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Glucocorticoid-induced impairment of long-term memory retrieval in female rats: Influences of estrous cycle and estrogen

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ABSTRACT

Using an inhibitory avoidance (IA) task, the effects of glucocorticoids on memory retrieval in intact and ovariectomized (OVX) female rats were investigated. Young adult female rats were trained in a one trial IA task (1-mA, 3-s footshock). The latency to reenter the dark compartment of the apparatus was recorded in the retention test performed 48 h after training. Pre-retrieval injection of corticosterone (CORT, 1, 3, and 10 mg/kg) to OVX rats impaired memory retrieval at all doses tested. Similar administration of CORT (3 mg/kg) in intact female rats impaired memory retrieval in the estrus phase (when endogenous plasma levels of estrogen are low) but not in the proestrus phase (when endogenous levels of estrogen are high). Concurrent administration of CORT (3 mg/kg) and 17-β-estradiol (15 μg/kg) in both proestrus and estrous phases impaired memory retrieval. Our findings indicate that the effects of corticosterone on memory retrieval are modulated by the estrous cycle and 17-β-estradiol.

1. Introduction

Stress levels of glucocorticoids (cortisol in humans and corticosterone in rodents) are known to induce impairment in long-term memory retrieval both in humans and experimental animals (Roozendaal, 2002). Systemic or intra-cerebral administration of glucocorticoids impairs retrieval of previously acquired information, including contextual/spatial information, in animals (Dominique, Roozendaal, & McGaugh, 1998; Rashidy-Pour, Sadeghi, Taherain, Vafaei, & Fathollahi, 2004). In humans, acute administration of cortisol impairs memory retrieval in both sexes (Dominique, Roozendaal, Nitsch, McGaugh, & Hock, 2000; Het, Ramlow, & Wolf, 2005).

The actions of glucocorticoids on cognitive functions are mediated through two distinct receptor types: the mineralocorticoid receptors (MR) and the glucocorticoid receptors (GR). GRs have low affinity for corticosterone and are occupied only during stress and at the circadian peak, when circulating levels of glucocorticoids are high. In contrast, MRs show a 10-fold higher affinity for corticosterone and are nearly saturated under basal conditions (De Kloet, Ratka, Reul, Sutanto, & Van Eekelen, 1986). Although it was reported glucocorticoid-induced memory impairment is mediated by GRs, recent findings have revealed that MRs may have played a role in this effect (Dorey et al., 2011; Khaksari, Rashidy-Pour, & Vafaei, 2007).

Female gonadal hormones can influence cognitive functions depending on the phase of the estrous cycle. In rats and mice, a single estrous cycle lasts 4–5 days and consists of four phases, namely the proestrus, estrus, metestrus, and diestrus phase (Ter Horst, De Kloet, Schächinger, & Oitzl, 2012). During the proestrus phase, concentrations of estrogen and progesterone are high. In the ensuing estrus phase, circulating concentrations of both sex hormones decline. In the metestrus and diestrus phases, a slight increase in the progesterone level occurs, which returns to baseline at the end of the diestrus phase. Females in the proestrus phase are less emotional than when they are in other estrous phases and show improvement in learning simple cognitive tasks, such as object recognition. In contrast, they show difficulties in learning complex tasks, such as the Morris water maze and the radial arm maze (Shors, 2002; Ter Horst et al., 2012). Remarkably, the estrous cycle is associated with a rapid fluctuation of neural spinal density in the CA1 region of the hippocampus. The spine density and the number of synapses reaches a

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peak during the proestrus phase or when exposed to exogenous estrogen, then declines rapidly in the estrus phase, and rises again moderately in the diestrus phase (Shors, 2002; Shors, Chua, & Falduto, 2001). These findings suggest that the estrous cycle and, thus, the concentrations of sex hormones results in significant changes in the morphology and function of the hippocampus in female rats.

Stress response differs according to sex. Endogenous levels of glucocorticoids are tightly controlled by sex hormones. In many species, both under stress-free and stressed conditions, glucocorticoid levels are higher in females than males (Critchlow, Liebelt, Bar-Sela, Mountcastle, & Lipscomb, 1963; Viau & Meaney, 1991). Additionally, in rats, glucocorticoid levels are higher during the proestrus phase than in other phases of the estrous cycle (Atkinson & Waddell, 1997; Critchlow et al., 1963). Stressful experience can also elicit markedly different behavioral responses in males and females. For instance, it was found that in response to an acute stressful event, which depends on the presence of glucocorticoids, male rats acquired an associative learning skill faster than females, and produced more conditioned responses (Shors, 2001; Shors, Weiss, & Thompson, 1992). In contrast, female rats exposed to the same acute stressful event exhibited learning impairment and produced fewer conditioned responses (Wood, Beylin, and Shors, 2001; Wood & Shors, 1998). This difference may reflect a biological difference between the sexes. It has been reported that compared to males or females in other phases of the estrous, in females under proestrus phase, there is a greater density of apical dendritic spines on pyramidal neurons of the CA1 area of the hippocampus (Woolley et al., 1990). Stressful experience has opposite effects on this sex-difference in the spine density. In response to an acute stressful event of intermittent tail shocks, spine density increased in the male hippocampus, but declined in the female hippocampus (Shors et al., 2001). Although estradiol exerts a significant effect on the dendritic spine growth within the hippocampus, studies have shown that dendritic spine changes may not be correlated with learning and memory, and may not be the prime site for memory storage (Segal, 2005). Additionally, it has been reported that in adult rats, the increase in dendritic spine density on pyramidal neurons of the CA1 region of hippocampus following spatial learning is minimal (Moser, Trommald, & Andersen, 1994). A number of studies have shown that increases in dendritic spines following learning are transient, with no permanent increase in the synapse number observed following a learning event (Nimchinsky, Sabatini, & Svoboda, 2002). However, the possible role of dendritic spines in the hippocampus in learning and memory is suggested by others (Engert & Bonhoeffer, 1999; Sutton & Schuman, 2006).

Several studies on hippocampus-dependent tasks have shown that stress impairs memory performance in male rats (Conrad, Galea, Kuroda, & McEwen, 1996; Luine, Villegas, Martinez, & McEwen, 1994; Park, Campbell, & Diamond, 2001), but facilitates memory performance in female rats (Bowman, Ferguson, & Luine, 2002; Bowman, Zrull, & Luine, 2001; Conrad, Grote, Hobbs, & Ferayorni, 2003). Although it has been reported that acute stress and stress levels of glucocorticoids impair memory retrieval in male rats (Roozendaal, 2002), their effects on memory retrieval in female rats are not well documented. Glucocorticoids may interact with estrous cycle and estrogens to influence memory retrieval in female rats. Therefore, we sought to determine the effects of glucocorticoids on the retrieval of inhibitory-avoidance memory in ovariectomized (OVX) rats, in intact adult female rats in the estrus and proestrus phases of the estrous cycle, and in intact rats spending in the estrus and proestrus phases of their estrous cycle after receiving 17-β-estradiol.

2. Materials and methods

2.1. Animals

Adult female Wistar rats, weighting 200–250 g, obtained from the breeding colony of Semnan University of Medical Sciences (Semnan, Iran), were used in the study. Rats were initially housed in large cages (50 × 26 × 25 cm), 5 animals per cage, under 12-h light/dark cycles (6 am lights on–6 pm lights off). Food and water were provided *ad libitum*. The cages were maintained at 22 ± 2 °C under a relative humidity of 50–60%. All experiments were performed between 09:00 h and 12:00 h during the light cycle. The experimental protocol was approved by the Ethics Review Board of the Semnan University of Medical Sciences (Iran). All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

Corticosterone (CORT, 2-hydroxypropyl-β-cyclodextrin complex) (1, 3, or 10 mg/kg; 2 mL/kg) or vehicle (NaCl, 0.9%) was intraperitonealy (i.p.) administered 30 min before the behavioral test. These doses of CORT give rise to plasma levels of CORT that resemble its levels under moderate to severe stress (Abrari, Rashidy-Pour, Semnanian, & Fathollahi, 2008; Dominique et al., 1998; Pugh, Tremblay, Fleshner, & Rudy, 1997).The hormone 17-β-estradiol (15 μg/kg, Sigma) was dissolved in 4% ethanol/saline solution, and was administered (2 ml/kg, i.p.) 30 min before the CORT injection. This dose of 17-β-estradiol results in a circulating level of estradiol that is approximately fivefold higher than that observed in the morning of proestrus phase (Scharfman et al., 2007), and is close to the threshold for influencing the spine synapses in CA1 (MacLusky, Luine, Hajszan, & Leranth, 2005). This concentration of estradiol is sufficient to enhance some, but not all, components of estrogen-sensitive cognitive behavior in the female rats (Luine, Jacome, & MacLusky, 2003).

2.3. Ovariectomy

The rats were bilaterally ovariectomized by removing the ovaries after making a dorsal incision under general anesthesia induced using a combination of ketamine (Sigma, England; 75 mg/kg) and xylazine (Sigma, England; 10 mg/kg). Behavioral experiments started after a 20-day of post-operative recovery period.

2.4. Determination of normal estrous cycle

The phases of estrous cycle were determined by examining the vaginal smear as described elsewhere (Marcondes, Bianchi, & Tanno, 2002). Briefly, vaginal secretion was collected by inserting the tip of a plastic pipette, filled with 10 μL of normal physiological saline, into the rat vagina. One drop of the smear was collected with a clean tip from each rat. The vaginal fluid was then placed on glass slides, dyed using crystal violet and the stained samples were examined under a light microscope fitted with 10× and 40× objective lenses. The presence of large, round, nucleated cells indicated the proestrus phase. Estrus phase was characterized by the homogeneous presence of cornified cells in the smears. Vaginal smear was collected immediately after the retention test. To insure that all female rats were cycling regularly, in experiments 2 and 3, the phase of the estrous cycle was determined for a minimum of 10 days before treatment.