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Estradiol valerate and tibolone: Effects on memory

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Abstract

This study investigated the effects of estradiol valerate (EV) and tibolone (TB) treatments on some memory parameters of ovariectomized young (2 months), adult (8 months) and old (20 months) female rats. A Sham-operated group was used as control and the animals were treated daily, by oral gavage, with saline (Sham and placebo NR group), EV (0.3 mg/kg) or TB (0.5 or 1 mg/kg, TB1 and TB2, respectively). In step-down inhibitory avoidance task, the latency of old TB2-treated females in the short-term test was significantly inferior ($p<0.05$), compared to TB2 adults. In the elevated plus maze, adult NR females spent significantly less time ($p<0.05$) in the open arms as compared with EV and TB2-treated animals. Additionally, adult TB2-treated females spent significantly less time in the closed arms compared to Sham, NR and TB1 groups. Finally, in the water maze retention test, young TB1-treated animals performed worse when compared to Sham, EV and TB2 females. In the old animals, EV treatment hampered subject performance as compared to all other treatments. Taken together, these results indicate that ovarian hormones differently affect female memory in an age-dependent manner.

Keywords: Ovarian steroids; Memory; Tibolone; Estradiol valerate; Ovariectomy; Behavior

1. Introduction

Mammals exhibit various types and rates of changes throughout the aging process. In most cases, females show a significant diminution in reproductive capacity with a consequent reduction of gonad hormone production. This pattern is largely distributed in the mammalian group, with rare exceptions (Finch, 1990).

The loss of synaptic connections in the hippocampus is one of the principal modifications of the aging brain (Squire and Kandel, 1999). Also, a physiological decline in gonadal steroids, which occurs during middle-age, greatly affects brain functions. Indeed, age-related deficits in memory function have already been associated with a significant reduction in estrogen levels, which occurs during the aging process. These deficits may be associated with the estradiol modulatory role on cholinergic innervations in the hippocampus, an important brain structure for several memory processes (McEwen, 1999).

Memory is usually divided into two types: short-term memory (STM) and long-term memory (LTM) and estrogens may influence these types of memories through two main pathways: non-genomic rapid and genomic slow paths. The STM is a protein and RNA synthesis-independent type of memory, formed within 1–3 h after training and usually lasting for hours. It may be affected by non-genomic steroidal effects. The LTM is a protein and RNA synthesis-dependent type of memory, formed over several hours to days and lasting weeks, or even longer. In this type of memory steroids may act through slow genomic pathways (Izquierdo et al., 1998; Squire and Kandel, 1999; Izquierdo and McGaugh, 2000; Chakraborty and Gore, 2004).

The effect of estrogens on the performance of laboratory animals during learning and memory tasks has been widely studied, but many doubts still remain. Some authors attribute beneficial effects to estradiol in the performance of female rats in spatial and reference memory tasks, as well as in working

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memory tests (Bowman et al., 2002; Frick et al., 2002; Daniel et al., 2006), while other researchers reported damaging, or no effect at all, of estrogens in the same types of memory tasks (Fader et al., 1999; Chesler and Juraska, 2000; Fernandez and Frick, 2004).

Also, there are several articles suggesting a possible connection between estrogen and emotional disturbances in humans. Significant levels of anxiety have been associated with low levels of estrogen in postmenopausal women (Thomson and Oswald, 1977; Soares et al., 2001). But the results obtained from experiments with animals are controversial, since anxiogenic and anxiolytic effects of estradiol have been registered depending upon subject age and stage of their reproductive cycle, amongst other factors (Lund et al., 2005).

In the investigation of estrogen effects, oral administration is a practical and widely used method and estradiol valerate (EV) is a commonly used form of the hormone. When administered orally, EV is rapidly split into valerate and estradiol, increasing the serum concentration of the latter. Part of this estrogen is metabolized into estrone and estrone sulfate. Estrone serum levels reach high concentrations, but it is a weak estrogen acting more as an inactive hormonal reservoir (Kuhl, 2001). Estradiol bioavailability after oral administration of EV is very low in humans (about 3%) and even lower in rats (0.5%) (Kuhnz and Putz, 1989). A 2 mg treatment with this steroid generates, in women, a serum concentration below 0.4 nmol/l after both 1 and 21 days of oral treatment (Kuhl, 2001). It is thus logical to conclude that oral administration of EV generates a dose-dependent (but time-independent) supraphysiological blood concentration.

Meanwhile, tibolone (TB) (7α, 17α)-17-hydroxy-7-methyl-19-norpregn-5(10)-en-20-yn-3-one; LIVIAL®, N. V. ORGANON) is a 7α-methyl derivative of norethynodrel and, like this substance, has mild androgenic/progestogenic and marked estrogenic effects (Kuhl, 2001). These metabolites have different affinities for the steroid receptors (Gooyer et al., 2003): 3α- and 3β-hydroxytibolone bind to estrogen receptors, triggering estrogen-like responses, while the Δ^4-isomer has a high affinity for the progesterone and androgen receptors. The blood levels resulting from multiple dosages of tibolone do not significantly differ from those generated by a single dose (Timmer and Houwing, 2002). Due to its characteristic of producing estrogen-, progesterone- and androgen-like responses, TB could be a practical hormone for use in the investigation of combined gonadal steroid actions.

There are many studies regarding the effects of tibolone treatment on bone mass and blood parameters and several studies show the action of tibolone metabolites in different tissues (Cagnacci et al., 1997; Yoshitake et al., 1999; Cetinkaya et al., 2002; Gooyer et al., 2003) but reports of the specific effects of this steroid hormone on cognition are lacking (Fluck et al., 2002; Davis, 2002), and only in the last few years has there been an increase in the number of investigations referent to the effects of tibolone on brain biochemistry (Gibbs et al., 2002; Celik et al., 2005; Genazzani et al., 2006).

Considering the still incompletely defined role of estrogens and the paucity of information on the action of tibolone on memory and anxiogenesis processes, we have investigated the effects of long-term chronic treatment, using estradiol valerate (EV; Primogyna®, SCHERING BRAZIL) and tibolone, in the performance of ovariectomized young (2 months), adult (8 months) and old (20 months) female rats during different memory tasks.

2. Materials and methods

2.1. Animals

All the procedures involving animal subjects were reviewed and approved by the Institutional Research Ethics Committee from the Fundação Universidade Federal do Rio Grande (Proc. 23116.006192/2005–89).

Female Wistar rats (*Rattus norvegicus*, $n=105$) were obtained from the Fundação Universidade Federal do Rio Grande (Rio Grande, RS, Brazil). They were housed five per cage, under a 12 h light/dark cycle, temperature of 21±2 °C with water and food *ad libitum*. A soy-free rat chow (Supra®, ALISUL, São Leopoldo, RS, Brazil) was used to avoid phytoestrogen interference. After a week of habituation, the animals were submitted to surgical procedures.

2.2. Surgical procedure

Female rats were bilaterally ovariectomized (OVX) under deep Nembutal (Abbot Labs, Illinois, USA) anesthesia (40 mg/kg i.p.). Control groups underwent a Sham surgery, including bilateral incisions, but without gonad removal. All animals were allowed to recover for 7 days before treatment started.

2.3. Experimental groups and treatments

Females were divided into three age groups ($n=35$ each): Young (2 months, 210±5 g), adult (8 months; 273±13 g) and old (20 months, 300±10 g). Within the ages there were 5 treatment groups ($n=7$ each) identified as the following: 1) Sham, intact control submitted to a surgical process without gonads removal; 2) no replacement (NR), ovariectomized group; 3) EV, ovariectomized group treated with estradiol valerate; 4) and 5) TB1 and TB2 ovariectomized groups treated with tibolone.

Each day, in the morning, the animals received their treatment by oral gavage. Sham and NR female rats received saline (0.9% NaCl). The EV group received 0.3 mg/kg/day (according to Stomati et al., 2002) of estradiol valerate; TB1 and TB2 received, respectively, 0.5 and 1 mg/kg/day of tibolone (according to Yoshitake et al., 1999).

The animals received a minimum of 7 days treatment before the beginning of the memory tests. Treatment was continuous during the entire process.

2.4. Behavior and memory tasks

2.4.1. Step-down inhibitory avoidance task

The task is described elsewhere (Izquierdo et al., 1997) and was performed after a week of continuous treatment. Briefly, in

training session, the animals were placed on the apparatus platform and upon stepping down, received a 0.4 mA shock for 2 s. After 90 min and 24 h from the training session, the female rats were placed in the apparatus and the latency for stepping down with all 4 legs was registered (to a maximum time of 180 s) to evaluate STM and LTM, respectively.

2.4.2. Open field

After the second week of continuous treatment, animals were placed in a rectangular apparatus with a divided floor (12 quadrants) and observed for 5 min. The number of quadrant crossings was registered to evaluate locomotor activity.

2.4.3. Elevated plus maze (EPM)

This task is described elsewhere (Pellow et al., 1985) and was performed in the beginning of the third week of continuous treatment. Briefly, each rat was placed in the central square of the EPM facing a closed arm, and its behavior was observed for 5 min. The times spent in open and closed arms were scored as a measure of anxiety. For timing counts, the animal had to enter with all 4 paws inside the arm. The time the females spent with only half of their body inside the arm was not considered. Testing took place in a room lit by dim light and the maze was cleaned after each trial.

2.4.4. Object recognition test

This task was performed as described elsewhere (Lima et al., 2005), after 3 weeks of continuous hormone replacement therapy. In the training session, the animal is presented to two identical objects and allowed to explore them for 5 min. After 90 min, one object is replace by a new object and the animal is again allowed to explore both, the old and the new, objects in order to evaluate STM. Twenty-four hours later, the different object from the STM test is replaced by a third object to evaluate LTM. The results are expressed as recognition indexes (RI), calculated using the exploration time on each objects:

$$\mathrm{RI} = \frac{\text{time in the novel object}}{(\text{time in the novel} + \text{time in the known})}$$

2.4.5. Morris water maze (MWM)

A modification of the spatial version described by Morris (1984) was used. Data was obtained through a tracking video system (EthoVision®, NOLDUS). The water maze consisted of a circular dark tank (168 cm diameter and 70 cm depth) filled with water. A hidden platform was fixed in one of the four virtual quadrants for all of the training sessions. Visual cues were placed in the water maze room. The water was kept at 24±1 °C. The retention test was performed after the females had completed 12 weeks of continuous treatment. On the first day (11 weeks of continuous treatment), the female rats faced a 4 trial training session of 120 s with a 70 s interval. For each trial, the animals started from a different position in the water maze. From the second day until the fourth day, the number of trials was raised to 5 per day. In the training sessions, learning progress was evaluated as the latency to reach the hidden platform (escape). On the retention test day, the platform was removed and the animals swam freely for 90 s. Time spent in the platform quadrant was registered as an indication of memory retention.

2.5. Statistical analyses

Treatment effects were compared for the same age group, while ages were compared within the same treatment group. When the assumption of variance homogeneity was verified, a one-way ANOVA was performed followed by Student–Newman–Keuls *post hoc* test. In the Morris water maze task, the learning phase was evaluated using repeated measures ANOVA. If the assumption of variance homogeneity was violated, a Kruskal–Wallis nonparametric ANOVA followed by Kolmogorov–Smirnov comparisons was performed. The significance level adopted was 5%. The results are presented as mean±SEM, except for the step-down inhibitory avoidance task, where they are presented as median and interquartile ranges (median, 25/75).

3. Results

3.1. Step-down inhibitory avoidance task

There was no significant difference in the latency to step down from the platform during the training session. The results for treatment groups in the STM and LTM tests are shown in Table 1. No differences between treatments were found in young, adult or old female rats. In the comparison between different age groups, under the same treatment, only the old TB2-treated group showed a significant decrease in the latency to step down compared to adult subjects.

3.2. Open field

Locomotor activity (Table 2) was not altered by the different treatments within the young and old groups. For adults, TB2

Table 1
Latency for step down in the step-down inhibitory avoidance task (in seconds)

Treatment	Young	Adult	Old
Short-term memory test (STM)			
SHAM	76 (18.5/180)	180 (170/180)	168 (131/180)
NR	158 (28/180)	180 (144/180)	180 (31/180)
EV	180 (85.2/180)	180 (92/180)	103 (14/180)
TB1	156.5 (57/180)	180 (180/180)	169 (9/180)
TB2	180 (137/180)	180 (180/180)	32.7 (7/119)*
Long-term memory test (LTM)			
SHAM	180 (96.5/ 180)	180 (180/180)	180 (180/180)
NR	84.95 (16.7/170)	160 (88/180)	180 (104/180)
EV	180 (81.5/180)	180 (103/180)	180 (180/180)
TB1	173 (47/180)	180 (180/180)	163 (95/180)
TB2	180 (64/180)	180 (180/180)	134 (58.9/180)

Results are presented as medians and interquartile range (25/75).
Sham (intact gonad+saline); NR (OVX+saline). EV (OVX+estradiol valerate 0.3 mg/kg/day); TB1 (OVX+tibolone 0.5 mg/kg/day); TB2 (OVX+tibolone 1 mg/kg/day).
* TB2 old females showed a significant ($p<0.05$) decrease in the latency to step-down, compared to adult TB2-treated females.

Table 2
Number of crossings in the open field task

Treatment	Young	Adult	Old
SHAM	54.88±7.54	58.4±9.34	45.67±6.8
NR	56.43±8.07	33±7.9	53.83±6.8
EV	75.12±7.54*	36.28±7.9	28.28±6.29
TB1	59.25±7.54	40.43±7.9	35.57±6.29
TB2	74±7.54*	21.14±7.9**	31.17±6.8

The results are presented as mean±SEM.
Sham (intact gonad+saline); NR (OVX+saline). EV (OVX+estradiol valerate 0.3 mg/kg/day); TB1 (OVX+tibolone 0.5 mg/kg/day); TB2 (OVX+tibolone 1 mg/kg/day).
* Young EV and TB2 females presented significant higher crossing numbers compared to adult and old female rats under the same treatment ($p<0.05$).
** TB2 adult females showed a significant ($p<0.05$) lower number of crossing compared to adult Sham control.

females (21.14±7.89 crossings) showed a lower number of crossings compared to Sham control (58.40±9.34 crossings) ($p<0.05$), but no other differences were found. For EV and TB2 groups, young animals made significantly more crossings ($p<0.05$) compared to adult and old female rats (see Table 2).

3.3. Elevated plus maze (EPM)

No significant differences in the time spent inside the open and closed arms were found among the treatments, in the young female rats (Table 3). For adult females, EV and TB2-treated animals spent significantly more time inside the open arms (Fig. 1A) and less time in the closed arms (Fig. 1B), compared with Sham, NR and TB1 female rats ($p<0.05$) (see Table 3). Old NR females (131.33±22 s) spent more time inside open arms compared with EV (29.64±20.28 s) and TB2 (39±24) treated rats (Fig. 1A and Table 3).

Table 3
Time spent inside the arms of the elevated plus maze (in seconds)

Treatment	Young	Adult	Old
Open arms			
SHAM	106±18	73±25	79±24
NR	100.57±19	34±21‡	131±22
EV	80.38±18	171±25*	30±20
TB1	88.12±17.91	60.57±19.27	110±23.99
TB2	112.62±17.91	196±25.49*	39±23.99
Closed arms			
SHAM	144.25±17.77	208.5±20.99	156.6±29.3
NR	154.86±19	182±17.14	118.33±26.75
EV	161.62±17.77	88±20.99#	193.57±24.76
TB1	154.5±17.77	191.57±15.86	131.8±29.3
TB2	133.25±17.77	52.25±20.99#	138.2±29.3

The results are presented as mean±SEM.
Sham (intact gonad+saline); NR (OVX+saline). EV (OVX+estradiol valerate 0.3 mg/kg/day); TB1 (OVX+tibolone 0.5 mg/kg/day); TB2 (OVX+tibolone 1 mg/kg/day). ‡NR adult females spent less time in the open arms, compared to young and old females under the same treatment. *Adult females spent more time in the open arms, compared to young and old female rats under the same treatment. #Adult females spent less time in the close arms, compared to young and old female rats under the same treatment ($p<0.05$).

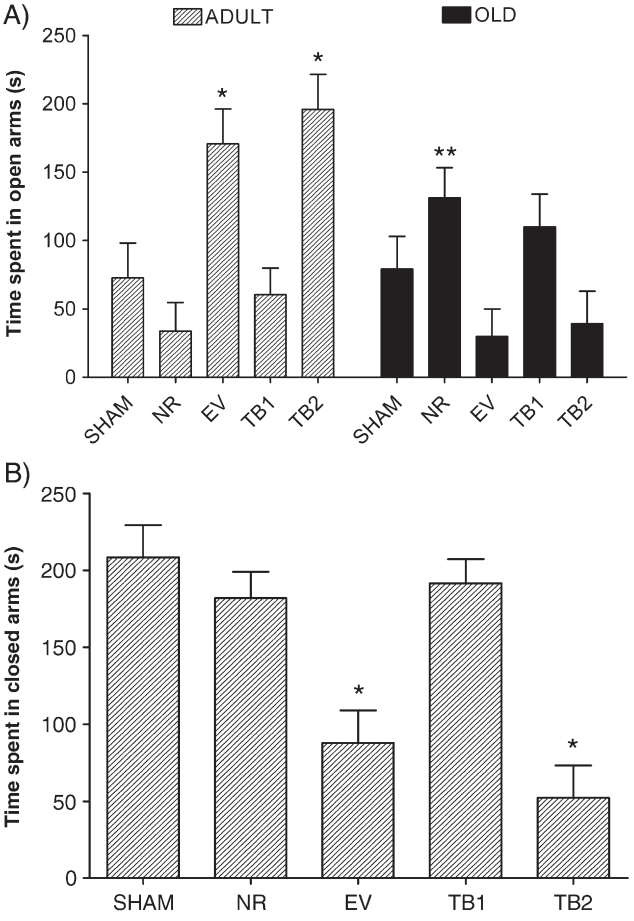

Fig. 1. Effect of ovariectomy and ovarian hormone treatment on behavior of female rats in the elevated plus maze task. Sham (intact gonad+saline); NR (OVX+saline), EV (OVX+estradiol valerate 0.3 mg/kg/day); TB1 (OVX+tibolone 0.5 mg/kg/day); TB2 (OVX+tibolone 1 mg/kg/day). A) Time in the open arms. *Adult EV and TB2-treated females spent significantly more time in the open arms, compared to all other groups. **For old animals, EV and TB2-treated females spent less time in open arms, compared with NR females. B) Time in the closed arms. EV and TB2 treatments significantly reduced the time spent inside the closed arms, compared with all other treatments ($p<0.05$).

When comparing ages undergoing the same treatment, Table 3 shows that the EV-treated adults spent significantly more time inside the open arms compared with young and old female rats. It also shows that treatment with TB2 resulted in a significant increase in the time that adults spent inside open arms, compared with young and old animals (which also significantly differ from each other). EV and TB2 treatments resulted in a lower permanence time of adult females inside the closed arms compared with young and old subjects ($p<0.05$) (Table 3).

3.4. Object recognition

During the training session, no differences ($p>0.05$) were found in the exploration time between the identical objects (data not shown). Results are presented in Table 4. Neither STM nor LTM performances were altered by the treatments or ages in this experiment.

Table 4
Values of object recognition indexes

Treatment	Young	Adult	Old
Short-term memory test (STM)			
SHAM	0.62±0.04	0.78±0.04	0.79±0.05
NR	0.65±0.04	0.78±0.04	0.73±0.05
EV	0.73±0.04	0.79±0.05	0.72±0.05
TB1	0.78±0.04	0.67±0.04	0.79±0.06
TB2	0.77±0.04	0.73±0.04	0.81±0.05
Long-term memory test (LTM)			
SHAM	0.65±0.04	0.72±0.05	0.77±0.05
NR	0.71±0.05	0.74±0.04	0.75±0.04
EV	0.74±0.04	0.79±0.04	0.85±0.04
TB1	0.71±0.04	0.72±0.04	0.78±0.04
TB2	0.71±0.04	0.80±0.04	0.85±0.05

The results are presented as index mean±SEM.
Sham (intact gonad+saline); NR (OVX+saline). EV (OVX+estradiol valerate 0.3 mg/kg/day); TB1 (OVX+tibolone 0.5 mg/kg/day); TB2 (OVX+tibolone 1 mg/kg/day). No significant differences were found.

3.5. Morris water maze (MWM)

No significant differences in the escape latency among the age groups, were found in the learning phase of this task (Fig. 2), except in the first day of training, where young females performed better than adult and old animals ($p<0.05$). A significant difference between the first and the last training day was also registered, showing that all groups had learned the task. In the retention test, the time of permanency in the platform quadrant was influenced by age and hormonal treatment. Table 5 shows that treatment with TB1 resulted in a reduction of the time young female rats spent in the platform quadrant when compared with Sham, EV and TB2 ($p<0.05$). No differences were found for the adult females (see Table 5). Treatment with EV reduced the time of permanence of old animals (19.62±2.05 s) in the platform quadrant compared to all other treatments (Sham=30.88±2.29 s; NR=32.45±1.87 s; TB1=26.85±2.04 s; TB2=33.5±2.04 s) ($p<0.05$). When comparing animals of different ages undergoing the same treatment, old female rats treated with EV showed a significant decrease ($p<0.05$) in the time spent in the platform quadrant compared to young and adult EV-treated animals (see Table 5). Also, a significant difference between young and adult Sham groups was found.

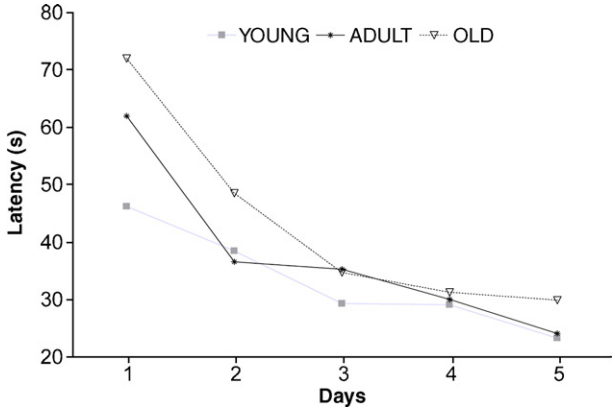

Fig. 2. Effect of ovariectomy and ovarian hormone treatment on the acquisition phase of spatial learning in the Morris water maze. Sham (intact gonad+saline); NR (OVX+saline), EV (OVX+estradiol valerate 0.3 mg/kg/day); TB1 (OVX+tibolone 0.5 mg/kg/day); TB2 (OVX+tibolone 1 mg/kg/day). Values are shown as the time required to find a submerged platform in the water maze (escape latency). There was a significant difference in the escape latency between the first and the fifth acquisition day ($p<0.05$), indicating successful learning for all age groups.

Table 5
Time spent in the platform quadrant during the water maze retention test (in seconds)

Treatment	Young	Adult	Old
SHAM	36.06±2.04	24.60±4.63	30.88±2.29
NR	29.82±2.04	37.83±4.14	32.45±1.87
EV	34.50±2.04	37.45±3.78	19.62±2.05*
TB1	25.70±2.04	32.22±3.78	26.85±2.05
TB2	33.18±2.04	33.66±3.78	33.50±2.05

The results are presented as mean±SEM.
Sham (intact gonad+saline); NR (OVX+saline). EV (OVX+estradiol valerate 0.3 mg/kg/day); TB1 (OVX+tibolone 0.5 mg/kg/day); TB2 (OVX+tibolone 1 mg/kg/day).
* Old EV-treated females spent less time in the platform quadrant, compared to younger females under the same treatment ($p<0.05$).

4. Discussion

The results of the present work reinforce the concept that gonadal steroid hormones have differential effects regarding female performance in memory tasks. Estradiol valerate, as well as tibolone, proved to interfere in some memory and behavioral patterns. However, the most interesting results were those that showed a significant difference in the way that treatment with gonadal steroid hormones affects the performance of animals of different ages.

In the step-down inhibitory task, the reduction of latency was only significant when comparing TB2-treated old females with adults, in the STM test (90 min post-training), not LTM (24 h post-training). Information regarding a biological explanation for this interaction is scarce. Savonenko and Markowska (2003) showed that an estrogen absence or its reposition for short periods, did not affect the escape behavior of middle-aged and old females in the inhibitory avoidance STM test. But, Foster et al. (2003) noticed a significant impairment of old ovariectomized rats (compared to middle-age animals) in an LTM test of inhibitory avoidance. Based on the inconsistent nature of published reports, it is difficult to understand the role of estrogenic hormones with respect to fear-based learning in tests like inhibitory avoidance. We observed that the higher tibolone concentration (1 mg/kg) may produce, in the hippocampal milieu in old female rats, an impairment of the STM formation. Short-term memory is dependent upon NMDA receptor and protein kinase activations (Liberman and Mody, 1999; Izquierdo and McGaugh, 2000) and TB2 might interfere somehow in this pathway.

Different treatments and ages in the present work, did not affect female performance in object recognition memory tests. However, Fernandez and Frick (2004), when studying estradiol

effects on middle-aged mice, reported that estrogen (8 weeks of continuous treatment) improves recognition memory. In much the same way, Vaucher et al. (2002) found beneficial effects of estrogen, but they also found that in mice, age does not reduce animal performance. These differences in the results found in the literature may reflect differences in the hormone administration protocols. Walf et al. (2006) found that immediate post-training hormone administration improved object recognition memory, but if the administration was delayed 1 h, no enhancement was observed.

In the present experimental conditions, TB2 treatment significantly decreased the locomotor activity in adult female rats, when compared to Sham control within this same age group. On the other hand, TB2 and EV-treated young females made more crossings than adult and old rats within the same treatment, thus characterizing an age-dependent anxiolytic activity of these hormones. In agreement with the results found in the present work, most reports attribute estrogen with the augmentation of locomotor activity in young mice and rats (Ogawa et al., 2003; Papalexi et al., 2005).

In addition, the EPM test is a widely used experimental model to assess the state of anxiety in laboratory animals. Young animals with different treatments did not reveal changes in anxiety scores. However, the lower permanence of NR adult females and EV and TB2 old females inside the open arms suggests an increase in anxiety; contrarily, a decrease of anxiety was observed in EV and TB2-treated adult females. The anxiolytic effect of gonadal steroid hormones is well known. Marcondes et al. (2001), working with young female rats, associated lower anxiety levels with the high steroid hormone level verified in proestrus, since females were significantly more anxious during diestrus. On the other hand, these authors reported that treatment of diestrus females with estradiol abolished this difference. Pandaranandaka et al. (2006) also found an anxiolytic effect in adults treated with estradiol.

It is known that estradiol can exert its actions through specific receptors: ERα and ERβ. It has also been demonstrated that ERβ has significant anxiolytic effects on ovariectomized young female rats during the EPM task (Lund et al., 2005), so the expression of this receptor in the amygdala could be the key-factor regarding the anxiolytic effects verified, by the present work, in the EV and TB2-treated adult groups. However, with the decrease of ERβ expression during aging (Chakraborty et al., 2003) the anxiogenic effects of estrogenic hormones could be accentuated. Indeed, ERβ deficient mice have shown an increase in anxiety (Krezel et al., 2001). Also, it is possible that ERs have antagonistic roles (Toufexis et al., 2006), thus if ERβ is known to evoke anxiolytic responses, the activation of ERα may be implicated in an anxiogenic response.

Results from studies on the action of estradiol with respect to spatial reference memory are variable. Markham et al. (2002) showed that the reposition of ovarian hormones, chronic or acute, is capable of preventing forgetfullness during the acquisition phase in the water maze task. Nevertheless, Chesler and Juraska (2000) found a negative action of estrogen plus progesterone in young ovariectomized rats, increasing the latency of the animals to reach the platform in the acquisition phase of the Morris water maze. They also found that estradiol alone decreased the time females spent in the target quadrant during the retention test. The present work shows contrasting results. Treatment with EV only reduced the time that old females spent in the platform quadrant during the retention test while treatment with TB1 decreased the time young ovariectomized females spent in the platform quadrant during the retention test.

Recently, Fernandez and Frick (2004) reported that estrogen impairs reference memory in middle-aged female mice tested in the water maze and that aging, per se, reduced the animal performance in this task. The results obtained in the present study support the idea that ovarian hormones may impair female performance in tasks which demand reference memory utilization in young and old female rats. Contrarily, Foster et al. (2003) found that no replacement for young, low estradiol dose for middle-aged and high estradiol dose for aged animals improved their scores in a spatial task. The complexity of the interactions between memory or behavioral task and age in determining the effect of hormonal treatments on animal responses suggests that the effect of estrogen on behavior is likely to involve a number of brain systems (Foster et al., 2003). For example, impairment in the water maze performance could be due to an effect of this steroid hormone on level of anxiety or stress (Lund et al., 2005) rather than a direct effect in the pathways of spatial memory formation in the brain. In fact, old EV-treated females showed more anxiety in the open field and elevated plus maze results which may explain their lower performance in the water maze test.

However, other factors could produce a differential response to estrogen in the aging brain (Chakraborty and Gore, 2004). For example, the diminution of ERβ expression during aging may not only affect anxiety, but also other behavioral or even memory aspects of the female brain. In this case, even with hormonal reposition, no treatment effect would be expected, since it has been reported that this receptor's expression is not altered by estrogen reposition (Chakraborty et al., 2003). Indeed, an opposite effect would not be a surprise as the reposed estrogen may activate ERα receptors, reported to provoke an ERβ antagonistic effect (Toufexis et al., 2006).

In summary, our work is a meaningful contribution to the characterization of behavioral, memory and anxiety responses evoked by gonadal steroid hormones, in different ontogenic stages. In this regard, we state that EV and TB2, administered daily in ovariectomized rats, induce an increase in anxiety in old female rats, and play an anxiolytic role in adult female rats. Thus, even though hormonal replacement therapy (HRT) may be one of the greatest conquests in the battle for quality of life for women, there remain many factors to be considered when choosing an HRT. The results of the present work bring into focus important facts to be evaluated in this choice, such as the different age-related emotional and cognitive responses to gonadal steroid hormones. With all the known changes that come with menopause, such as memory limitations, mood changes, irritability and increased anxiety, the risk of choosing an HRT that aggravates these symptoms must not be ignored. All of these factors are important in maintaining the quality of life and should thus be carefully considered.

It is likely that the great variability of results in the literature is due to experimental conditions and the myriad pathways involved in behavioral and memory mechanisms (Izquierdo et al., 1998; Liberman and Mody, 1999; Squire and Kandel, 1999; Izquierdo and McGaugh, 2000; Chakraborty and Gore, 2004). Even so, our study clearly shows that hormonal reposition is capable of interfering in the behavioral and memory processes in ovariectomized female rats and that this interference varies across aging.

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