Original Article

DOI: 10.5582/ddt.2020.03117

Therapeutic effects of sertraline on improvement of Ovariectomy-induced decreased spontaneous activity in mice

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SUMMARY We have already reported that ovariectomized (OVX) rats reduced the spontaneous activity during the dark period due to the decease of serotonin release in the amygdala. In this study, we examined the potential of sertraline, a selective serotonin reuptake inhibitor, on the recovery of less spontaneous activity seen in mice with OVX-induced despair-like behaviors. Female 9-week old ICR mice were underwent either OVX or sham surgery. Sertraline (10 mg/kg/day, *s.c.*) or saline were started to administer to each group for 8 weeks (6 times/week) from the 8th week after OVX. Each spontaneous activity of mouse was evaluated during the dark period (19:00-07:00) using an infrared sensor. Moreover, mRNA expression levels of tryptophan hydroxylase (TPH) and X-box binding protein 1 (XBP1) were measured in the hippocampus and prefrontal cortex using by a real-time PCR method. We found out that the OVX-induced despair-like behaviors were improved by the continuous administration of sertraline. After treatment of OVX, our real-time PCR data showed that sertraline significantly suppressed the upregulation of XBP1 expression levels in both hippocampus and prefrontal cortex, although this suppression of the downregulation of TPH expression levels was seen in only hippocampus. These results suggest that sertraline improves the decrease in spontaneous activity induced by OVX assessed by the hippocampus suppressing decreased serotonin synthesis in the serotonergic neuron.

Keywords Sertraline, ovariectomized mice, spontaneous activity, hippocampus, serotonergic neuron, tryptophan hydroxylase

1. Introduction

There are gender differences in the incidence of depressive symptoms and mood disorders, and it has been reported that the lifetime prevalence of depression in women is about twice that in men (*1*). Furthermore, women often exhibit anxiety, emotional instability, and depression during the menstrual cycle in the case of premenstrual syndrome and psychiatric symptoms such as anxiety, depression, fear, and fatigue during menopause (*2*). It is thought that estrogen, a female hormone, may be involved in the onset of these specific women's psychiatric symptoms, although the causes of these symptoms induced by changes in estrogen levels have not been elucidated, yet. There are many reports that estrogen administration inhibits anxiety and depressive symptoms in animal experiments, although there is also a conflicting report that estrogen increases symptoms (*3*); therefore, a consensus has not been reached. In clinical practice, hormone replacement therapy (HRT) is used as a treatment of menopause disorder, but the efficacy of HRT is limited for treating vasomotor symptoms such as hot flashes and sweating (*4*), and there is no effective treatment of depression. Furthermore, estradiol administration has been reported to increase the risk of breast and endometrial cancer (*5,6*), and there is a need for the development of treatments of estrogen-dependent psychiatric symptoms. In particular, psychiatric symptoms during menopause are likely to develop into major depression, which is one of the factors contributing to the high suicide rate among older women (*7*); thus, prompt elucidation of the mechanism of estrogen-dependent psychiatric symptoms is desirable.

It has been reported that monoamine neurotransmitters

in the brain are involved in the onset of symptoms such as depression and anxiety due to decreased estrogen levels, suggesting involvement of the serotonergic neuronal system (*8,9*). We also reported that the significant reduction of estradiol after ovariectomy (OVX) suppresses the spontaneous activity during the dark period and that brain serotonin is involved in such effect in rats (*10*). In addition, we previously reported that the specific serotonin uptake inhibitor (SSRI), fluvoxamine, counteracts the decrease in spontaneous activity in an OVX rat model (*11*).

At present, the efficacy of SSRIs and serotonin and norepinephrine reuptake inhibitors (SNRIs) has been clinically demonstrated for the main treatment for symptoms of depression during menopause (*12*). Furthermore, it is well known that there are gender differences in the therapeutic response to antidepressants, and women have been reported to show better response to sertraline, a typical SSRI, than the tricyclic antidepressant imipramine (*13*). In addition, the results of a non-clinical study showed that female transgenic mice deficient in aromatase are more responsive to sertraline than males (*14*). Thus, sertraline may be highly effective against the female depression-like symptoms. Sertraline is widely known increasing the serotonin concentration in synaptic cleft by selectively inhibiting serotonin reuptake, while Kim *et al.* reported that long-term administration of sertraline increases tryptophan hydroxylase (TPH) mRNA of rate-limiting enzyme for serotonin synthesis (*15*). In addition, the expression of transcription factor X-box protein 1 (XBP1) increases in stress-induced rats and is associated with the endoplasmic reticulum (ER) stress response (*16*), suggesting a correlation between depression and ER stress response.

In the present study, we evaluate whether sertraline, which is a potential clinical treatment of estrogen-dependent psychiatric symptoms, recover the behavioral changes in OVX model mice. In order to resolve this questionnaire, we investigated both gene expression levels of TPH, which is the rate-limiting enzyme for serotonin synthesis, and XBP1, which is involved in the ER stress response, in OVX mice.

2. Materials and Methods

2.1. Animals and rearing conditions

A total of thirty-two ICR female mice (8 weeks old, Japan SLC, Shizuoka, Japan), which had undergone one week of preliminary rearing, were used for the study. The breeding environment was set to room temperature (24 ± 1°C), 12-h light/dark cycles (light period: 07:00-19:00; dark period: 19:00-07:00). Mice were allowed free access to a standard rodent diet (Labo MR stock, Nosan Corporation, Kanagawa, Japan) and water during the entire experimental period. The present study was approved by the Animal Experiment Committee of the Yokohama University of Pharmacy (approval number: 2017-021), and care was taken to the welfare of laboratory animals.

2.2. OVX and administration

After 1 week of preliminary rearing, the animals were divided into 4 groups to minimize the difference in weight. Under inhalation anesthesia with isoflurane, one of the groups underwent sham surgery (sham group), and the other 3 groups underwent OVX. Drug administration was initiated 8 weeks after OVX. Eight weeks after OVX, sertraline hydrochloride (Tokyo Chemical Industry, Tokyo, Japan) was administered (10 mg/kg *s.c.*) to the OVX + sertraline administration group, and β-estradiol (Sigma Sigma-Aldrich St. Louis, MO, USA) was administered (20 μg/kg *s.c.*) to the OVX + β-estradiol administration group. The Sham and OVX groups were administered saline by *s.c.*. Administration was performed at 10 mL/kg, 5 days per week, over a period of 8 weeks.

2.3. Measurement of uterine weight

Eight weeks after administration of test drugs (16 weeks after OVX), the mice were euthanized, the uterus was removed, and the weight of the uterus was measured.

2.4. Measurement of spontaneous activity

After 8 and 16 weeks following OVX, the mice were placed in individual cages using a spontaneous activity-measuring device (NS-AS01, Neuroscience, Tokyo, Japan), and the amount of activity per hour was measured with an infrared sensor. The measurement was performed during the 12-h dark period (19:00-07:00), and the total activity was compared.

2.5. Open field test

Eight weeks after administration, the behavior of the mice was observed for 10 min using an open field test device. The device features a 50 cm^2 shape at the bottom and a wall height of 30 cm; the bottom and side walls are made of gray plastic. Each mouse was released at the center of the device, their behavior was observed for 10 min, and the track length and duration were then measured. After each test, any excrement was removed, and the device was wiped clean with 10% ethanol. The square at the bottom of the device was divided into central (25 cm × 25 cm) and outer areas, and the ratio of the track length to the central area and the duration was calculated.

2.6. TPH, serotonin transporter (5-HTT), and XBP1 mRNA expression levels

To extract total RNA, Isogen (Nippon Gene, Tokyo,

Japan) was added to the hippocampus and frontal lobe specimens extracted from each mouse and homogenized with POLYTRON PT 1300 D (Central Scientific Commerce, Tokyo, Japan). Chloroform (Nacalai Tesque Inc., Kyoto, Japan) was added to the homogenized samples, the samples were centrifuged, and the supernatant was collected in a new tube. Isopropanol (Nacalai Tesque Inc.) was added to the supernatant, and the precipitate was collected by centrifugation. The precipitate was then resuspended in sterile water, the RNA concentration was measured, and cDNA was synthesized using the SuperScript VIRO cDNA Synthesis kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) protocol, with an incubation for 10 min at 25°C, 60 min at 42°C, and 5 min at 85°C. For real-time PCR, LightCycler 480 II (F. Hoffmann-La Roche, Basel, Switzerland) was used with a primer pair for each marker (Table 1), and the TaqMan probe method was followed for 40 to 50 cycles, where each cycle consisted of 95°C for 10 min, 95°C for 10 s, and 60°C for 20-30 s.

2.7. Statistics

Data are shown as the mean ± standard error. Comparison of the data between groups was performed using Fisher's protected least significant difference (PLSD) method. Stat View (SAS institute Inc., version 5.0) was used as a statistical software. The significance level was set as $p < 0.05$ or $p < 0.01$.

3. Results

3.1. Effects on the uterus

The weights and entities of representative uteri, 16 weeks after OVX, are shown in Figure 1. The uterine weight significantly decreased in the OVX group in comparison with the sham group. The decrease in uterine weight induced by OVX was significantly mitigated by β-estradiol administration, but no effect on the uterus was observed in the sertraline administration group.

3.2. Spontaneous activity

The results of the measurement of spontaneous activity during the dark period, 8 weeks after OVX, revealed that there was no difference in circadian rhythm. However, there was a significant decrease in total activity during the dark period in the OVX group compared with the sham group (Figure 2a).

Eight weeks after administration (16 weeks after OVX), there was no difference in spontaneous activity circadian rhythm, consistent with the observations 8 weeks after OVX. In contrast, the OVX group showed a significant decrease in total activity during the dark period in comparison with the sham group. The decrease in total activity in the OVX group was significantly improved in the sertraline and β-estradiol administration groups (Figure 2b).

3.3. Open field test

The results of the open field test revealed that there was no significant difference in the track length across all areas in the device between the different administration groups; however, the OVX group showed a decreasing trend compared with the sham group, and an improvement was observed in the sertraline and β-estradiol administration groups (Figure 3b). The ratio of the track length from the outer area to the center increased significantly in the OVX group compared with the sham group, and a tendency for the increase to be reduced was observed in the sertraline and β-estradiol administration groups (Figure 3c). The ratio of duration in the outer area to the central part was significantly increased in the OVX group in comparison with the sham group (Figure 3d). Moreover, the increase led to a significant recovery in the sertraline and β-estradiol administration groups (Figure 3d).

3.4. mRNA expression levels of TPH, 5-HTT, and XBP1

Changes in mRNA expression levels in the mouse

Table 1. List of all primers used for real-time PCR

Gene	Universal Probe Livrary Probe No.	Sequence (5'→3')
GAPDH	#9	Forward:agcttgtcatcaacgggaag Reverse: tttgatgttagtggggtctcg
TPH	#21	Forward: cacagttcagatcccctctaca Reverse: gaacgtggcctaggagttca
5-HTT	#62	Forward: acctggacactccattccac Reverse: cctggagtccctttgactga
XBP1	#39	Forward: gctggatcctgacgaggtt Reverse: gccaccagccttactccac

GAPDH: *Mus musculus* glyceraldehyde 3-phosphate dehydrogenase, TPH: *Mus musculus* tryptophan hydroxylase, 5-HTT: *Mus musculus* serotonin transporter, XBP1: *Mus musculus* X box-binding protein 1.

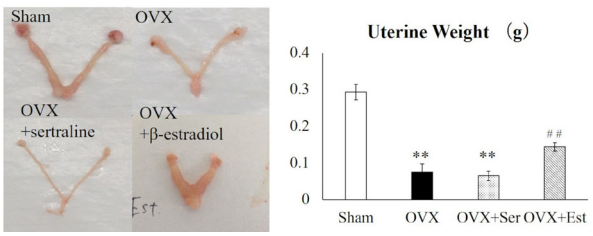

Figure 1. Effects of β-estradiol and sertraline on the uterine weight at 16 weeks. The images are of representative uteri from the different treatment groups. The data are shown as the mean ± standard error. $^{**}p < 0.01$ compared with the sham group. $^{\#\#}p < 0.01$ compared with the OVX group. Sham: sham surgery group; OVX: ovariectomy surgery group; Est: β-estradiol administration group; Ser: sertraline administration group.

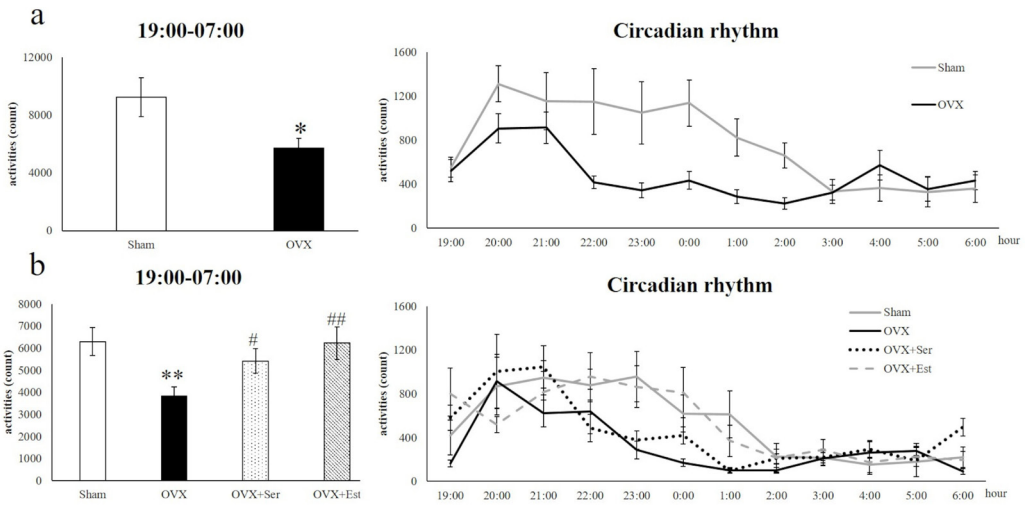

Figure 2. Spontaneous locomotor activities of mice in the sham, OVX, OVX with β-estradiol, and OVX with sertraline groups. (a) Eight weeks after ovariectomy and **(b)** 8 weeks after administration. The amount of spontaneous locomotor activities was measured from 15:00 to 10:00, and the amount of activity for 12 hours from 19:00 to 7:00 was calculated. The data are shown as the mean ± standard error. $^{**}p < 0.01$ or $^{*}p < 0.05$ compared with the sham group; $^{\#\#}p < 0.01$ or $^{\#}p < 0.05$ compared with the OVX group. Sham: sham surgery group; OVX: ovariectomy surgery group; Est: β-estradiol administration group; Ser: sertraline administration group.

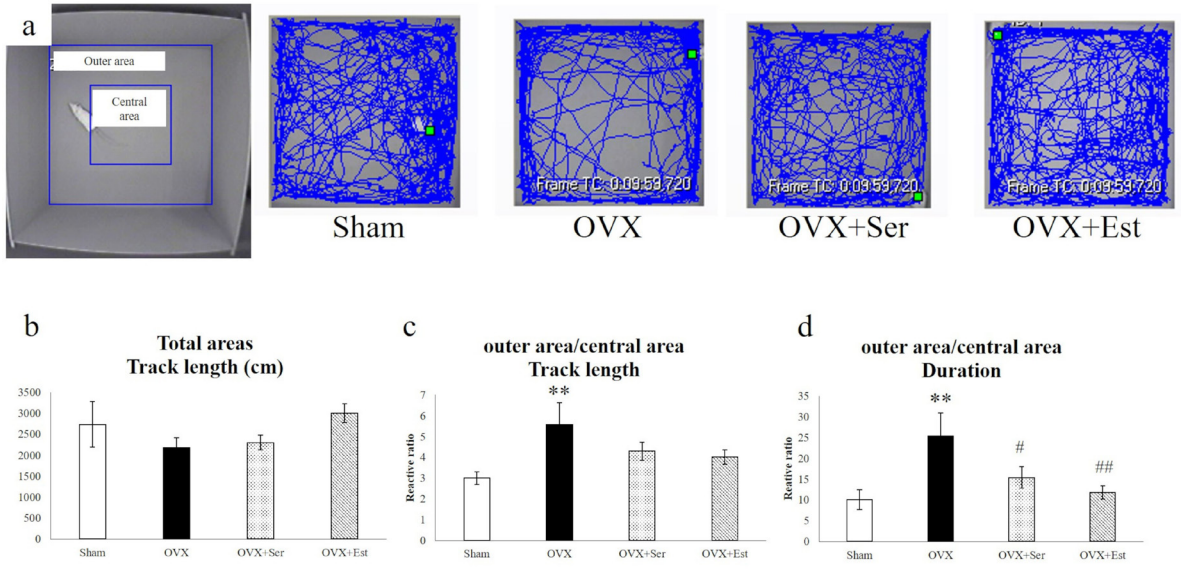

Figure 3. Open field test of mice in the sham, OVX, OVX with β-estradiol, and OVX with sertraline groups. (a) The images of representative from the different treatment, **(b)** Track length in total areas, **(c)** the ratio of the track length (outer area / central area) and **(d)** the ratio of the duration (outer area/central area). The data are shown as the mean ± standard error. $^{**}p < 0.01$ compared with the sham group; $^{\#\#}p < 0.01$ or $^{\#}p < 0.05$ compared with the OVX group. Sham: sham surgery group; OVX: ovariectomy surgery group; Est: β-estradiol administration group; Ser: sertraline administration group.

hippocampus and frontal lobe were examined by real-time PCR. In the hippocampus, the *TPH* gene expression level was significantly decreased in the OVX group compared with the sham group; this decrease was significantly counteracted in the sertraline administration group but no effect was noted in the β-estradiol administration group (Figure 4a). No significant difference in the *TPH* gene expression level in the frontal lobe was noted (Figure 4b). While the *5-HTT* gene expression levels showed a decreasing trend in both the hippocampus and frontal lobe in the OVX group in comparison with the sham group, no significant differences were noted, and the β-estradiol administration group showed a significant increase compared with the OVX group. The *XBP1* gene expression levels were significantly higher in the hippocampus and frontal lobe in the OVX group compared with the sham group; this increase was significantly diminished in the sertraline

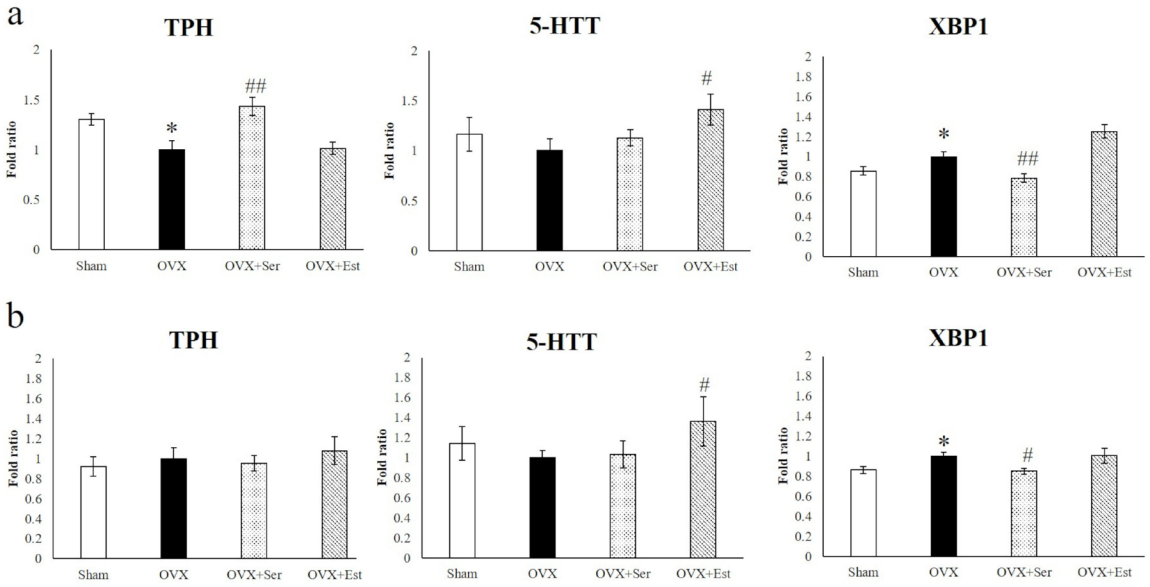

Figure 4. Effects of sertraline on the mRNA expression levels of TPH, 5-HTT, and XBP1. Expression levels in the **(a)** hippocampus and **(b)** prefrontal cortex. The data are shown as the mean ± standard error. $^{*}p < 0.05$ compared with the sham group; $^{\#\#}p < 0.01$ or $^{\#}p < 0.05$ compared with the OVX group. Sham: sham surgery group; OVX: ovariectomy surgery group; Est: β-estradiol administration group; Ser: sertraline administration group.

administration group, but no effect was observed in the β-estradiol administration group (Figure 4).

4. Discussion

4.1. Influence on the uterine

The present study focused on the effects of sertraline, a SSRI, on psychiatric symptoms specific to women, using OVX model mice. In current clinical practice, HRT is mainly performed for the menopausal disorders in women. However, it has been reported that estrogen administration induced uterine cancer and breast cancer (*5,6*). In the present study, the uterine of OVX model group showed a significant atrophy compared to that of the sham group. Moreover the atrophy was significantly enlarged after administration of β-estradiol (Figure 1). And also our study found out the hyperplasia of uterine in the β-estradiol treatment group. The previously reported paper supported that the continuous administration of β-estradiol cause the hyperplasia in rats (*17*). However, administration of sertraline showed no effect on the uterus, pathologically. These results suggest that administration of sertraline does not cause any risks to the uterus but not β-estradiol administration.

4.2. Behavioral experiments

We previously reported that the spontaneous activity in OVX model rats decreased during the dark period as measured for 24 hours (*10*). Similarly, the present study using mice showed a significant decrease in spontaneous activity during the dark period was observed for all OVX mice (n = 24) 8 weeks after OVX (Figure 2a). This data also showed that the decreased estrogen due to OVX reduced the amount of activity during the active period (dark period) in mice. We have reported that the treatment of fluvoxamine, another SSRI, just after OVX significantly inhibited the reduction of spontaneous behavior in rats induced by OVX (*11*). Saloua *et al.* reported that administration of sertraline just after OVX induced antidepression-like behavior (*18*). These reports have shown that SSRIs have been administered at the same time as OVX surgery and have a preventive effect. However, in clinically, SSRIs are clinically used after symptoms of depression during menopause was occurred. In this study, β-estradiol and sertraline administrated 8 weeks after OVX and its therapeutic effect was examined. The result showed that the decrease in spontaneous activity due to OVX was significantly recovered by the administration of either β-estradiol or sertraline for 8 weeks (Figure 2b). Few reports are available on administration from a state in which the amount of the spontaneous activity was reduced by OVX, and the results of this study are new findings. These results suggest that the recovery of decreased spontaneous behavior induced by OVX relates to serotonergic neuron.

In the open field test, OVX showed a significant increase in the amount of outer area activity (track length and duration) (Figure 3c). Administration of either β-estradiol or sertraline significantly suppressed decrease of duration in outer area by OVX (Figure 3d). The open field test is one of the widely used methods for measuring anxiety-related emotional behavior (*19*).

The mouse prefers to stay adjacent to the wall and move around the outer area, which is described "thigmotaxis". It is reported thigmotaxis significantly increases as anxiety levels rise in mice (*19,20*). This study showed that sertraline suppress increase of thigmotaxis due to OVX. These results suggest that sertraline improved anxiety-like behavior in OVX model mice.

In the present study, sertraline improved the behavioral changes caused by OVX in both the spontaneous activity and open field tests. As a result, sertraline appeared to ameliorate psychiatric symptoms such as anxiety, depression, and fatigue induced by decreased estrogen levels. Benmansour *et al.* also reported that sertraline administration, initiated at the same time as OVX surgery in OVX model rats, resulted in improved behavioral changes induced by OVX after 2 weeks of administration (*21*). In the present study, we investigated sertraline administration after decreased spontaneous activity due to OVX, and the results revealed that sertraline has a therapeutic effect on estrogen-dependent behavioral changes in OVX model mice.

4.3. mRNA levels for related the serotonergic cell functions in the brain

The involvement of brain monoamine neurotransmitters such as noradrenaline and serotonin has been reported in the case of psychiatric symptoms such as depression and anxiety induced by decreased estrogen levels (*22,23*). We previously showed that serotonin release in the amygdala is decreased in OVX model rats and that the serotonin nervous system is involved in the decrease in spontaneous activity induced by OVX (*10,11*). In the present study, the *TPH* gene expression level in the hippocampus was decreased by OVX, and sertraline significantly inhibited this decrease (Figure 4a). However, the *5-HTT* gene expression levels did not differ between the sham and OVX groups. TPH is a rate-limiting enzyme for serotonin synthesis, catalyzing the conversion of l-tryptophan to 5-hydroxytryptophan. It is well known that sertraline increases the serotonin concentration in the synaptic cleft by selectively inhibiting serotonin reuptake, and Kim *et al.* report that long-term administration of sertraline increases TPH mRNA and protein levels (*15*). The results of the present study are consistent with this report, suggesting that long-term administration of sertraline may increase serotonin release by increasing serotonin synthesis.

Sertraline significantly inhibited the OVX-induced increase in the expression of the *XBP1* gene, which is involved in the ER stress response and unfolded protein response (UPR), in both the hippocampus and frontal lobe (Figure 4). ER stress has been implicated in depression-like symptoms, and it has been reported that ER stress-related gene expression levels increase in the hippocampus of rats subjected to restraint stress or electric shock (*16,24*). Moreover, the relationship between ER stress and estrogen has been reported (*25,26*), but there have been no reports on UPR in OVX model mice. In the present study, OVX significantly increased the gene expression level of *XBP1*, suggesting that a decrease in estrogen may induce an excessive ER stress response. Sertraline administration significantly inhibited the OVX-induced increase in *XBP1* gene expression. These results suggest that UPR may well be involved in improvement the OVX-induced decrease spontaneous activity by sertraline, but there are some limitations in this study. UPR pathway is under the control of the PKR-like endoplasmic reticulum kinase (PERK) pathway, inositol requiring enzyme-1-XBP1 pathway, and activating transcription factor (ATF) 6 pathway. Further studies also need to analysis of other ER stress and UPR markers such as C/EBP homologous protein, ATF6, PERK, glucose-regulated protein 78.

In conclusion, the present study demonstrated that sertraline ameliorates the behavioral changes induced by the decrease of estrogen levels and that sertraline can expect as therapeutic effects on OVX model mice. In addition, these results suggest that sertraline improves suppressing serotonin synthesis in the serotonergic neuron in the hippocampus.

Funding: None.

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Newhouse P, Albert K. Estrogen, stress, and depression: A neurocognitive model. JAMA Psychiat. 2015; 72:727-729.
2. Schiller CE, Johnson SL, Abate AC, Schmidt PJ, Rubinow DR. Reproductive steroid regulation of mood and behavior. Compr Physiol. 2016; 6:1135-1160.
3. Morgan MA, Pfaff DW. Effects of estrogen on activity and fear-related behaviors in mice. Horm Behav. 2001; 40:472-482.
4. Maclennan AH, Broadbent JL, Lester S, Moore V. Oral oestrogen and combined oestrogen/progestogen therapy versus placebo for hot flushes. Cochrane Db Syst Rev. 2004; 4:CD002978.
5. Beral V, Bull D, Reeves G. Endometrial cancer and hormone-replacement therapy in the Million Women Study. Lancet. 2005; 365:1543-1551.
6. Samavat H, Kurzer MS. Estrogen metabolism and breast cancer. Cancer Lett. 2015; 356:231-243.
7. Weiss SJ, Simeonova DI, Kimmel MC, Battle CL, Maki PM, Flynn HA. Anxiety and physical health problems increase the odds of women having more severe symptoms of depression. Arch Women Ment Hlth. 2016; 19:491-499.
8. Nomura M, Akama KT, Alves SE, Korach KS, Gustafsson JA, Pfaff DW, Ogawa S. Differential distribution of estrogen receptor (ER)-alpha and ER-beta in the midbrain raphe nuclei and periaqueductal gray in male mouse: Predominant role of ER-beta in midbrain serotonergic systems. Neuroscience. 2005; 130:445-456.

9. Hiroi R, McDevitt RA, Neumaier JF. Estrogen selectively increases tryptophan hydroxylase-2 mRNA expression in distinct subregions of rat midbrain raphe nucleus: Association between gene expression and anxiety behavior in the open field. Biol Psychiat. 2006; 60:288-295.
10. Izumo N, Ishibashi Y, Ohba M, Morikawa T, Manabe T. Decreased voluntary activity and amygdala levels of serotonin and dopamine in ovariectomized rats. Behav Brain Res. 2012; 227:1-6.
11. Ishibashi Y, Izumo N, Iwata K, Morikawa T, Kameyama T, Watanabe Y, Manabe T, Matsuzaki H. Fluvoxamine reverses estrogen-dependent decline in voluntary activitise and decreased and amygdala levels of serotonin in ovariectomized rats. J Brain Sci. 2016; 46:5-19.
12. Altshuler LL, Cohen LS, Moline ML, Kahn DA, Carpenter D, Docherty JP. The expert consensus guideline series. Treatment of depression in women. Postgrad Med. 2001; 1-107.
13. Kornstein SG, Schatzberg AF, Thase ME, Yonkers KA, McCullough JP, Keitner GI, Gelenberg AJ, Davis SM, Harrison WM, Keller MB. Gender differences in treatment response to sertraline versus imipramine in chronic depression. Am J Psychiatry. 2000; 157:1445-1452.
14. Ma L, Xu Y, Jiang W, Li Y, Zhang X, Wang G, Li R. Sex differences in antidepressant effect of sertraline in transgenic mouse models. Front Cell Neurosci. 2019; 13:1-11.
15. Kim SW, Park SY, Hwang O. Up-regulation of tryptophan hydroxylase expression and serotonin synthesis by sertraline. Mol Pharmacol. 2002; 61:778-785.
16. Timberlake M, Prall K, Roy B, Dwivedi Y. Unfolded protein response and associated alterations in toll-like receptor expression and interaction in the hippocampus of restraint rats. Psychoneuroendocrino. 2018; 89:185-193.
17. Zhu L, Pollard JW. Estradiol-17beta regulates mouse uterine epithelial cell proliferation through insulin-like growth factor 1 signaling. Proc Natl Acad Sci U S A. 2007; 104:15847-15851.
18. Saloua B, Opeyemi SA, Anthony AP, Alan F. Effects of long-term treatment with estradiol and estrogen receptor subtype agonists on serotonergic function in ovariectomized rats. Neuroendocrinology. 2016; 103:269-281.
19. Seibenhener ML, Wooten MC. Use of the open field maze to measure locomotor and anxiety-like behavior in mice. J Vis Exp. 2015; 96:e52434.
20. Kraeuter AK, Guest PC, Sarnyai Z. The open field test for measuring locomotor activity and anxiety-like behavior. Methods Mol Biol. 2019; 1916:99-103.
21. Benmansour S, Adeniji OS, Privratsky AA, Frazer A. Effects of long-term treatment with estradiol and estrogen receptor subtype agonists on serotonergic function in ovariectomized rats. Neuroendocrinology. 2016; 103:269-281.
22. Borrow AP, Cameron NM. Estrogenic mediation of serotonergic and neurotrophic systems: implications for female mood disorders. Prog Neuro-Psychoph. 2014; 54:13-25.
23. Gu S, Jing L, Li Y, Huang JH, Wang F. Stress induced hormone and neuromodulator changes in menopausal depressive rats. Front Psychiatry. 2018; 9:1-8.
24. Timberlake M, Dwivedi Y. Altered expression of endoplasmic reticulum stress associated genes in hippocampus of learned helpless rats: Relevance to depression pathophysiology. Front Pharmacol. 2016; 6:319.
25. Crider A, Nelson T, Davis T, Fagan K, Vaibhav K, Luo M, Kamalasanan S, Terry AV Jr, Pillai A. Estrogen receptor β agonist attenuates endoplasmic reticulum stress-induced changes in social behavior and brain connectivity in mice. Mol Neurobiol. 2018; 55:7606-7618.
26. Raina K, Noblin DJ, Serebrenik YV, Adams A, Zhao C, Crews CM. Targeted protein destabilization reveals an estrogen-mediated ER stress response. Nat Chem Biol. 2014; 10:957-962.

Received December 29, 2020; Revised January 17, 2021; Accepted February 14, 2021.

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Released online in J-STAGE as advance publication February 24, 2021.