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Chronic treatment with estradiol restores depressive-like behaviors in female Wistar rats treated neonatally with clomipramine

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ABSTRACT

Neonatal administration of clomipramine (CMI) induces diverse behavioral and neurochemical alterations in adult male rats that resemble major depression disorder. However, the possible behavioral alterations in adult female rats subjected to neonatal treatment with clomipramine are unknown. Therefore, the aim of this study was to explore the effect of neonatal treatment with CMI on adult female rats in relation to locomotion and behavioral despair during the estrus cycle. Also evaluated was the effect of chronic treatment with E2 on these female CMI rats. We found no effects on spontaneous locomotor activity due to neonatal treatment with CMI, or after 21 days of E2 administration. In the FST, neonatal treatment with CMI increased immobility and decreased active swimming and climbing behaviors. Influence of the ovarian cycle was detected only in relation to climbing behavior, as the rats in the MD phase displayed less climbing activity. Chronic E2 administration decreased immobility but increased only swimming in CMI rats. These results suggest that neonatal treatment with CMI induces despair-like behavior in female rats, but that chronic E2 administration generates antidepressant-like behavior by decreasing immobility and increasing swimming, perhaps through interaction with the serotonergic system.

1. Introduction

Depression is a common mental illness worldwide that is not exclusive to any age group or social or income level (Bromet et al., 2011; Lépine and Briley, 2011). In 2015, 322 million of people in the world suffered depression. This disorder is ranked as the most disabling medical conditions in terms of years lost to disability and the major contributor to suicide deaths (WHO, 2017). Depression is characterized by the loss of interest and enjoyment (anhedonia), together with other symptoms that include variations in weight, loss of appetite, sleep disturbances, psychomotor symptoms such as restlessness or retardation, feelings of low self-esteem and hopelessness, lack of interest in sex, and suicidal thoughts, among others (American Psychiatric Association, 2013). Interestingly, women are twice as likely as men to suffer from depression (Bromet et al., 2011; Kessler et al., 2003). Among other factors, hormonal influences have been found to contribute to these sex differences (Desai and Jann, 2000). For instance, periods characterized by hormonal deficiency – *e.g.* postpartum, menopause, climacteric – have been shown to be closely-related to an increased risk of depression in women (see Halbreich and Kahn, 2001; Solomon and Herman, 2009; Sundström Poromaa et al., 2017).

For studies in this field, animal models constitute important tools for improving our understanding of the pathophysiological mechanisms of depression, and for predicting effective therapeutic targets for future treatments (Valvassori et al., 2013). Such models have employed different stressors to induce depressive-like behaviors, though some have involved long-term manipulations that promote a predisposition to developing depression, such as the neonatal administration of clomipramine (CMI) model (Willner and Mitchell, 2002). Neonatal exposure to tricyclic antidepressants is considered a neurodevelopmental model for studying depression (Willner and Mitchell, 2002) due to their ability to produce depressive-like pathophysiology during adulthood (Feng and Ma, 2003; Justel et al., 2011; Vijayakumar and Meti, 1999; Vogel et al., 1990a; Yannielli et al., 1998). This model induces the desired behavior by administering clomipramine, a tricyclic antidepressant that inhibits serotonin and noradrenaline reuptake in the neonatal stage (8–21 PN), a pharmacological manipulation that induces behavioral alterations, such as decreased sexual behavior (Bonilla-Jaime et al., 2003, 1998; Feng et al., 2001), greater aggressiveness (Vogel et al., 1990a), less pleasure-seeking behavior (*e.g.* intracranial self-stimulation), alterations in REM sleep parameters, and cognitive alterations that may

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affect learning and memory (Bhagya et al., 2008; Feng and Ma, 2002; Savelyev et al., 2012; Vogel et al., 1990b). There are reports that neonatal CMI treatment also produces exaggerated immobility in the FST, manifested in less swimming behavior compared to control animals given saline neonatal treatment. These results are interpreted as showing despair or low motivation (Bhagya et al., 2008; Bonilla-Jaime et al., 1998; Savelyev et al., 2012; Vázquez-Palacios et al., 2005; Velázquez-Moctezuma and Diaz Ruiz, 1992). In addition, CMI rats show neurochemical alterations in both the noradrenergic and serotonergic systems (Andersen et al., 2002; Feenstra et al., 1996; Hansen and Mikkelsen, 1998; Yannielli et al., 1999; Yavari et al., 1993), as well as hyperactivity in the HPA axis (Bonilla-Jaime et al., 2010; Prathiba et al., 1998). Some behavioral abnormalities in these rats (sexual, aggressive, motor and despair-related) begin to normalize with the onset of antidepressant treatments, such as the administration of antidepressants or hormone replacement therapy, or after REM sleep deprivation (Limón-Morales et al., 2014; Martínez-González et al., 2002; Vázquez-Palacios et al., 2005; Vogel et al., 1990a). However, it is important to mention that these alterations have been observed mainly in male rats, which is rather strange, since the incidence of depression-like disorders is significantly higher in females than males. In this context, it is widely-reported that sex differences in behavioral and neurochemical aspects exist in different animal models of depression (Dalla et al., 2011; Kokras and Dalla, 2014). Focusing research on males, therefore, may result in a failure to detect important findings related to mechanisms that could affect depression in women (Dalla et al., 2011, 2009).

Little is known about exposure to CMI in early-life in female rats, though Andersen et al. (2002) reported that CMI treatment has been seen to increase anxiety-like behaviors, but without affecting locomotor activity. Other studies have reported that early-life exposure to selective serotonin reuptake inhibitors (SSRI) increased depression-related behavior in both female rats and mice evaluated using the forced swimming test (FST) (Boulle et al., 2016; Glover et al., 2015; Lisboa et al., 2007; Sprowles et al., 2016). Therefore, it could be proposed that neonatal treatment with CMI will also induce depressive-like behavior in female rats, and that its effect may be influenced by the estrous cycle. This behavior could be reflected in the FST, which has been shown to be effective in detecting despair-like behavior in rodents (Borsini and Meli, 1988; Detke et al., 1995; Porsolt et al., 1978, 1977) and, moreover, is sensitive to a wide range of antidepressants (Borsini and Meli, 1988; Petit-Demouliere et al., 2005), including steroid hormones, such as estrogens, that produce antidepressant actions (Estrada-Camarena et al., 2003; Galea et al., 2001; Rachman et al., 1998). For instance, studies have shown that 17-β-estradiol (E2) decreases immobility behavior and increases swimming behavior in ovariectomized female rats; while in males, an E2 dose of 10 μg/rat reduced immobility and increased climbing behavior, and a dose of 20 μg/rat increased swimming (Martínez-Mota et al., 2008). Thus, our second objective was to analyze whether chronic treatment with estradiol can reverse manifestations of depressive-like behavior possibly observed in female rats treated neonatally with CMI.

2. Materials and methods

2.1. Animals and neonatal clomipramine treatment

All procedures involving animals were performed in strict accordance with the Official Mexican Standard NOM-062-ZOO-1999 (Norma Oficial Mexicana NOM-062-ZOO-1999, 2001). Pregnant Wistar rats were obtained from the vivarium at the Universidad Autónoma Metropolitana. Three days after delivery, female pups were separated from their biological mothers and lodged with a foster mother, maintaining the same litter size (n = 6) as described previously (Bonilla-Jaime et al., 1998, 2003; Neill et al., 1990; Vázquez-Palacios et al., 2005; Vogel et al., 1990a, 1990b). Male pups were set aside for a different experiment and cross-fostered with different mothers to those used for the females. From 8 to 21 days of age, all pups received treatment as follows: the females in the experimental group were treated with clomipramine (CMI, 15 mg/kg; 0.1 ml, twice-daily, 09:00 and 21:00 h, sc), a dose that has been used previously and generated behavioral abnormalities in rats (Andersen et al., 2002; Bonilla-Jaime et al., 1998; Hartley et al., 1990; Vázquez-Palacios et al., 2005; Velázquez-Moctezuma et al., 1993; Vogel et al., 1990a, 1990b); while those in the control group received twice-daily subcutaneous injections of saline in the same volume (09:00 and 21:00 h). On postnatal day 25, the pups were separated from their foster mothers and housed in groups (6 rats from the same treatment regimen per cage) in Plexiglas boxes (45 × 30 × 30 cm). All animals were maintained under a reversed 12/12 h light/dark cycle (lights on at 19:00 h) with *ad libitum* access to food and water. At the age of three months, both groups of female rats (250–300 g) were subjected to the following experimental conditions.

2.2. Experimental design

Experiment 1 was designed to analyze the effect of neonatal clomipramine administration on spontaneous locomotor and despair-like behaviors and the influence of the estrous cycle. Female CTRL (n = 15) and CMI (n = 15) rats performed the open-field (OFT) and forced swimming tests (FST) (see Fig. 1a). First, they were subjected to a 5-min session to permit familiarization with the OFT arena. Immediately after this pre-test, they were subjected to a second pre-test session that consisted of 15 min of the FST, designed to induce despair (Porsolt et al., 1977). These sessions were not included in the statistical analysis. Twenty-four hours later, the same female rats were evaluated again on the same behavioral tests, but 5 min before beginning, vaginal smears were taken to determine the phase of the estrous cycle and define two sub-groups for statistical analysis: proestrus-estrus (PE) and metestrus-diestrus (ME). After this procedure, the rats were evaluated in the OFT and then the FST, but considering the estrus phase. We continued this procedure until completing 15 animals per sub-group, since the despair effect lasts for at least 16 days (Alcaro et al., 2002; Mezadri et al., 2011). Both behavioral tests had a duration of 5 min.

A second experiment was then conducted to analyze the effect of chronic administration of pharmacological doses of 17-β-estradiol (E2) on despair behavior in the CMI rats (Fig. 1b). Another group of animals was used in this experiment. At the age of 3 months, these CTRL (n = 12) and CMI (n = 12) rats were ovariectomized, a common method employed to decrease circulating gonadal hormones, including estrogens (Zhao et al., 2005). Hormone treatment began 3 weeks after surgery. Regardless of group (n = 6 per treatment), all rats were treated with E2 (10 μg per rat) or a vehicle (corn oil, 0.1 ml per rat, sc). Administration was performed once per day at 10:00 a.m. for 21 days. On day 20 of treatment, the rats were subjected to a 5-min pre-test in the OFT arena. At the end of that pre-test, they were subjected to a pre-test (15 min) of the FST. Next, 1 h after the final injection on day 21, the rats were evaluated in the OFT followed by the FST (5 min each). The dose of E2 and chronic administration were chosen in light of their previously demonstrated effectiveness in producing antidepressant-like action in female rats (Bekku and Yoshimura, 2005; Estrada-Camarena et al., 2003; Okada et al., 1997).

2.3. Vaginal cytology

Before initiating the aforementioned behavioral tests, vaginal smears were obtained daily. Only females with two continuous regular cycles (4–5 days) were included in this phase of the study. Smears were obtained gently 5 min before behavioral testing. Vaginal secretions were collected with a glass medicine dropper filled with saline solution (NaCl 0.9%) by gently – not deeply – inserting the tip into the rat's vagina. Smears were placed on microscope slides and observed under light microscopy using 10× and 40× objective lenses (Marcondes et al.,