Effects of gonadal status and the estrogen milieu on hypothalamic oxytocin gene expression and serum oxytocin levels in female rats

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ARTICLE INFO

ABSTRACT

Oxytocin (OT) and its receptor (OTR) play various roles in the central and peripheral regulation of appetite and body weight. Previously, we have shown that the administration of OT markedly decreased appetite and body weight gain in ovariectomized (OVX) obese rats. In addition, recent studies have shown that the endogenous OT system is also affected by endogenous or exogenous estrogen. In this study, we showed that ovariectomy decreased rats' hypothalamic OT/OTR mRNA and serum OT levels, but did not affect their visceral fat OTR mRNA levels. The chronic administration of estradiol (E2) abrogated these ovariectomy-induced changes; i.e., it increased the rats' hypothalamic OT/OTR mRNA and serum OT levels, and may be associated with reductions in food intake and body weight gain. In addition, acute E2 administration increased the rats' hypothalamic OTR mRNA and serum OT levels, but did not affect their hypothalamic OT mRNA levels. Taken together, these results suggest that endogenous OT and/or OTR expression might be positively regulated by E2 and that the suppressive effects of E2 on appetite and body weight gain might be mediated, at least in part, by the OT system. Thus, we consider that OT might be a target hormone to pursue subsequent interventions of menopause for menopause-induced metabolic disorders.

1. Introduction

It has been revealed that oxytocin (OT), which is a 9-amino acid neuropeptide, plays various roles in the central and peripheral regulation of metabolism, appetite, and body weight (McCormack et al., 2020). Oxytocin receptor (OTR)-deficient mice exhibit late-onset obesity accompanied by increased amounts of visceral fat, and the intracerebroventricular (i.c.v.) injection of an OT antagonist increased food intake in mice, suggesting that endogenous OT may help to prevent obesity by modulating appetite (Takayanagi et al., 2008; Zhang et al., 2011). In addition, i.c.v., intraperitoneal (i.p.), or subcutaneous injection of OT and the intranasal administration of OT have been shown to decrease food intake in experimental animals and humans (McCormack et al., 2020), indicating that exogenous OT also affects central appetite regulation systems. Moreover, exogenous OT directly and indirectly promoted lipolysis in adipose tissue via the OTR and decreased fat mass in the experimental animals and humans (McCormack et al., 2020). Interestingly, it has been reported that such effects of exogenous OT on

appetite and fat mass are more prominent in obese individuals (Altirriba et al., 2014; Maejima et al., 2017; Plante et al., 2015) and that chronic OT administration alleviated leptin resistance in diet-induced obesity (DIO) mice (Labyb et al., 2019; Lawson et al., 2020). In contrast, it has been shown that leptin administration activated hypothalamic OT neurons in both normal and DIO rats (Blevins et al., 2004; Perello and Raingo, 2013). Although these findings suggest that OT might interact with leptin to regulate appetite and body weight appropriately, it remains unclear whether the interactions are a pharmacological phenomenon or ones that have physiological importance.

The menopausal loss of ovarian function induces hyperphagia and decreased energy expenditure (Lovejoy et al., 2008), and these changes can cause various metabolic disorders, such as obesity, diabetes, and hyperlipidemia (Carr et al., 2004; Ferrara et al., 2002; Palmisano et al., 2017; dos Reis et al., 2003; Tchernof and Despres, 2000). Some of these menopause-induced changes in humans, such as hyperphagia and obesity, can be reproduced in ovariectomized (OVX) rodents (Liang et al., 2002; Meli et al., 2004; Richard, 1986; Rogers et al., 2009; Wade

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and Gray, 1979). Although estrogen replacement might be an effective treatment for these conditions, the long-term use of estrogen can have adverse effects (Rossouw et al., 2002). As OT has more marked effects in obese individuals (Altirriba et al., 2014; Maejima et al., 2017; Plante et al., 2015; Thienel et al., 2016), we considered that OT may be useful for menopause-induced metabolic disorders. Thus, in previous studies we evaluated the effects of the peripheral administration of OT on body weight, appetite, adiposity, and central and peripheral metabolism-related factors in OVX rats and aged female rats with irregular estrous cycle (Iwasa et al., 2019; Erdenebayar et al., 2021). It was found that the administration of OT attenuated appetite and body weight gain and decreased the amount of body fat without having any apparent adverse effects. Similarly, it has been shown that the daily administration of OT normalizes body weight and fat deposition, and reverses osteopenia, in ovariectomized mice (Beranger et al., 2014). On the other hand, none of these studies evaluate whether loss of ovarian function affects endogenous OT levels.

Therefore, in the present study the relationships between ovarian status/the estrogen milieu and endogenous OT/OTR were evaluated. Firstly, we examined the effects of ovariectomy on hypothalamic OT gene expression and serum OT levels in female rats. Secondly, we investigated the effects of chronic estrogen administration on these factors in OVX rats to assess the underlying causes of ovariectomy-induced changes. Thirdly, we evaluated the effects of acute estrogen administration in OVX rats to confirm the direct effects of estrogen in rats with similar body weights. We also measured serum leptin levels in the first and second experiments because, as noted above, it has been reported that leptin might activate hypothalamic OT neurons (Perello and Raingo, 2013).

2. Materials and methods

2.1. Animals

Eight- to 9-week-old Wistar female rats were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan), and housed in a room under controlled temperature (24 °C) and light (12 h light, 12 h darkness; lights turned on at 0800 and turned off at 2000) conditions with free access to food and water. In total, 40 rats were used in this study. After a one-week habituation period, surgery was performed (at 9–10 weeks of age). Ovariectomy was carried out under sodium pentobarbital-induced anesthesia (60–80 mg/kg, i.p.). Alternatively, a sham operation, in which the ovaries were touched with forceps, was performed. After undergoing surgery, the rats were individually housed. The implantation of the estradiol (E2)-containing tubes and tissue sampling were carried out under sevoflurane-induced anesthesia. All animal experiments were conducted in accordance with the ethical standards of the institutional animal care and use committee of the University of Tokushima.

2.2. Effects of ovariectomy on central and peripheral OT levels

Eleven rats were randomly assigned to either the OVX or sham-operated (sham) group (n = 5 or 6 per group) at 10 weeks of age. At 6 weeks after surgery (at 16 weeks of age), the rats were weighed, anesthetized with sevoflurane and then decapitated. Thereafter, the weights of the rats' visceral (the parametrial, perirenal, and mesenteric depots) and subcutaneous fat (the inguinal depot) depots were measured, and the brain, blood, and a piece (approximately 300–400 mm^3) of visceral (parametrial) fat were collected from each rat. Serum was separated by centrifugation and stored at −20 °C, and tissue samples were stored at −80 °C.

2.3. Effects of chronic E2 administration in OVX rats

Fourteen rats were OVX and then weighed and randomly assigned to

groups that were (OVX + E2) or were not administered E2 (OVX) (n = 7 per group) at four weeks after the ovariectomy procedure (at 13 weeks of age). In the OVX + E2 group, a silastic tube (inner diameter: 3 mm, outer diameter: 5 mm, length of the filled part: 3 mm) (As One Co., Ltd., Tokyo, Japan) filled with crystalline 17-β estradiol (Sigma, St. Louis, MO, USA) was implanted into each rat under sevoflurane anesthesia, whereas in the OVX group an empty tube was implanted into each rat. It has been shown that this protocol maintains physiological serum E2 levels for at least three weeks in rats (Le et al., 2014). Two weeks after the implantation procedures (at 15 weeks of age), body weight and cumulative food intake measurements were obtained prior to sacrifice. A fixed amount of normal food was placed in the food receptacle at the top of the cage, and the weight of the remaining food was measured. In addition, the wood-chip bedding was changed 7 days after implantation, and any bits of food in the bedding were collected at this time point and after sacrifice. Their weights were added to the remaining food weight. As the rats' body weights at tube implantation were highly variable and food intake might be affected by individual body weight, cumulative food intake values that had been normalized based on body weight at implantation were used for comparison. Thereafter, rats were anesthetized with sevoflurane and decapitated. The visceral (the parametrial, perirenal, and mesenteric depots) and subcutaneous fat (the inguinal depots) depots of the rats were weighed, and the brain, blood, and samples of parametrial and subcutaneous fat (approximately 300–400 mm^3) were collected from each rat. The samples were stored as noted above.

2.4. Effects of acute E2 administration in OVX rats

Fifteen rats were OVX and then were weighed and randomly assigned to groups that were (OVX + E2) or were not (OVX) administered E2 (n = 7 or 8 per group) at four weeks after the ovariectomy procedure (at 13 weeks of age). In the OVX + E2 group, the rats were subcutaneously injected with E2 (10 μg/0.1 mL peanut oil) daily for 2 consecutive days at 0700, whereas the rats in the OVX group were injected with peanut oil alone. This E2 injection schedule was used in our previous study (Iwasa et al., 2014). At 6 h after the second injection, body weight measurements were obtained, and then the rats were sacrificed by decapitation under sevoflurane-induced anesthesia. The brain and blood were collected from each rat, and the samples were stored as noted above.

2.5. Hormone assay

The rats' serum E2 levels were measured by a commercial laboratory (SRL, Tokyo, Japan) using an electrochemiluminescence immunoassay (Roche Diagnostics GmbH). Their OT levels were measured by commercial laboratories (ASKA Pharma Medical Co., Ltd., Kanagawa, Japan) using a chemiluminescent enzyme immunoassay. In brief, serum was diluted with mixture of buffer containing bovine serum albumin and 0.1% trifluoroacetic acid and extracted using solid phase extraction column. Thereafter, elution was evaporated and reconstituted. Standards or test samples are added to goat anti-rabbit IgG antibody precoated well, along with alkaline phosphatase conjugated-oxytocin and rabbit antibody specific to oxytocin. After washing, chemiluminescent assay of alkaline phosphatase was carried out. The limit of detection of the whole system of measurement including extraction operation is 7.5 pg/mL, and intra- and inter-assay coefficient of variation are 3.5–8.9% and 4.7–5.0%, respectively. Cross reactions with [Arg]-vasopressin and neurophysin 1 are 0.08% and 0%, respectively. Each sample was measured in duplicate. The samples used for the E2 and OT measurements both had volumes of 250 μl.

2.6. Quantitative real-time polymerase chain reaction (PCR)

Whole hypothalamic explants were dissected out via an anterior

coronal cut at the anterior border of the optic chiasm, a posterior cut at the posterior border of the mammillary bodies, parasagittal cuts along the hypothalamic fissures, and a dorsal cut 2.5 mm from the ventral surface. Total RNA was isolated from the hypothalamic explants using a TRIzol® reagent kit (Invitrogen Co., Carlsbad, CA, USA) and an RNeasy® mini kit (Qiagen GmbH, Hilden, Germany). Then, cDNA was synthesized with oligo (deoxythymidine) primers at 50 °C using the SuperScript III first-strand synthesis system for real-time PCR (Invitrogen Co.). The PCR analysis was performed using the StepOnePlus™ real-time PCR system (PE Applied Biosystems, Foster City, CA, USA) and FAST SYBR® green. The mRNA levels of OT and the OTR were measured. The mRNA expression level of each factor was normalized to that of GAPDH or 18S rRNA. Dissociation curve analysis was also performed for each gene at the end of the PCR. Each amplicon generated a single peak. The relevant primer sequences, product sizes, and annealing temperatures are shown in Table 1. The PCR conditions were as follows: initial denaturation and enzyme activation were performed at 95 °C for 20 s, followed by 45 cycles of denaturation at 95 °C for 3 s, and annealing and extension for 30 s.

2.7. Statistical analyses

All results are presented as mean ± standard error of the mean (SEM) values. Student's *t*-test for parametric data or the Mann-Whitney *U* test for non-parametric data was used for comparisons between groups. p-Values of <0.05 were considered significant. Cohen's d (small effect = 0.2, medium effect = 0.5, large effect = 0.8) and r (small effect = 0.1, medium effect = 0.3, large effect = 0.5) are reported when analyses were undertaken by Student's *t*-test and the Mann-Whitney U test. The Spearman's rank correlation test was used for correlation analyses.

3. Results

3.1. Effects of ovariectomy on central and peripheral OT levels

At the sampling point, the rats in the OVX group were significantly heavier than those in the sham group (Table 2). Similarly, the subcutaneous fat weight to body weight (SF:BW) ratio was significantly greater in the OVX group than in the sham group, whereas the visceral fat weight to body weight (VF:BW) ratio did not differ between the OVX and sham groups. The mean serum E2 level of the OVX group was significantly lower than that of the sham group.

The hypothalamic OT and OTR mRNA expression levels of the OVX group were significantly lower than those of the sham group (OT; 1.0 ± 0.05 vs 1.34 ± 0.14, $p < 0.05$, $r = 0.61$, OTR; 1.0 ± 0.06 vs 1.37 ± 0.17, $p < 0.05$, $r = 0.72$), whereas the OTR mRNA expression levels in visceral fat did not differ between the OVX and sham groups (1.0 ± 0.15 vs 0.77 ± 0.29, $p = 0.27$, $r = 0.33$) (Fig. 1A). The mean serum OT level of the OVX group was significantly lower than that of the sham group (16.1 ± 5.6 vs 70.2 ± 20.1, $p < 0.05$, $d = 1.43$), whereas the mean serum leptin level of the OVX group was significantly higher than that of the sham group (12.9 ± 1.5 vs 4.67 ± 0.77, $p < 0.05$, $d = 0.83$). There weren't correlations between hypothalamic OT mRNA level and serum OT and leptin levels (Fig. 1B, C).

Table 1
Primer sequences, product sizes and annealing temperature.

Primer	Sequence	Annealing T (°C)
OT forward	GAACACCAACGCCATGGCCTGCCC	62
OT reverse	TCGGTGCGGCAGCCATCCGGGCTA	
OTR forward	CGATTGCTGGGCGGTCTT	67
OTR reverse	CCGCCGCTGCCGTCTTGA	
GAPDH forward	ATG GCA CAG TCA AGG CTG AGA	70
GAPDH reverse	CGC TCC TG GAA GAT GGT GAT	
18S rRNA forward	GAC GGA CCA GAG CGA AAG C	64
18S rRNA reverse	AAC CTC CGA CTT TCG TTC TTG A	

Table 2
Body weight, visceral and subcutaneous fat weight, and serum E2 level in OVX or Sham operated rats.

	OVX	Sham
Body weight at sampling (g)	407.2 ± 11.2**	328.6 ± 10.4
Visceral fat weight (g/100 g body weight)	4.4 ± 0.3	4.4 ± 0.2
Subcutaneous fat weight (g/100 g body weight)	1.9 ± 0.1**	1.1 ± 0.1
Serum E2 level (pg/mL)	53.4 ± 29.0	347.2 ± 120.7*

* $P < 0.05$.

** $P < 0.01$ vs Sham group.

3.2. Effects of chronic E2 administration in OVX rats

Body weight at the sampling point and the body weight change induced by the chronic administration of E2 were significantly lower in the OVX + E2 group than in the OVX group (Table 3). During the administration of E2, body weight decreased in the OVX + E2 group, whereas it increased in the OVX group. Similarly, cumulative food intake was lower in the OVX + E2 group than in the OVX group. The VF: BW and SF:BW ratios were greater in the OVX group than in the OVX + E2 group. The mean serum E2 level of the OVX + E2 group was significantly higher than that of the OVX group, indicating that the implanted E2-containing tubes worked properly.

The hypothalamic OT and OTR mRNA expression levels of the OVX + E2 group were significantly higher than those of the OVX group (OT; 1.88 ± 0.13 vs 1.0 ± 0.09, $p < 0.01$, $d = 3.00$, OTR; 2.09 ± 0.16 vs 1.0 ± 0.05, $p < 0.01$, $r = 0.84$), whereas no differences in the OTR mRNA expression levels in visceral (0.47 ± 0.18 vs 1.0 ± 0.97, $p = 0.60$, $d = 0.29$) or subcutaneous fat (1.18 ± 0.42 vs 1.0 ± 0.53, $p = 0.80$, $d = 0.14$) were seen between the OVX + E2 and OVX groups (Fig. 2A). The mean serum OT level of the OVX + E2 group was significantly higher than that of the OVX group (42.6 ± 10.7 vs 12.7 ± 2.4, $p < 0.05$, $d = 1.46$), whereas the mean serum leptin level of the OVX + E2 group was lower than that of the OVX group (6.40 ± 0.66 vs 13.1 ± 1.5, $p < 0.01$, $d = 2.16$). There weren't correlations between hypothalamic OT mRNA level and serum OT and leptin levels (Fig. 2B, C).

3.3. Effects of acute E2 administration in OVX rats

Body weight at sampling and the body weight change that occurred between the point at which the first dose of E2 was administered and the sampling point did not differ between the OVX + E2 and OVX groups (Table 4). The mean serum E2 level of the OVX + E2 group was significantly higher than that of the OVX group. The hypothalamic OT mRNA levels of the OVX + E2 and OVX groups did not differ significantly (0.97 ± 0.11 vs 1.0 ± 0.07, $p = 0.85$, $d = 0.1$), while the mean hypothalamic OTR mRNA level of the OVX + E2 group was significantly higher than that of the OVX group (1.69 ± 0.10 vs 1.0 ± 0.05, $p < 0.01$, $d = 3.18$) (Fig. 3). The mean serum OT level of the OVX + E2 group was significantly higher than that of the OVX group (33.3 ± 6.4 vs 12.8 ± 1.7, $p < 0.05$, $d = 1.49$).

4. Discussion

Recently, many researchers have focused on the relationships between OT and metabolism, appetite, or body weight regulation systems. As was found in animals with DIO and obese humans, administering exogenous OT to OVX rats suppressed their appetite and body weight without having any apparent adverse effects (Iwasa et al., 2019; Erdenebayar et al., 2021). Thus, we suggest that OT is a target hormone to investigate for menopause-induced metabolic disorders. However, it is important to clarify how the cessation of ovarian function and change of
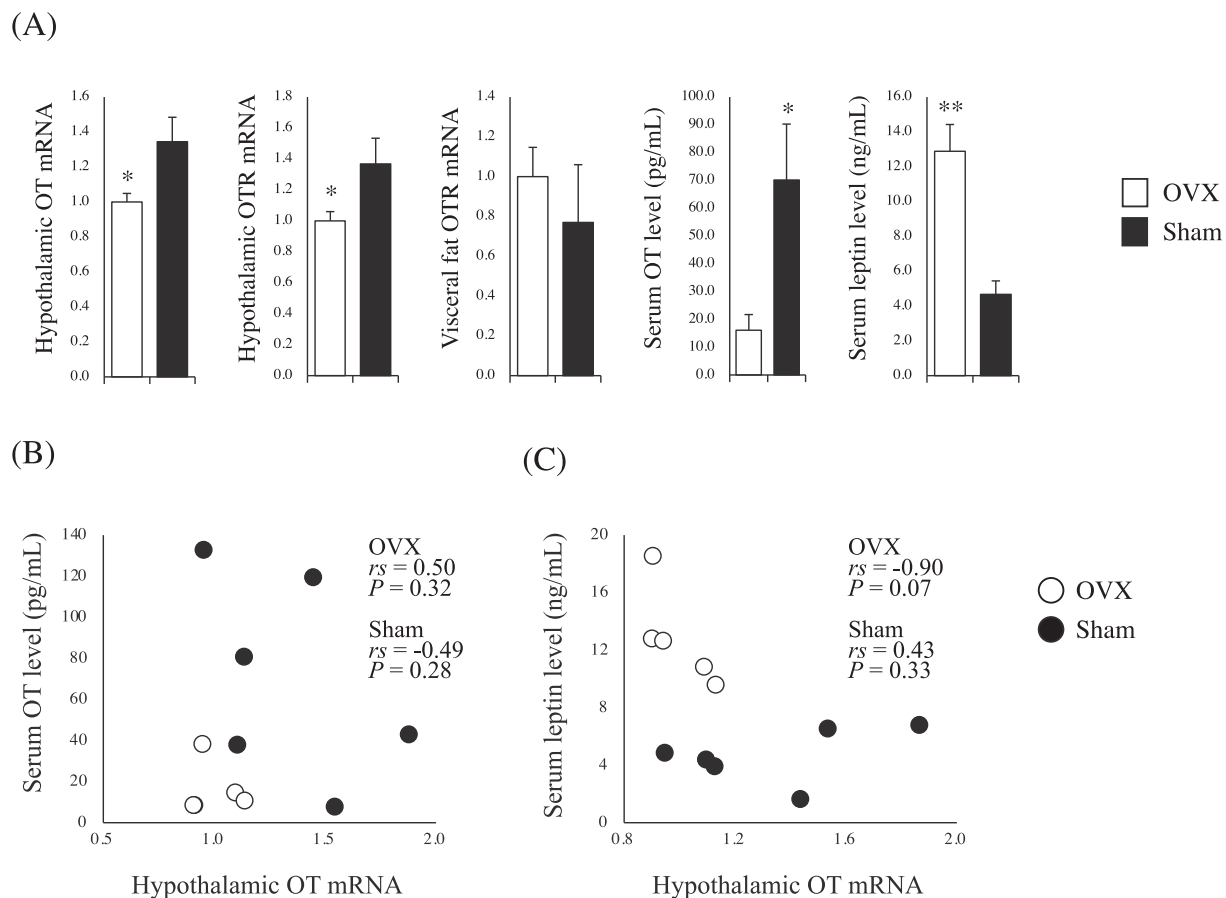
(A)

(B)   (C)

Fig. 1. (A) Oxytocin (OT) and/or oxytocin receptor (OTR) mRNA levels in hypothalamus and visceral fat, and serum OT and leptin levels in ovariectomized (OVX) (□) and sham-operated (■) rats. (B) Correlation between hypothalamic OT mRNA and serum OT levels, and (C) correlation between hypothalamic OT mRNA and serum leptin levels in OVX and sham-operated rats. The mRNA levels of OT and OTR were normalized to that of GAPDH or 18S rRNA. Data are expressed as means ± SEM. *p < 0.05, **p < 0.01.

Table 3
Body weight change, food intake, and visceral and subcutaneous fat weights in OVX rats with or without chronic E2 administration.

	OVX	OVX + E2
Body weight at implantation (g)	343.7 ± 5.1	338.0 ± 2.7
Body weight at sampling (g)	371.7 ± 6.5	305.2 ± 4.7**
Body weight as percentage of baseline (%)	108.1 ± 0.7	90.3 ± 1.2**
Cumulative food intake (g/100 g body weight at implantation)	99.7 ± 2.4	73.5 ± 1.0**
Visceral fat weight (g/100 g body weight at sampling)	3.8 ± 0.2	2.5 ± 0.1**
Subcutaneous fat weight (g/100 g body weight at sampling)	1.8 ± 0.1	1.0 ± 0.1**
Serum E2 level (pg/mL)	17.1 ± 2.0	127.4 ± 34.4**

** P < 0.01 vs OVX+E2 group.

estrogen milieu affects the endogenous OT system before evaluations of the clinical use of exogenous OT treatment are performed. Therefore, in this study, we evaluated the relationships between ovarian status/the estrogen milieu and endogenous OT/OTR expression at the peripheral and central levels.

In our first experiment, we showed that ovariectomy reduced hypothalamic OT and OTR mRNA levels and the serum OT level and caused concomitant increases in body weight and the amount of subcutaneous fat in rats. On the other hand, ovariectomy did not affect the OTR mRNA level in visceral fat. As far as we know, this is the first study to show that ovariectomy suppresses the OT system at both the central and peripheral levels. This study supports the findings of a previous human study, which showed that the serum OT levels of postmenopausal women were lower than those of premenopausal women (Maestrini et al., 2018). As described above, exogenous OT administration markedly decreased food intake and body weight gain in OVX rats in our previous study (Iwasa et al., 2019). Based on the results obtained in our present and previous studies, it can be assumed that reductions in hypothalamic and/or peripheral OT activity induce hyperphagia and obesity in OVX animals and menopausal women. Interestingly, the changing patterns of OT and OTR expression observed in OVX animals are different from those observed in other obesity models and humans. It has been shown that in mice with DIO, Zucker fatty rats, and obese humans serum OT levels are decreased (Gajdosechova et al., 2014; Qian et al., 2014; Yuan et al., 2016; Zhang et al., 2011), while OTR mRNA/protein expression are upregulated in the hypothalamus and adipose tissue (Maejima et al., 2018). In addition, as far as we know, there are no data about obesity-induced changes in hypothalamic OT levels, indicating that DIO and genetically induced obesity might not have marked effects on hypothalamic OT levels. We assume that OT is strongly associated with ovariectomy-induced obesity, in which both central and peripheral OT levels are decreased, and OTR expression is not upregulated, and suggest that exogenous OT is theoretically an ideal drug for preventing ovariectomy-induced metabolic disorders.

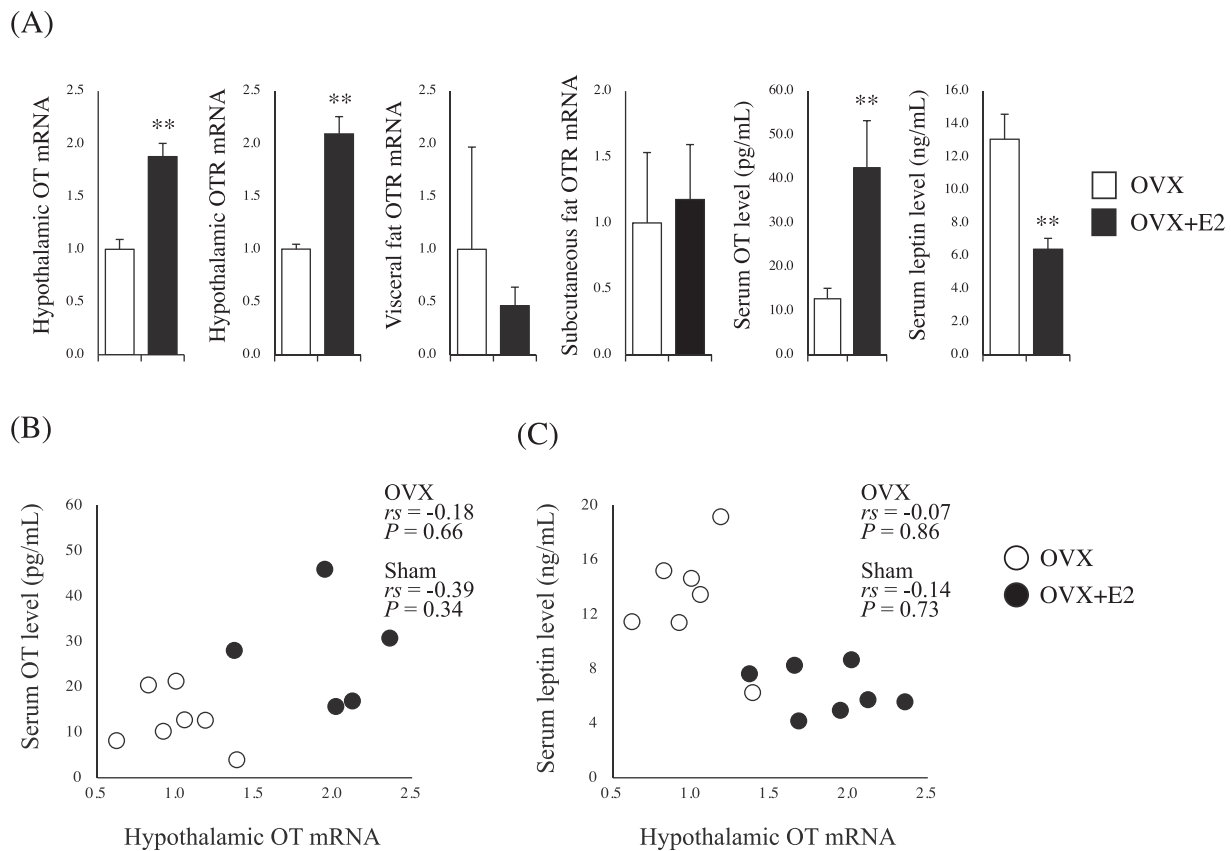In our first experiment, we could not detect any relationship between

(A)

(B)   (C)

Fig. 2. (A) Oxytocin (OT) and/or oxytocin receptor (OTR) mRNA levels in hypothalamus and visceral and subcutaneous fat, and serum OT and leptin levels ovariectomized (OVX) (□) and OVX with chronically E2-administered (■) rats. (B) Correlation between hypothalamic OT mRNA and serum OT levels, and (C) correlation between hypothalamic OT mRNA and serum leptin levels in OVX and OVX with chronically E2-administered rats. The mRNA levels of OT and OTR were normalized to that of GAPDH or 18S rRNA. Data are expressed as means ± SEM. **p < 0.01.

Table 4
Body weight change and serum E2 level in OVX rats with or without acute E2 injection.

	OVX	OVX + E2
Body weight at first injection (g)	343.1 ± 6.1	347.4 ± 6.7
Body weight at sampling (g)	351.1 ± 6.0	356.4 ± 6.8
Body weight as percentage of baseline (%)	102.3 ± 0.6	102.6 ± 0.3
Serum E2 level (pg/mL)	50.5 ± 33.8	3770.0 ± 813.2**

** P < 0.01 vs OVX+E2 group.

hypothalamic OT mRNA levels and serum OT levels. In addition, we could not detect any relationship between hypothalamic OT mRNA levels and serum leptin levels. In this experiment, tissue samples were randomly collected from rats in the sham group, and the estrous stage of the rats was not monitored. As it is possible that hypothalamic OT levels and their effects are influenced by the estrous stage (Liu et al., 2020), a more precise experiment performed under stable estrogen conditions is needed to identify the underlying causes of the observed ovariectomy-induced alterations.

In our second experiment, we showed that chronic E2 administration increased hypothalamic OT and OTR mRNA levels and serum OT levels and caused concomitant reductions in body weight, food intake, and visceral and subcutaneous fat levels. These results agree with the findings of a previous human study, which showed that the serum OT levels of women were increased by chronic and acute estrogen administration (Amico et al., 1981). On the other hand, ovariectomy did not affect the OTR mRNA levels of visceral or subcutaneous fat. These results suggest that the changes in hypothalamic OT and OTR mRNA levels and serum

OT levels observed in the OVX rats in the first experiment were caused by reduced estrogen levels. They also suggest that the suppressive effects of E2 on body weight and food intake in OVX rats may, at least in part, be mediated by upregulation of the OT system. As was demonstrated in the present study, the acute administration of E2 increased hypothalamic OT mRNA and protein levels in female rats, and the concomitant administration of an OT antagonist reversed the suppressive effects of E2 on food intake (Sloan et al., 2018). In addition, although the central administration of OT decreases food intake in male and female rats, these effects are less pronounced in females (Liu et al., 2020). Further-more, the effects of central OT administration on food intake are reduced in the proestrus stage, in which the serum E2 level is elevated (Liu et al., 2020). We assumed that central OT levels may be upregulated under hyperestrogenic conditions and that the additional effects of exogenous OT may be less marked under these conditions. As mentioned above, it has been reported that chronic OT administration alleviates leptin resistance (Labyb et al., 2019; Lawson et al., 2020) and that the administration of leptin activates centrally and peripherally projecting hypothalamic OT neurons (Blevins et al., 2004; Perello and Raingo, 2013; Velmurugan et al., 2013). Therefore, these stimulatory effects of leptin on oxytocin cell activity seem to be part of a regulatory feedback mechanism, which likely contributes to the normal homeostasis of body weight. On the contrary, hypothalamic OT mRNA levels were decreased, and serum leptin levels were increased in our second experiment. This suggests that the effects of leptin-based feedback regulation on hypothalamic and serum OT levels were not strong enough to overwhelm the effects of estrogen deficiency.

In our third experiment, we found that the acute injection of E2 increased hypothalamic OTR mRNA and serum OT levels, but did not
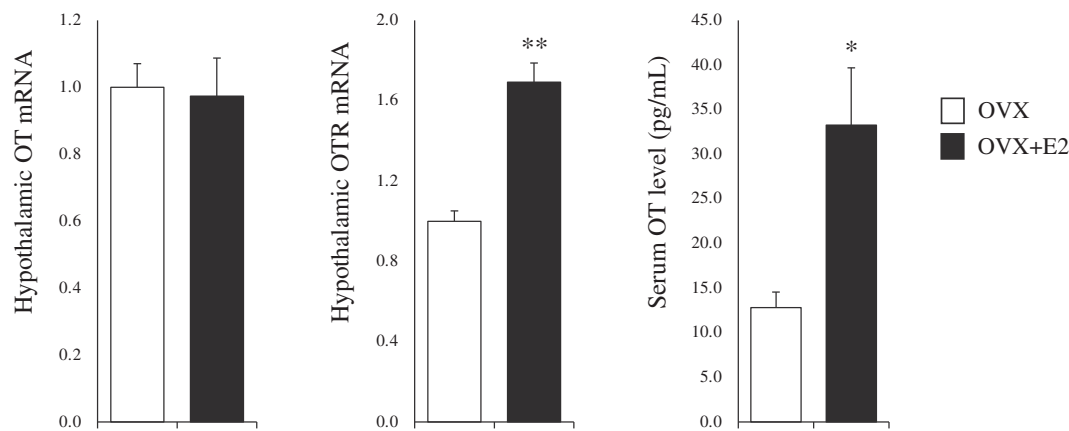
Fig. 3. Oxytocin (OT) and oxytocin receptor (OTR) mRNA levels in hypothalamus, and serum OT level in ovariectomized (OVX) (□) and OVX with acutely E2-administered (■) rats. The mRNA levels of OT and OTR were normalized to that of GAPDH. Data are expressed as means ± SEM. *p < 0.05, **p < 0.01.

alter hypothalamic OT mRNA levels. These results partially differ from those obtained in the first and second experiments in terms of the absence of changes in hypothalamic OT mRNA levels. Similarly, these results also differ from those of a previous study that showed that hypothalamic OT levels were increased at 72 h after the administration of E2 (Sloan et al., 2018). One possibility is that 30 h; i.e., the length of time from the first injection to the tissue sampling in the present study, is not long enough for E2 to exert direct effects on hypothalamic OT levels. Another possibility is that the changes in hypothalamic OT levels might be induced, at least in part, by indirect effects of E2, such as reductions in food intake and body weight gain.

Nonetheless, there are limitations to this study which must be considered. In the second and third experiments, the rats were allowed to recover for four weeks before they were subjected to chronic or acute E2 supplementation. In addition, chronic E2 administration was achieved through the implantation of an E2-containing silastic tube in the second experiment. On the other hand, in previous studies the rats were allowed a different recovery period; i.e., one week after surgery, and were subjected to different administration methods, e.g., intermittent injections that mimicked the fluctuations in estrogen levels or the implantation of E2-containing silastic tubes of a different size from those used in the present study. It is possible that these differences between the experimental protocols may have caused variations in the observed responses among studies. In addition, because whole hypothalamic blocks were used in this study, which may have precluded detecting more subtle site-specific differences of OT and OTR mRNA levels. Further precise experiments are needed to clarify these matters.

5. Conclusion

In summary, we have shown that ovariectomy decreased hypothalamic OT and OTR mRNA levels and serum OT levels and that the chronic administration of E2 abrogated these changes and might cause concomitant reductions in food intake and body weight gain. These results indicate that endogenous OT and OTR are affected by E2 and that the suppressive effects of E2 on appetite might be mediated, at least in part, by OT systems. We consider that OT may be a target hormone to pursue subsequent interventions of interventions for menopause-induced metabolic disorders.

Funding

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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