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Estrogen levels modify scopolamine-induced amnesia in gonadally intact rats

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ABSTRACT

Previous studies suggested that estrogen plays a role in cognitive function by modulating the cholinergic transmission. However, most of the studies dealing with this subject have been conducted using ovariectomized rats. In the present study we evaluated the effects of physiological and supra-physiological variation of estrogen levels on scopolamine-induced amnesia in gonadally intact female rats. We used the plus-maze discriminative avoidance task (PMDAT) in order to evaluate anxiety levels and motor activity concomitantly to the memory performance. In experiment 1, female Wistar rats in each estrous cycle phase received scopolamine (1 mg/kg) or saline i.p. 20 min before the training session in the PMDAT. In experiment 2, rats in diestrus received estradiol valerate (1 mg/kg) or sesame oil i.m., and scopolamine (1 mg/kg) or saline i.p., 45 min and 20 min before the training, respectively. In experiment 3, rats in diestrus received scopolamine (1 mg/kg) or saline i.p. 20 min before the training, and estradiol valerate (1 mg/kg) or sesame oil i.m. immediately after the training session. In all experiments, a test session was performed 24 h later. The main results showed that: (1) scopolamine impaired retrieval and induced anxiolytic and hyperlocomotor effects in all experiments; (2) this cholinergic antagonist impaired acquisition only in animals in diestrus; (3) acute administration of estradiol valerate prevented the learning impairment induced by scopolamine and (4) interfered with memory consolidation process. The results suggest that endogenous variations in estrogen levels across the estrous cycle modulate some aspects of memory mediated by the cholinergic system. Indeed, specifically in diestrus, a stage with low estrogen levels, the impairment produced by scopolamine on the acquisition was counteracted by exogenous administration of the hormone, whereas the posttraining treatment potentiated the negative effects of scopolamine during the consolidation phase of memory.

1. Introduction

In the last decades, numerous studies have addressed the role of estrogen and its replacement in some neurodegenerative disorders, mainly Alzheimer's disease (Panidis et al., 2001; Zec and Trivedi, 2002). Estrogen has been shown to reduce cognitive impairments through different mechanisms, such as modulation of neurotransmitters, synaptic plasticity, increase of cerebral blood flow, protection against apoptosis, anti-inflammatory actions and antioxidant properties (for review, see Cholerton et al., 2002). In the brain, the estrogen receptors are located in several structures, including the hippocampus and the amygdala of both humans (Osterlund et al., 2000) and rodents (McEwen and Alves, 1999).

Estrogens are a group of steroid hormones that includes three biologically significant compounds: estrone, estradiol and estriol. Estradiol valerate (EV), an analog of estradiol, was shown to improve some types of memory (Hosseini et al., 2010; Vázquez-Pereyra et al., 1995). Besides their classical genomic actions, estrogens also activate non-genomic mechanisms through intracellular signaling pathways that are critical

Abbreviations: PMDAT, plus-maze discriminative avoidance task; SCO, scopolamine; EV, estradiol valerate; SAL, saline; VEH, vehicle sesame oil; MET, metestrus; DIE, diestrus; PRO, proestrus; EST, estrus; %TAV, percentage of time in aversive arm; %TOA, percentage of time in open arm; NO, nitric oxide; ER, estrogen receptor; WHI, Women's Health Initiative; NAV, non-aversive arm; AV, aversive arm; OA, open arm; RIA, radioimmunoassay; MW, molecular weight.

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for memory processes (Fitzpatrick et al., 2002; Kuroki et al., 2000; Mannella and Brinton, 2006; Sawai et al., 2002). These mechanisms are probably involved with the short-term beneficial effects of estradiol on memory acquisition (Levin, 2005; Walf and Frye, 2008). This rapid effect of the hormone on memory can be achieved during a two-hour time window (Luine et al., 2003; Packard, 1998).

Many cellular and behavioral acute effects appear to require physiological concentrations of estrogen higher than normal (Cornil, 2009). Behavioral studies have also revealed that rapid changes in estrogen bioavailability, resulting from a single injection of a high dose of estradiol, modify the expression of some behaviors (sexual behavior and pain threshold) within less than 1 h. These data indicate that the action of estrogen in the brain can occur in different time ranges (short to long-term), possibly combining non-genomic and genomic actions (Balthazart et al., 2006).

In animal models, sexual hormones have shown to influence memory mainly through neuronal pathways related to the hippocampus (McEwen, 2002). Particularly, estrogen influences learning and memory evaluated in hippocampus-dependent tasks (McEwen, 2002; Zec and Trivedi, 2002). In addition, this effect is possibly associated with increased acetylcholine release in the hippocampus (Dumas et al., 2006, 2008). Other investigations have suggested that a long-term deprivation of estrogen decreases the functional status of the basal forebrain cholinergic projections to the cortex and hippocampus (Gibbs, 1998). Further, both the activity of choline acetyltransferase and the release of acetylcholine in the neocortex, hippocampus and basal forebrain are enhanced by estrogen (Gibbs, 2000; Marriott and Korol, 2003). Lastly, studies showed that estrogen increases the acetylcholine release stimulated by potassium in the hippocampus (Gibbs et al., 1997) and potentiates the effects of cholinergic agonists in avoidance tasks when administered directly into the hippocampus (Farr et al., 2000). All these findings indicate that estrogen may mediate memory processes through modulation of the cholinergic system.

It has been suggested that a previous impairment has to be present for the estrogen's beneficial effects to occur (Gibbs and Aggarwal, 1998; Tinkler and Voytko, 2005). In this respect, scopolamine (SCO) is a muscarinic receptor antagonist that classically induces cognitive impairments in behavioral tests (Silva et al., 1999; Wallenstein and Vago, 2001). Wink and coworkers described that the loss of cholinergic stimuli produced by the administration of SCO is comparable to the one underlying the aging processes (Wink et al., 2006). Furthermore, the replacement with estrogen in ovariectomized rats enhances memory acquisition and counteracts the negative effects of SCO on working and spatial memory tasks (Fader et al., 1999; Gibbs, 1999). In this respect, studies in animal models are usually conducted with ovariectomized animals, and do not take into account the natural hormonal changes in gonadally intact females (Frick, 2009).

Estrogen has also been shown to influence fear and anxiety-related behaviors. For example, endogenous changes in estrogen levels may increase women's susceptibility to anxiety disorders (Walf and Frye, 2006). In this respect, the literature shows that memory can be dependent on affective contents, such as anxiety level (Silva and Frussa-Filho, 2000) and the emotional valence (negative or positive) of the events (Labar and Cabeza, 2006). Further, the neural circuit for emotional reinforcement includes brain regions that are also related to memory formation, i.e. amygdala, hippocampus and prefrontal cortex (Labar and Cabeza, 2006; Mathews, 1990). Thus, the hypothesis that estrogen can influence female rats' performance in cognitive tasks with emotional contexts by modulating anxiety-like behavior should be considered (Frye and Walf, 2004).

The aim of the present study was to investigate the effects of physiological and supra-physiological variations of estrogen levels on SCO-induced amnesia in gonadally intact female rats. We used the plus-maze discriminative avoidance task (PMDAT; Silva et al., 1999; Silva and Frussa-Filho, 2000) in order to evaluate anxiety levels and motor activity concomitantly to the learning and memory performances.

2. Methods

2.1. Animals

Three-month-old female Wistar rats (250–300 g) were housed with free access to food and water, in a number of 4 or 5, under controlled conditions of temperature (23–24 °C) and a 12 h light/12 h dark cycle (lights on 06:30 am). The rats were handled according to the Brazilian law for the use of animals in scientific research (Law Number 11.794) and all procedures described were approved by the local ethical committee (CEUA/UFRN no. 036/2010). Prior to the procedures all the animals were gently handled for 10 min/day for 3 days.

2.2. Drugs

Scopolamine hydrobromide (SCO, Sigma, USA) was diluted in saline solution and given i.p. at 1 mg/kg. This dose choice was based on previous studies showing scopolamine-induced impairment in rodent memory models (Barbosa et al., 2010; Claro et al., 2006), including the task used here (Claro et al., 1999; Silva et al., 1999). Estradiol valerate (EV, Sigma, USA) was dissolved with absolute ethanol and sesame oil and left overnight for evaporation. Afterward, the compound was diluted to the correct concentration with sesame oil and given i.m. at 1 mg/kg. Estradiol levels are considered supra-physiological at doses above 0.5 mg/ml in rodents (Cornil et al., 2006). Saline solution (SAL) (0.9% NaCl) or vehicle sesame oil (VEH) in a volume of 1 ml/kg were administered to controls for SCO or EV treatments, respectively.

2.3. Estrous cycle

Before the beginning of the experiments, the estrous cycle was monitored by the analysis of vaginal smears under a light microscopy for three complete cycles of 4–5 days. Vaginal secretions were collected by the gentle introduction of plastic pipettes with distillated water in a volume of 0.1 ml. Only animals with regular cycling were included in the experiments (approximately 95%). The four stages of estrous cycle were determined according to the following characteristics: estrus (EST) — predominance of cornified cells; proestrus (PRO) — predominance of epithelial nucleated cells; diestrus (DIE) — predominance of leucocytes and metestrus (MET) — similar proportion of nucleated cells, leucocytes and cornified cells (Marcondes et al., 2001; Pompili et al., 2010).

2.4. Plus-maze discriminative avoidance task (PMDAT)

The apparatus employed was a modified elevated plus-maze, made of wood, containing two enclosed arms (50 × 15 × 40 cm), an aversive (AV) and a non-aversive (NAV), opposite to two open arms (OA; 50 × 15 cm). The task consisted of two sessions: training and testing, each lasting 10 min. In the training session, each rat was placed in the center of the apparatus with body orientation toward the intersection between the open arms. Every time the animal entered with the four paws into the aversive enclosed arm, aversive stimuli were turned on until the animal left the arm. The aversive stimuli were a 100-W light and an 80 dB noise produced by a lamp and speakers placed over the aversive enclosed arm. In the test session, the animals were again placed in the apparatus, but without presentation of the aversive stimulation (Fig. 1). All behavior experiments were performed between 1:00 and 5:00 p.m. The sessions were recorded with a digital camera placed over the apparatus and the behavioral quantification was performed by a video-tracking software (Anymaze®, Stoelting, USA).

The percent time spent in aversive arm [%TAV = time in NAV / (time in NAV + AV) × 100] was used to assess aversive memory (%TAV overall, when the entire session was considered, and %TAV in time blocks, when the variable was analyzed throughout the sessions). The whole sessions durations were divided into three blocks: initial (from start to third minute), middle (fourth to seventh minute) and final (eighth to