Hormones and Behavior 61 (2012) 76–83

Contents lists available at SciVerse ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Chronic administration of tibolone modulates anxiety-like behavior and enhances cognitive performance in ovariectomized rats

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ARTICLE INFO

Article history:
Received 29 June 2011
Revised 17 October 2011
Accepted 25 October 2011
Available online 29 October 2011

Keywords:
Tibolone
Learning
Memory
Anxiety
Choline acetyl transferase
Tryptophan hydroxylase
Hippocampus

ABSTRACT

Hormone replacement therapy (HRT) may be prescribed to prevent the symptoms of menopause. This therapy may include estrogenic and/or progestin components and may increase the incidence of endometrial and breast cancers. Tibolone (TIB), which is also made up of estrogen and progestin components, is often used to reduce the impact of HRT. However, the effect of TIB on the processes of learning, memory and anxiety has yet to be fully elucidated. The aim of this study was to evaluate the long-term effect on learning, memory processes and anxiety in ovariectomized rats caused by different doses of TIB (0 mg/kg, 0.01 mg/kg, 0.1 mg/kg 1.0 mg/kg and 10 mg/kg, administered daily via the oral route for 18 weeks). Two behavioral animal models, the autoshaping and T maze models were employed. The concentrations of acetyl choline transferase (ChAT) and tryptophan hydroxylase (TPH) in the hippocampus were directly measured by Western blot. No significant changes were observed in the autoshaping model and spontaneous activity test. In the T maze, increased latency was observed with TIB doses of 1 and 10 mg/kg compared to the vehicle. We observed that the ChAT content decreased with increasing doses of TIB, whereas TPH content increased with doses of 1 and 10 mg/kg of TIB. These data indicate that high doses of TIB improved emotional learning, which may be related to the modulation of the cholinergic and serotonergic systems by TIB.

Introduction

Recent studies have suggested that HRT enhances specific cognitive processes (Haskell et al., 1997; Keenan et al., 2001; Sherwin, 1998) and reduces the risk of Alzheimer's-related dementia (Lerner, 1999; Skoog and Gustafson, 1999) in postmenopausal women. However, the effects of combined estrogen/progesterone (P4) therapy are less well-known. The mechanisms responsible for the effects of estrogen on cognitive processes and the risk for Alzheimer's disease (AD) are also unknown, although potential mechanisms have been proposed (Brinton, 2001; McEwen, 1999).

Cholinergic neurons from the basal forebrain, including those in the nucleus basalis magnocellularis (NBM), the septum medial (MS) and the vertical and horizontal limbs of the diagonal band of Broca (DB), project to the cortex and hippocampus and have been implicated in learning, memory, arousal and attention processes (Gibbs, 2000; Wenk, 1997). The loss of cholinergic neurons may be partially responsible for the cognitive decline associated with aging and neurodegenerative disorders such as AD (Luine, 1985; Decker and McGaugh, 1991).

Some studies have suggested that the loss of gonadal function in women contributes to a decrease in cholinergic and serotonergic function and that this effect is reversible with appropriate HRT (Gibbs, 2000; Genazzani et al. 2007; Lasiuk and Hegadoren, 2007).

Ovariectomy (Ovx) and menopause result in behavioral, neurochemical, and molecular deficits, which are partially normalized by estrogen treatment (Sherwin, 2003; Chakraborty and Gore, 2004). In addition, menopausal women have shown signs of anxiety, depression and impaired cognitive function (Halbreich, 1997; Sherwin, 1998), which improved with estrogen replacement therapy (ERT). Similarly, in experimental animals, proestrous rats that were influenced by the highest level of endogenous estrogen (Sherwin, 1998; Diaz-Veliz et al., 1997) and the Ovx rats that were supplemented with chronic estrogen (Frye et al., 2000; Koss et al., 2004; Pandaranandaka et al., 2006) showed anxiolytic-like behaviors when tested using an elevated plus-maze (EPM).

TIB (7-α, 17- α-17-hydroxy-7-methyl-19-norpregn-5[10]-en-20-ψn-3-one), a synthetic steroid, is widely prescribed to treat menopausal

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doi:10.1016/j.yhbeh.2011.10.005

symptoms (such as hot flushes, vaginal dryness and sexual dysfunction) and to prevent bone loss. TIB is metabolized by humans and non-human primates into three biologically active metabolites: 3-α-hydroxyestrogenic metabolite, 3-β-hydroxyestrogenic metabolites, and Δ-4 ketoisomer, which display progestogenic and androgenic effects (Campisi and Marengo, 2007; Kloosterboer, 2001). TIB metabolites circulate in inactive forms, and their conversion to bioactive forms depends on tissue-specific desulfation (Verheul et al., 2007).

Previous reports have indicated that the 3-hydroxy TIB is the predominant free metabolite in brain tissues of Ovx cynomolgus monkeys (Verheul and Kloosterboer, 2006). In hypothalamic neurons of guinea pigs, TIB administration rapidly attenuates the $GABA_B$ response (Qiu et al., 2008). In the cortex and hippocampus of young, adult and old Ovx rats, TIB has decreased lipid hydroperoxide levels, produced higher total antioxidant capacity (Aguiar et al., 2008) and improved memory in a step-down inhibitory avoidance task (Aguiar et al., 2006).

In premenopausal women, TIB administration has reversed the cognitive damage caused by leuprolide acetate and improves mood and quality of life in patients receiving this gonadotropin-releasing hormone agonist for uterine leiomyomas treatment (Palomba et al., 2008). However, the effect of chronic administration of TIB on the regulation of key enzymes in the synthesis of serotonin (5-HT) and acetylcholine (ACh), and its correlation with the behavior of memory, learning processes and anxiety are unknown. Therefore, in this work we analyzed the effect of long-term administration of different doses of TIB in learning and anxiety using two behavioral animal models, the autoshaping and T maze models, and its correlation with the expression of key enzymes in the synthesis of neurotransmitters involved in modulating these behaviors.

Methods

Animals

Ovx female Sprague–Dawley rats (200–250 g, 2 months old) were group housed, under a 12 h light–dark cycle (lights on at 9:00 p.m.) and provided water and food ad libitum. All procedures were conducted following the Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999). All efforts were made to reduce both animal suffering and the number of animals in each experiment.

Treatments

All animals included in this study were Ovx through a dorsal incision while under anesthesia (80 mg/kg ketamine and 5 mg/kg xylazine, administered intraperitoneally). The complete extraction of the ovaries was corroborated by visual inspection. At the time of surgery, rats were randomly divided into five subgroups (n = 10 per group) and daily, for the subsequent 18 weeks, received 1) vehicle (Veh) (water), 2) 0.01 mg/kg, 3) 0.1 mg/kg, 4) 1.0 mg/kg or 5) 10 mg/kg of TIB treatment by oral gavage. We decided to administrate these doses during this period of time because, in previous studies, it has been reported that TIB exerts effects on cognitive processes from the first two months of treatment with doses of 0.5 and 1 mg/kg (De Aguiar et al., 2006; Wu et al., 2008). On the other hand, it has been reported that estrogens have a different effect at low doses compared to higher doses (Espinosa-Raya et al., 2011); therefore, we wanted to evaluate the effect of TIB on memory, learning and anxiety at different doses, so we performed a logarithmic curve to analyze this effect at low and high doses.

Autoshaping learning task (ALT)

ALT has been validated as a useful tool to explore associative learning and some aspects of instrumental memory. As occurs in Pavlovian autoshaping, a conditioned stimulus (CS) and an unconditioned stimulus (US) are independent of the behavior of the animal because autoshaped behavior is minimally affected by instrumental responses. Furthermore, this task is almost completely automated, thus reducing human intervention, and is sensitive to small increases or decreases in various behavioral parameters, including sign tracking and goal tracking. The latter parameter is quite important, as it allows for the study of bidirectional expression of an improved or impaired memory formation (Meneses, 2003).

The protocol used was a modification of that previously described (Meneses, 2003). Standard Skinner boxes (WMPC 1999; Med Associates Inc. USA), which consisted of a lighted lever in the middle of one wall, 4 cm above the floor, were used. A food tray was located 5 cm to the right of the lever. Twelve hours before the test, food was withdrawn from the cage. On the training day, each rat was placed into an experimental chamber and given time to acclimate to the experimental environment (~20 min). During this time, animals were left in the chambers, where they found and ate 45 food pellets (45 mg each) placed on the tray. The trial immediately began and consisted of the presentation of an illuminated lever (CS) for 60 s during which time the rat could obtain a pellet each time it pressed, which is known as conditioned response (CR). When the light turned off, one pellet (US) was immediately delivered. There was an inter-trial interval of 5 s. An increase or decrease in the number of CRs was considered an enhancement or impairment in the consolidation of learning, respectively. The protocol consisted of three sessions, one every 24 h, with the first and second sessions comprising 20 trials, and the third session comprising 10 trials. Data are expressed as the mean number of CRs during the last session (lever press responses during CS/10 trials).

Elevated T-maze (ETM)

The ETM, an ethologically based test, has been used to investigate the effects of anxiolytic drugs on memory and the relationships between neural systems involved in such modulation. This test allows the measurement in the same rat of two kinds of aversively motivated behaviors, i.e. learned (or conditioned) anxiety, represented by inhibitory avoidance behavior, and innate (or unconditioned) fear, represented by one-way escape (Graeff et al., 1993; Viana et al., 1994; Zangrossi and Graeff, 1997).

We used the method previously described by De-Mello and Carobrez (2002). The ETM consisted of three arms of equal dimensions (30 cm × 5 cm) elevated 45 cm above the floor. One of these was enclosed by lateral walls (15 cm high) and stood perpendicular to the opposite open arms. The rat's behavior was video recorded. After each trial, the maze was cleaned with ethanol solution (10% v/v).

On the training day, rats were placed at the distal end of the enclosed arm, facing the intersection of the arms, and they were allowed to explore the enclosed area. The trial ended when the rat, leaving the enclosed arm, placed four paws onto one of the open arms or remained in the enclosed arm for a maximum of 300 s (avoidance criterion). During the 30 s intertrial interval, the animals were returned to their cage.

In addition, when the rat stretched out from the enclosed arm, placing one, two or three paws on to one of the open arms and then returning to its original position, this was recorded as a try. A risk assessment index was determined according to the following formula:

$$\text{Risk index} = \text{frequencies of tries/open arm entry} + \text{frequency of tries}$$

where a risk index (RI) that equals to 1 represents no successful open-arm entry and where an RI that equals 0 indicates that every movement towards the open arm was successful.