Systemic administrations of β-estradiol alleviate both conditioned and sensitized fear responses in an ovariectomized rat model of post-traumatic stress disorder

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ABSTRACT

Although no single widely accepted animal model of PTSD has been established to date, the single prolonged stress (SPS) animal model has been partially validated as a model for PTSD. SPS rats mimic the pathophysiological abnormalities and behavioral characteristics of PTSD, such as enhanced fear response to the traumatic cue (conditioned fear response) and hyper arousal (the sensitized fear response). In the present study we are looking at PTSD-like symptoms in rats. We examined whether Systemic administrations of β-estradiol could alleviate PTSD-like symptoms that are induced by SPS model.

In this study, electric foot shocks (two 4s, 1 mA with an interval of 30 s) were given to Adult ovariectomized rats 1 day after SPS procedures. Additionally, β-estradiol (45, 90, and 180 μg/kg) or sesam oil (vehicle) were injected immediately after foot shock and before Tests 2 and 3. After different incubation times, one (Test 1), two (Test 2), and three (Test 3) weeks later, the conditioned or sensitized fear responses were measured (Percent of freezing during test) by re-exposing the stressed rats to the shock chamber or a neutral tone in a novel environment. Three other groups were shock, control and sham groups. Ovariectomized rats of Shock group received shocks conducted through the procedure described below on. Animals in control (Ovariectomized rats) and Sham groups (Only submitted to surgery without removal of the ovaries), neither were exposed to the SPS procedure nor received an electrical shock. Also, these three groups were tested for fear responses three times.

Findings indicated that rats who received electric shock the day after SPS exhibited both enhanced conditioned and sensitized fear responses in comparison to the control group. β-estradiol in 45 μg/kg dose could reduce both types of fear responses. β-estradiol exert an inhibitory influence on contextual fear conditioning (hippocampal-dependent) and on sensitized fear conditioning (amygdala-dependent). Single injection of this dose is enough for CFR alleviation but at least twice injections are necessary to reduce sensitized fear response. Overall our data demonstrate that multiple injections of β-estradiol dose dependently, could alleviate both SPS induced conditioned and sensitized fear responses, as signs of PTSD.

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1. Introduction

Post-traumatic stress disorder (PTSD), an anxiety disorder defined by Diagnostic and Statistical Manual of Mental disorders, fourth edition (American Psychiatric Association, 1994), can develop following the exposure to a severe traumatic event(s) like what occurs during a war or combat (Kulka et al., 1990; Sorg & Kalivas, 1995). PTSD has proven to be related to exaggerated implicit fear memory, resulting from associative fear conditioning and non-associative sensitization processes (Charney, Deutch, Krystal, Southwick, & Davis, 1993; Foa, Zinbarg, & Rothbaum, 1992; Sorg & Kalivas, 1995). Accordingly, the symptoms of this psychiatric disorder can be divided into those that clearly relate to the memory of the trauma (re-experiencing, avoidance of and exaggerated response to cues reminding of the trauma) and others which lack this overt relation, such as hyper-arousal, irritability, increased startle, social withdrawal (Gewirtz, McNish, & Davis, 1998; Siegmund & Wotjak, 2006). There are many animal models for PTSD. Although these animal models presented behavioral alterations resembling PTSD, they failed to show the most consistent neuroendocrinologic characteristic observed in PTSD patients. Several clinical neuroendocrinologic studies showed that dysfunction of the
hypothalamo–pituitary–adrenal (HPA) axis is one of the core neuroendocrine abnormalities related to the disorder (de Kloet, Vermetten, Geuze, et al., 2006). This neuroendocrine finding specific to PTSD has served as the basis for animal models that are useful for elucidating the pathophysiology of PTSD (Yehuda, 2005). Single-prolonged stress (SPS) (Liberson, Krstov, & Young, 1997), was shown to induce enhanced inhibition of the HPA axis and also exhibit behavioral abnormalities (enhanced anxiety) that mimic the symptoms of PTSD (Iwamoto et al., 2007). Then, SPS paradigms have been extensively developed and employed in the investigation of PTSD (Iwamoto et al., 2007).

SPS shows behavioral and physiological conditioned fear responses to trauma-related cues, as well as sensitized responses to novel stressful stimuli, including acoustic startle, long after the trauma has ended (Wang, Peng, Tan, Chen, & Zhang et al., 2010; Stam, 2007; Francati, Vermetten, & Bremner, 2007). Trauma is common in women; five out of ten women experience a traumatic event. Women tend to experience traumas different from those of men. While both men and women report the same symptoms of PTSD (hyper-arousal, re-experiencing, avoidance, and numbing), some symptoms are more common for women or men. It was found that men and women differed in lifetime rates of PTSD. Surprisingly, women were twice as likely as men to have a diagnosis of PTSD at some point in their lifetime. The estimated prevalence of lifetime PTSD was 7.8% in the general population. Specifically, women (10.4%)were more than twice as likely as men (5%) to have PTSD at some point in their lives (Breslau, Davis, Andreski, Peterson, & Schultz, 1997; Kessler, Sonnega, Bromet, et al., 1995). Women are more likely than men to suffer from stress-related mental disorders, such as major depression, social anxiety disorder, and PTSD (Kessler et al., 2003; Nestler et al., 2002).

Rachael Yehuda, discussed the neuroanatomic, neuroendocrine, and immune-related bases for various gender differences observed in PTSD (Yehuda, 1999; Yehuda, 2001). In individuals with PTSD, the HPA axis response is deregulated, they have low circulating levels of cortisol. These HPA axis parameters are affected by the gonadal hormones in individuals with PTSD. A number of animal studies have demonstrated a greater acute response of the HPA axis to stress in females versus males (Ogilvie & Rivier, 1997; Yehuda, 2001). It appears that this increase may be estrogen-mediated because ovarioctomized females have a stress response which is similar in magnitude to the response of males. There are also changes in the stress response during different phases of the menstrual cycle. The data indicated that women had lower baseline cortisol levels than men, but PTSD status was not gender related. In other words, women with and without PTSD had lower cortisol as compared to men with and without PTSD. There was greater suppression in women than in men, indicating greater deregulation of the glucocorticoid receptors (Goldney, Wilson, Dal Grande, Fisher, & McFarlane, 2000; Ogilvie & Rivier 1997; Yehuda, 2001). It is believed (Burgess & Handa 1992; Carey et al.,1995) that estrogen acts centrally to modulate the neuroendocrine responses to stress. The central sites and mechanisms responsible for estrogen actions on the stress-induced glucocorticoid surge remain elusive. However, there is evidence that altered neuronal activity in the PVN might be involved in mediating estrogen effects on HPA axis responses to stress. In male rats, systemic injections of estrogen enhance the expression of the immediate early gene c-fos in response to novelty (Yukhananov & Handa, 1997) and to restraint (Lund, Munson, Haldy, & Handa, 2004). However, the relevance of these findings to HPA axis activity in females is not clear, especially considering pronounced organizational and activity gender differences in the rodent brain (Figueredo, Dolgas, & Herman, 2002). Thus, despite the evidence in support of the role of E2 in modulating PVN responses to stressful stimuli, the precise coordinated involvement of central sites in stress-induced corticosterone hypersecretion in females remains unclear.

A number of studies have demonstrated decreases in hippocampal volume in individuals with PTSD. Studies that examined gender differences associated with this finding have demonstrated fairly consistently that hippocampal volume is more decreased in men than in women (Conrad, Galea, Kuroda, & McEwen, 1996). In addition, women with PTSD have less memory loss and impairment in cognitive function than their male counterparts. There are also differences between men and women in the presentation of PTSD. Women are more likely to have symptoms of numbing and avoidance and men are more likely to have the associated features of irritability and impulsiveness. Men are more likely to have co-morbid substance use disorders and women are more likely to have co-morbid mood and anxiety disorders, although many disorders co-morbid to PTSD are commonly seen in both men and women. Studies in rodents also indicate sex differences in emotional behaviors. For example, male rats have been reported to exhibit significantly higher levels of contextual freezing response than female rats (Gupta, Sen, Diepenhorst, Rudick, & Maren, 2001; Kitraki, Kremmyda, Youlataos, Alexis, & Kittas, 2004; Maren, DeOca, & Fanselow, 1994) and women are more vulnerable to anxiety and depression (Kessler et al., 1994; Young & Korszun, 2010). Several reports indicate that understanding the stress response and cognitive ability in males may not extrapolate to females.

In conclusion there are some PTSD-specific gender differences in the biologic abnormalities seen in individuals with PTSD, but, in general, there are many more similarities than differences. Although there are gender differences in the stress response, many of these differences are not exaggerated or changed in individuals with PTSD.

In the brain, stress produces sexually dimorphic responses in both hippocampal morphology and function. For example, in male rats, restraint for 6 h/day/21 days retracts hippocampal dentritic arbor in the CA3 region, which corresponds to impaired hippocampal-dependent spatial memory (Conrad et al., 1996). Ovarian hormones and their fluctuations may modulate the effects of stress on hippocampal morphology and function in females (Sherwin, 1999; Mclaughlin, Baran, Wright, & Conrad, 2005). The interactions among stress, ovarian hormones, and hippocampal morphology and function are complex; thus, we designed an experiment using ovarioctomized female rats. This study is the first study to examine the influence of SPS in ovarioctomized rats on conditioned responses.

Investigators have in recent years conducted several pilot studies of pharmacotherapy to prevent PTSD. There are some different approaches in prevention of PTSD in patients, the main ones of which are administration of hydrocortisone (Aerni et al., 2004; Schelling et al., 2001), propranolol or temazepam (Mellman, Bustamante, & David, 2002). The rush over the last decade to elucidate the neuro-protective effects of estrogen in disease or injury of the nervous system was precipitated by a series of findings which forced recognition of the potential impact of gonadal steroid hormones in disturbances of the nervous system (Garcia-Segura, Azcoitia, & Don Carlos, 2001). Estrogen has been associated with a decreased risk, delayed onset and progression, or enhanced recovery from numerous traumatic or chronic neurological and mental diseases) (Filfit, 1994; Chang et al., 2009), some of which are discussed below. These span many different types of disease and trauma, ages, and hormonal status, and can be variously classified as dysfuction due to abnormal development (dyslexia, autism), abnormal neurotransmitter systems (depression; anorexia/bulimia), disorders caused by trauma (stroke, epilepsy, head injury), or disorders caused by abnormal immune (multiple sclerosis).
or cardiovascular (stroke, head injury) function) Kawas et al., 1997; Singh, Dykens, & Simpkins, 2006; Dribben et al., 2003; Irwin et al., 2008; Woolley et al., 1996; Lo & Wang, 2003). Sex steroid hormones are believed to provide women with endogenous protection against cerebrovascular events. Premenopausal women have a lower risk of stroke than men of the same age (Barret-Connor & Bush 1991), and the incidence of stroke in women increases rapidly after the menopause (Wenger, Speroff, & Packard 1993), coincident with diminished circulating levels of estrogen and progesterone. Several studies indicate that estrogen protects women against cerebral ischemia and other neurodegenerative diseases via various mechanisms. Recently, these have been proposed as: modification of cerebral blood flow and glucose transport; stabilization of neurons by inhibiting calcium currents; up-regulation of NGF and brain-derived neurotrophic factors (Garcia-Segura et al., 2001); stimulation of endothelial and neuronal nitric oxide synthase, which produces the vasodilator molecule nitric oxide (Fernandez-Tome et al., 1999); regulation of levels of inflammatory mediators post-ischemia (Liao, Chen, Kuo, & Chen, 2002); anti-apoptotic properties; protection from glutamate toxicity and one of the well documented effects of estrogen which is its antioxidant properties against oxidative stress (Garcia-Segura et al., 2001). Given the well-known neuro-protective effects of estrogens, it would be of interest to test whether β-estradiol can alleviate symptoms of PTSD in a rat model.

Although accumulating evidence from basic science studies using animal models suggests that estradiol plays a critical neuro-protective role against multiple types of neurodegenerative diseases and injuries, recent clinical studies have reported either inconclusive or untoward effects of hormone therapy on the brain, for several important biological reasons. Clearly, many factors, including the type of estrogen being used, the dose, the route of administration, and the age and previous hormonal and health status of the women being treated, must be taken into consideration when designing clinical studies and when interpreting the results. The estrogen is intimately associated with those of progesterone and androgen, making it difficult to consider each one independently with respect to neuroprotection. Also, because estrogen lowers cholesterol and alters immune function, the role of estrogen in neuroprotection may be indirect, through actions on other organs which then impinge upon the health of the brain.

It has been demonstrated that the rapid development of contextual memory is one of the major functions of the hippocampus (Phillips & LeDoux, 1992). It is hypothesized that hippocampal dysfunction may be associated with enhanced contextual freezing in SPS rats, though hippocampal-based contextual memory is regulated by the prefrontal–amygdala neural circuit (Sotres-Bayon, Cain, & LeDoux, 2006). Not only SPS rats should show clear conditioned responses but also their excessive behavioral and physiological responses should “spill over” to stimuli of sufficient strength that are unrelated to the trauma, a phenomenon called sensitisation (Shin, Rauch, & Pitman, 2006). The sensitized fear response was a general heighten emotional response. Thus the sensitized fear response might be indirectly related to the traumatic experience that is amygdala dependent. The main important points of this paper are the description of SPS as an animal model of PTSD, using ovariectomized female rats, determine whether exposure to SPS alters conditioned (CFR) and sensitized (SFR) fear responses and whether β-estradiol administration (multiple injections) following SPS could exert an influence on these responses. The utility of an animal model like this is that some data about the specificity of estradiol effects can be investigated when using tasks which tap into function of the hippocampus (CFR) versus the amygdala (SFR).

2. Materials and methods

2.1. Animals

A total of 80 wistar rats (200–250 g) were housed four into a cage, maintained on a 12-h light/dark cycle (light on from 08:00 to 20:00), and fed and watered ad libitum. Rats were obtained from the breeding colony of Damghan University of, Damghan, Iran. All procedures were conducted in agreement with the National Institutes of Health Guide for care and use of laboratory animals. Every effort was made to minimize the number of animals used per group and to minimize the suffering of animals used throughout all experimental procedures.

2.2. Experimental groups

Rats were randomly assigned to one of the following groups (8–10 animals in each group): SPS, Shock, Control and Sham group. Animals were ovariectomized by the surgical removal of both ovaries under ketamine anesthesia (90 mg/kg) and xylazine (10 mg/kg). The animals were allowed 2 weeks for recovery, during which they were housed in groups of two per cage (Chang et al., 2009). SPS group: Ovariectomized rats of this group were conducted in the procedures described in Sections 2.3 and 2.4. Shock group: Ovariectomized rats of this group received a shock that was conducted in the procedure described in Section 2.4 (Fig. 1A). Animals in control (Ovariectomized rats) and Sham groups (Only submitted to surgery without removal of the ovaries), neither were exposed to the SPS procedure nor received an electrical shock (They were placed in the shock chamber, without receiving electrical shock).

In the second part of experiments, animals who were exposed to the SPS procedure received multiple injections of vehicle or different doses (45, 90, and 180 μg/kg) of β-estradiol. The first injection was immediately after shock application, and the other two injections were 2 h before Tests 1 and 2.

2.3. Single prolonged stress procedure

SPS is an animal model of PTSD that was first proposed by Liberonz et al. (1997, 1999). Detailed SPS procedure has been described in previous studies (Liberonz et al., 1997; Wang, Liu, Zheng, Wang, & Jin et al., 2008). Briefly, rats were restrained for 2 h, immediately followed by forced swimming for 20 min in 24 °C water contained in a clear acrylic cylinder (24 cm in diameter and 50 cm in height). After 15 min of recuperation, animals were exposed to diethyl ether until they lost consciousness (for 1–2 min). Rats were exposed to ether for 1–2 min. Mean ± SD of exposure in total rats, was 1.255 ± 0.15. ANOVA analysis showed that there was no differences between groups (F4,79 = 1.359, P = 0.25). They lost their consciousness around 7–10 min. Although there were some individual differences in consciousness, but the differences were not significant (F4,79 = 0.728, P = 0.576). Rats in different groups had similar responses to the trauma.

2.4. Shock application and test of conditioned and sensitized fear responses

In a different fear conditioning paradigm, Iwamoto et al. (2007) demonstrated that 24 h after contextual fear conditioning (FC), SPS rats exhibited a significant increase in contextual freezing as compared with sham rats (Iwamoto et al., 2007). So, 1 day after SPS, stressed rats received the electrical foot shock within the shock chamber (45 cm × 45 cm × 47 cm) (Chamber A). They re-
ceived two 4s, 1 mA shocks with a 30 s interval, administered via the metal grid. Stressed rats were held in the shock chamber for another 60 s before being returned to the home cages. Those in the non-shock condition (sham and control groups) were placed in shock chamber without receiving a shock.

To test the CFR, 1, 2 and 3 weeks later, the rats were placed back in the chamber (Chamber A), for 3 min without further shock application. The duration of freezing (Absence of all visible movement except respiration) was evaluated. To test the SFR, animals were placed into the neutral test chamber (Chamber B). This chamber was the same size as the chamber A, but was different in that it had some toys placed on a wooden floor and some designs in the box. After 3 min, a neutral tone (80 dB, 9 kHz) was presented for 3 min. They were held in the test chamber for another 60 s before being returned to the home cages.

2.5. Drug treatment

β-estradiol was purchased from Sigma (USA). β-estradiol was prepared in sesame oil (vehicle, Sigma-USA), and was injected in volume of 2 ml/kg (S.C. 45, 90, and 180 µg/kg). Control animals were given vehicle only. The drug doses were mainly derived from pilot studies, and a survey of reports on these drugs (Dubal & Wise, 2001; Nunez & McCarthy, 2003; Sales, Ureshino, Santos Pereira, Oliveira, et al., 2010; Viau & Meaney, 1991; Wessa & Flor, 2007).

2.6. Statistical analysis

Data are presented as mean ± SEM. Data were analyzed by 1- and 2-way ANOVA for repeated measurements by means of SPSS 16.0. Tukey post hoc test was performed to determine the source of the detected significant differences. Values of $P < 0.05$ were considered significant.

3. Results

3.1. Experiment 1

The aim of this experiment was to investigate the conditioned and sensitized fear responses in female rats after post-traumatic stress disorder induction by SPS. Rats were randomly divided into the groups described in Section 2.4. One (Test 1), two (Test 2) and three (Test 3) weeks later, both CFR (Chamber A) and SFR (Chamber B) of all rats were tested according to the procedures described in Section 2.3 (Fig. 1A).

3.1.1. CFR results

Two-way ANOVA with repeated measures on freezing data showed a significant effect of groups ($F_{3,29} = 213.75, P = 0.000$), tests ($F_{2,58} = 10.65, P = 0.000$), and interaction between groups and tests ($F_{6,58} = 16.07, P = 0.000$). Post hoc comparisons indicated that SPS group showed significant increase in CFR as compared with sham, control and shock groups in all tests ($P = 0.00$). Animals in this group were likely to “freeze” for a longer time during the entire period of observation when compared to the above groups, and this result remained the same in all three Tests. Shock and control groups showed significant increase in conditioned fear response compared with sham group, in all three tests ($P = 0.00$). There were no differences between freezing behavior of control and shock groups (Fig. 2).

3.1.2. SFR results

Two-way ANOVA with repeated measures on freezing data showed a significant effect of groups ($F_{3,29} = 177.09, P = 0.000$), tests ($F_{2,58} = 5.42, P = 0.007$), and interaction between groups and tests ($F_{6,58} = 59.89, P = 0.000$). Post hoc comparisons indicated that SPS group showed significant increase in sensitized fear response...
as compared with sham, control and shock groups in Tests 2 and 3 ($P = 0.00$). In Test 1, there was a significant increase in freezing behavior of SPS group as compared with sham and control groups ($P = 0.00$), but not with shock group. Shock and control groups showed significant increase in sensitized fear response in comparison to sham group, in all three tests ($P = 0.00$). There were significant differences between freezing behavior of control and shock groups in Test 1 ($P = 0.00$), but not in Tests 2 and 3 (Fig. 3).

### 3.2. Experiment 2

The aim of this experiment was to test the effects of subcutaneous injections of different doses of β-estradiol on PTSD-like symptoms. Animals were exposed to the SPS procedure and received multiple injections of vehicle or different doses (45, 90, and 180 μg/kg) of β-estradiol following SPS (Fig. 1 B). The first injection was immediately after shock application, and the other two injections were 2 h before Test 1 and Test 2. One (Test 1), two (Test 2) and three (Test 3) weeks later, both conditioned and sensitized fear responses of all rats were tested according to the procedures described in Section 2.4.

#### 3.2.1. Effects of β-estradiol on CFR

Fig. 3 A shows the effect of treatment on CFR. Two-way ANOVA on freezing data showed a significant main effect of groups ($F_{3,28} = 14.97$, $P = 0.000$), tests ($F_{2,56} = 3.33$, $P = 0.043$) and interaction between groups and tests ($F_{6,56} = 8.53$, $P = 0.000$). Post hoc comparisons indicated that SPS rats that received 45 μg/kg subcutaneous multiple injection of β-estradiol showed a significant decrease in the percentage of freezing time as compared with control rats in all tests ($P = 0.00$). SPS rats which were treated with 90 and 180 μg/kg only showed a significant reduced conditioned freezing behavior in all tests ($P < 0.0001$, $P < 0.001$ as compared with the control group). Data are expressed as means ± SEM (n = 8 in each group).
response in Test 1 as compared with control group \( (P = 0.00) \) (Fig. 4).

### 3.2.2. Effects of \( \beta \)-estradiol on SFR

Two-way ANOVA on freezing data showed a significant main effect of tests \( (F_{2,56} = 33.38, P = 0.000) \), groups \( (F_{3,28} = 10.69, P = 0.000) \) and a significant interaction between groups and tests \( (F_{3,28} = 24.56, P = 0.000) \). Post hoc comparisons showed that \( \beta \)-estradiol at 45, 90, and 180 \( \mu \)g/kg reduced the percentage of freezing time as compared with control group, in Tests 2 and 3 \( (P = 0.000) \). In Test 1, Only SPS rats which were treated with 180 \( \mu \)g/kg of \( \beta \)-estradiol showed a significant reduction in sensitized fear response as compared with control group \( (P = 0.000) \) (Fig. 5).

### 4. Discussion

Using a fear conditioning paradigms in ovariectomized rats, we examine influence of SPS on fear behaviors, and the effects of \( \beta \)-estradiol on enhanced fear responses induced by SPS. This model covers both associative (CFR) and non-associative (SFR) fear components after the experience of a trauma. The principal findings of this study are: (1) SPS induced marked enhancement of both conditioned and sensitized fear responses in ovariectomized rats. (2) Repeated administration of \( \beta \)-estradiol, dose dependently, alleviated enhanced conditioned and sensitized fear responses in SPS rats. Overall, the data demonstrated that \( \beta \)-estradiol significantly decreased SPS induced conditioned and sensitized responses.

Previous studies show that SPS caused several numbers of changes similar to those described in PTSD, which marks it as a putative PTSD model (Iwamato, Morinobu, Takahashi, & Yamawaki, 2007; Liberzon et al., 1997). This model can produce more robust symptoms and enhanced conditioned and sensitized fear responses which mimic the characteristics of traumatically cued memory and hyper arousal, respectively (Wang et al., 2008; Dennis et al., 1993). These results are confirmed by the present findings indicating (Experiment 1) that SPS ovariectomized rats showed significant enhanced conditioned and sensitized fear responses as compared with control rats. This enhancement lasted even after three weeks.

Fluctuations in estradiol may modulate stress effects on the brain and subsequent behaviors (Lo & Wang, 2003). The interactions among stress, ovarian hormones, and hippocampal morphology and function are complex; thus, we designed an experiment using ovariectomized female rats (Mclaughlin et al., 2005). In the current study, ovariectomized control rats showed significant increase in CFR as compared with sham group. These findings are consistent with and further extend earlier work showing that estrogen has been reported to exert an inhibitory influence on contextual fear conditioning (hippocampal-dependent) in adult female rats (Gupta et al., 2001; Mclaughlin et al., 2005). Also, female rats showed significant increase in SFR as compared with control (Ovariectomized) and sham (Intact) groups. The present study is the first to demonstrate that ovariectomy exerts an excitatory influence on sensitized fear conditioning (amygdala-dependent).

Another finding of the present study was that multiple injections of \( \beta \)-estradiol could alleviate both enhanced conditioned and sensitized fear responses induced by SPS. In previous studies, the concentration of \( \beta \)-estradiol that produced protective effects in stroke models ranged from low physiological to high pharmacological levels. Neuroprotection is afforded with physiological doses of \( \beta \)-estradiol at the onset of an ischemic event (Dubal et al., 1998b), but the neuroprotective effects of pharmacological doses (100 \( \mu \)g/kg) of \( \beta \)-estradiol are demonstrated by the acute treatment at the time of or just before an ischemic event, as well as after its onset (Simpkins et al., 1997). In Experiment 2, we observed that single administration of \( \beta \)-estradiol in each doses alleviated the CFR, but it was only with 180 \( \mu \)g/kg dose that it reduced SFR. Repeated injection of \( \beta \)-estradiol showed that only the effect of 45 \( \mu \)g/kg dose on CFR was sustained for two weeks, but this effect did not last in other dosages. In other words, \( \beta \)-estradiol in 45 \( \mu \)g/kg dose could reduce the enhanced conditioned response induced by SPS, in all three Tests. Repeated administration of \( \beta \)-estradiol in all dosages had significant effect on SFR; it means that animals that were treated with all dosages showed significant decrease in percentage of freezing time in context B as compared with control group. Taken together, it is suggested that \( \beta \)-estradiol in 45 \( \mu \)g/kg dose could reduce both fear responses. Single injection of this dose is enough for CFR alleviation but at least twice injections are necessary to reduce sensitized fear response. So, \( \beta \)-estradiol dose influences both the hippocampal (CFR) and amygdala dependent fear memories (SFR) dependently. These findings agree with previous data indicating that repeated \( \beta \)-estradiol treatment influences the hippocampus and amygdala dependent memories differently (Galea et al., 1997; Fink, Sumner, Rosie, Grace, & Quinn, 1996; Walf, Paris, & Frye, 2009). Several reports indicated the ability of estradiol to modulate hippocampus-dependent and independent behaviors depend on dosage (Barker & Galea, 2010; Holmes, Wide, & Galea, 2002), age (Fink et al., 1996; Frick, 2009), continuous or intermittent exposure to estradiol (Gresack & Frick, 2006) and timing of exposure of estradiol in relation to learning (Packard, 1998; Frye, Duffy, & Walf, 2007).

Previous clinical and experimental data suggest that estrogen may act as a preventive agent for neurodegeneration, while the...
hormone is probably unable to act as a restorative agent in already degenerated neural tissue (Calakos & Scheller, 1994; Dubal & Wise, 2001), and findings indicated that estrogen is neuroprotective when administered before or at the same time as the neurodegenerative stimulus but is not neuroprotective when administered after the neurodegenerative stimulus (Garcia-Segura et al., 2001). It is likely that effectiveness of β-estradiol, in our study, depends on immediate administration after traumatic event.

What is the mechanism of β-estradiol’s effects? Clinical studies based on structural and functional neuroimaging studies implicated three brain areas in the pathophysiology of PTSD: the hippocampus (Vikas & Kuljeet, 2007; Iwamato et al., 2007; McEwen, 2004; Wignall, Dickson, & Vaughan, 2004; Yamamoto et al., 2008), amygdala, and medial prefrontal cortex (Iwamato et al., 2007; Kitayama, Vaccarino, Kutter, Weiss, & Bremner, 2005; Shin et al., 2006). These areas respond differentially to stress and in turn differentially regulate hypothalamic–pituitary–adrenal (HPA) activity (both positively and negatively) (Lei, Rulun, Xiaoli, Robert, & He, 2005; Li, Han, Liu, & Shi, 2010; Vlaau & Meaney, 1991). Connections between these structures have been implicated in determining the final fear responses (McClery & Harvey, 2004; Bisson, 2007). It might be that β-estradiol appears to play contradictory roles in the actions of stress on hippocampus, prefrontal cortex, and amygdala.

5. Conclusion

This manuscript used a single prolonged stress procedure as a model of post-traumatic stress disorder. Female rats were given SPS and then were shocked presumably to produce conditioned responses. They were then tested for sensitized fear and conditioned fear responses at 1, 2, and 3 weeks after the shock. In the present study, we found that Single prolonged stress model increased conditioned and sensitized fear responses in ovariectomized rats. Additionally some ovariectomized females were given estradiol to determine if it would help alleviate some of the conditioned or sensitized fear responses. Our data also indicate that multiple injections of β-estradiol, dose dependently, could alleviate fear responses induced by SPS. Although the underlying mechanisms remain to be determined, the present results show that when β-estradiol was administered following a traumatic event (SPS), subsequent expression of PTSD symptoms are considerably reduced. Even the best, well-designed clinical studies cannot benefit from the experimental advantages of many basic science studies, because studies performed with experimental animal models allow replication with adequate numbers of animals, controls with equivalent genetic backgrounds and previous exposure to similar environments, well controlled environments during the entire study, and lack of selection or recall bias. We think that our findings should help us develop therapeutic methods to prevent PTSD induction. Much more work is necessary before we fully understand the many ways through which estrogens exert beneficial actions.

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