticoid hypersecretion. This effect of estrogen is mediated by central as well peripheral mechanisms (Burgess & Handa, 1992; Carey, Deterd, De Koning, Helmerhorst, & De Kloet, 1995; Figueiredo et al., 2007). Also, progesterone is able to prevent the inhibitory effects of corticosterone on lordosis behavior (Madhuranath & Yajurvedi, 2011). These findings suggest an existence of interaction between glucocorticoids and female sex hormones on behavioral responses. For example, it has been shown that chronically stressed rats receiving estradiol showed *less* anxious behavior in an open field test and better performance in a radial arm maze than stressed rats receiving vehicle treatment (Bowman, Ferguson, & Luine, 2002). However, such a possible interaction between glucocorticoids and female sex hormones on CFC is not yet studied. Thus, this study was designed to investigate the effects of glucocorticoids on CFC in intact female rats in proestrus and estrus states as well as OVX female rats receiving different doses of 17β-estradiol.

2. Material and methods

2.1. Animals

Adult female Wistar rats, weighting 200–250 g, obtained from breeding colony of Semnan University of Medical Sciences (Semnan, Iran), were used in the study. Rats were initially housed 5 per cage in large cages (50 × 26 × 25 cm) and kept on a 12-h light/dark cycle (6 am lights on–6 pm lights off) with food and water available ad libitum. The ambient environment was maintained at constant temperature (22 ± 2 °C) and relative humidity (50–60%). The experimental protocol was approved by the Ethical Review Board of Semnan University of Medical Sciences (Iran). All of the experimental trials were conducted in agreement with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All experiments were performed between 08:00 and 12:00 h during the light cycle.

2.2. Drugs

Corticosterone (CORT, 0.3, 3 or 10 mg/kg, Sigma) and 17β-estradiol (1, 10 or 100 μg/kg, Sigma) were dissolved in 40% propylene glycol/saline, and 4% ethanol/saline solutions, respectively. These doses of CORT induce plasma levels of CORT resembling mild to severe stress (Abrari, Rashidy-Pour, Semnanian, & Fathollahi, 2008; Dominique, Roozendaal, & McGaugh, 1998; Pugh, Tremblay, Fleshner, & Rudy, 1997). Both drugs and control solutions were given by intraperitoneal (i.p.) injections at a volume of 2 ml/kg.

2.3. Ovariectomy surgery

The rats were bilaterally ovariectomized under chlorate hydrate anesthesia (300 mg/kg, i.p.) by removal of the ovaries with a dorsal incision. In sham operated rats, the ovaries were exteriorized but not removed. The subjects were allowed 7–8 days to recover from surgery (during which they were kept alone in a cage) before any experimental manipulations were initiated.

2.4. Determination of normal estrous cycle

The phases of estrous cycle were determined by examination of vaginal smear as described by others (Marcondes, Bianchi, & Tanno, 2002). In brief, vaginal secretion was collected with a plastic pipette filled with 10 mL of normal physiological saline by inserting the tip into the rat vagina, but not deeply. Vaginal fluid was placed on glass slides. One drop was collected with a clean tip from each rat. Slides were dyed using with methylene blue and stained slides were observed under a light microscope, with 10 and 40 × objective lenses. Vaginal secretion was collected between 8:00 and 10:00 am to maintain consistency, and rats were returned to their home cages for 60 min prior to the start of the experimental procedure.

2.5. CFC training and testing

2.5.1. Apparatus

An automated rodent fear conditioning system (TSE, Bad Hamburg, Germany) was used to study CFC of each rat as described in our recent study (Abrari et al., 2008). Training took place in a conditioning box (35 cm × 20 cm × 19.3 cm). The walls and the ceiling of the box were constructed of clear Plexiglass. The floor of the box was made of 25 stainless- steel rods (6 mm in diameter, 12 mm apart) through which foot shock could be delivered from a constant current source. The box was in an isolation cubicle (47 cm × 72 cm × 72 cm) containing a loud speaker, a ventilation fan providing fresh air and back ground noise (68 db) and a light bulb providing dim illumination the training box was cleaned with 5% ethanol before and after utilization. A software program was used to control the test in the box, and to collect, display and store all experimental data for "off-line" analysis.

2.5.2. Behavioral training and testing procedures

General procedures for CFC have previously been described (Abrari et al., 2008). Briefly, CFC took place in a conditioning box. Rats were habituated to the conditioning box for 10 min two days before training. For training, the rats were placed into the chamber and after 3 min received two footshocks at 120 s intervals. Each shock was 0.4 mA and 2 s duration. Rats were left in the conditioning box for 30 s after termination of the procedure and returned to their home cage. Two-day after contextual training, rats were placed for 5 min in the same context and the percentage of time animal spent freezing (characterized by the absences of all visible movement expect respiration) was measured using automated procedures. Time threshold for freezing behavior was set on 3 second and. Freezing was interpreted whenever the animal was not moving for more than this duration.