ORIGINAL

Comparison of endogenous hypothalamic and serum OT levels between young and middle-aged perimenopausal female rats

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Abstract : Oxytocin (OT) regulates food intake and body weight, particularly in obese individuals. Decreases in the effects of OT have recently been implicated in metabolic disturbances, and the administration of estradiol (E2) increased serum OT levels. Although weight gain is frequently observed in perimenopausal women, endogenous OT levels remain unclear. Therefore, we herein compared endogenous levels of hypothalamic and serum OT between young and middle-aged perimenopausal female rats and examined the relationship between serum estrogen and leptin levels. Body weight and visceral and subcutaneous fat weights were higher in middle-aged rats. Although no significant differences were observed in serum OT and E2 levels, serum leptin levels and hypothalamic mRNA levels of OT and the OT receptor (OTR) were significantly higher in middle-aged rats than in young rats. Serum OT levels did not correlate with hypothalamic OT mRNA levels or serum E2 levels. E2 maintains serum OT levels in perimenopausal rats, and other factors may elevate hypothalamic OT/OTR mRNA levels. Increases in body and fat weights in perimenopausal rats may be attributed to factors other than OT. Therefore, the administration of OT alone may not be sufficient to prevent metabolic disorders induced by the perimenopausal status. J. Med. Invest. 71 : 246-250, August, 2024

Keywords **:** *Oxytocin, obesity, perimenopause, estrogen*

INTRODUCTION

Oxytocin (OT) is a neuropeptide that is synthesized in the magnocellular neurons of the paraventricular nucleus and the supraoptic nucleus of the hypothalamus (1, 2). It induces contractions of the uterus during labor and milk ejection during lactation in females (3). Recent studies demonstrated that OT regulated metabolism, feeding behavior, and body weight in both sexes (4, 5). For example, OT receptor (OTR)-deficient mice developed late-onset obesity and increases in abdominal fat (6), and injections of an OT antagonist into the third ventricle of mice increased their food intake (7). In contrast, when exogenous OT was centrally or peripherally administered, appetite and/or body weight decreased (8, 9), lipolysis was promoted, and fat mass was reduced (5, 10). Furthermore, these effects of OT were more prominent in individuals with obesity.

The postmenopausal state is associated with weight gain, obesity, and several metabolic disorders, such as diabetes mellitus, hyperlipidemia, hypertension, and cardiovascular disease (11-14). Since ovariectomized (OVX) female rodents show similar metabolic phenotypes to menopausal women, they are often used in research as a menopausal model (15). We previously reported reductions in the hypothalamic gene expression of OT and OTR and serum levels of OT in OVX female rats and demonstrated that the administration of exogenous OT induced decreases in food intake, body weight gain, and fat mass (16). These findings implicate a decrease in the effects of OT in OVX-induced metabolic disturbances and indicate the potential of OT as a candidate anti-obesity drug for postmenopausal women. In another study, we found that supplementation with estradiol (E2) restored hypothalamic and serum OT levels in OVX rats, indicating a relationship between estrogen deficiency and decreases in the effects of OT under postmenopausal conditions (17). Moreover, we showed that food intake in ovary-intact old rats, a model of perimenopausal women, was reduced following the administration of OT, whereas body weight remained unchanged, suggesting no significant differences in the effects of exogenous OT between perimenopausal and postmenopausal women (18). In contrast, in OVX rats, central and peripheral OT levels and the relationship between OT and estrogen levels in perimenopausal rats remain unclear. In addition, the administration of exogenous leptin, an anorectic factor, activated hypothalamic OT (19). Based on these findings, the present study investigated the endogenous levels of hypothalamic and serum OT in perimenopausal female rats and examined the relationship between serum estrogen and leptin levels.

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MATERIALS AND METHODS

Animals

Seven 10-week-old (young) and seven 12-month-old (middle-aged) Sprague-Dawley female rats (Charles River, Kanagawa, Japan) were housed in a temperature-controlled room (24°C) with a 12-h light/dark cycle. It was previously confirmed that 12-month-old rats may be comparable to the perimenopausal status (i.e., irregular estrous cyclicity or acyclicity). All procedures performed in animal experiments followed the ethical standards of the Institutional Animal Care and Use Committee of the University of Tokushima. Food intake was measured for four weeks. After the induction of anesthesia with sevoflurane, rats were euthanized by decapitation. The brain, blood, and visceral fat (300–400 mm^3) were removed and visceral and subcutaneous

fat weights were evaluated. The visceral fat was dissected parametric, perirenal and mesenteric deposits, and subcutaneous fat were dissected inguinal deposits. The brain sections were dissected out via an anterior coronal 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. Serum and tissues were stored at –20 and –80°C, respectively.

Hormone assay

The measurement of serum OT levels was outsourced to a commercial laboratory (ASKA Pharmaceutical Medical Inc., Co., Ltd., Fujisawa city, Kanagawa, Japan) where assessments were performed using a chemiluminescent enzyme immunoassay according to a previously described method (20-22). The measurement of serum E2 levels were also outsourced to a commercial laboratory (SRL, Tokyo, Japan) where a chemiluminescence immunoassay kit with a detection limit of 10 pg/mL and coefficient of variation <7% was used for evaluations. A radioimmunoassay kit (multi-species leptin RIA kit, Linco Research Inc., MO, USA) with a sensitivity of 1.0 ng/ml and inter- and intra-assay coefficients of variation of 3.2 and 7.8%, respectively, was used to measure serum leptin levels.

Quantitative real-time polymerase chain reaction (PCR)

Following the dissection of whole hypothalamic explants from frozen brains using a previously described method (20), total RNA was isolated using a TRIzol® reagent kit (Invitrogen Co., Carlsbad, CA, USA) and RNeasy® mini kit (Qiagen Gmbh, Hilden, Germany). Total RNA was also isolated from visceral fat using the same method. The SuperScript III First-Strand Synthesis System for real-time PCR (Invitrogen Co.) was employed to synthesize cDNA with oligo (deoxythymidine) primers at 50°C. The StepOnePlusTM real-time PCR system (PE Applied Biosystems, Foster city, CA, USA) and FAST SYBR® green were used for PCR. Hypothalamic mRNA levels of OT and OTR and visceral fat mRNA levels of OTR were quantified and then normalized to that to GAPDH or the rRNA level of 18S. Each gene was subjected to a dissociation curve analysis after PCR, with each amplicon generating a single peak. Table 1 shows the relevant primer sequences, product sizes, and annealing temperatures. PCR was performed under the following conditions : initial denaturation and enzyme activation 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.

Statistical analysis

All results are shown as means ± SD. The Student's unpaired *t*-test was performed to examine the significance of differences. Correlation analyses were conducted using Pearson's correlation, whereas appropriate. Differences were considered to be significant at $P<0.05$.

RESULTS

Body weights were significantly higher in middle-aged rats than in young rats. However, cumulative food intake over four weeks was similar (Figure 1). Visceral and subcutaneous fat weights were significantly higher in middle-aged rats than in young rats, indicating that middle-aged rats were more likely to be obese (Figure 1). Serum E2 and OT levels did not significantly differ between middle-aged and young rats, whereas serum leptin levels were significantly higher in middle-aged rats than in young rats (Figure 2). Hypothalamic OT and OTR mRNA levels were significantly higher in middle-aged rats than in young rats, whereas visceral fat OTR mRNA levels were similar. Serum OT levels did not correlate with hypothalamic OT mRNA levels (Figure 2). Serum leptin levels correlated with serum OT levels and hypothalamic OT mRNA levels, whereas serum E2 levels did not (Figure 3, 4). According to these results, in middle-aged rats, serum E2 and OT levels did not decrease and hypothalamic OT and OTR mRNA levels were higher than in young rats.

DISCUSSION

Peri- and postmenopausal women are at an increased risk of developing obesity and associated metabolic disorders. However, there are currently no established medical interventions for these conditions. OT was recently shown to regulate metabolism, feeding behavior, and body weight gain in animals and humans, and the direct and indirect involvement of OT in lipid metabolism in adipose tissue has been demonstrated (4, 5). In our previous study, we found reductions in serum and hypothalamic OT levels in OVX female rats, a model of postmenopausal women, and also that the administration of exogenous OT decreased body weight, food intake, and fat weights (16, 17). In addition, serum OT levels were lower in postmenopausal women than in premenopausal women (23). Therefore, a decrease in the effects of OT appears to play a role in the development of obesity in postmenopausal cases, and OT has potential as a drug candidate for these conditions. Furthermore, we reported reductions in food intake, but not body or fat weight, following the administration of exogenous OT to middle-aged female rats, a model of perimenopausal women (18), indicating the weaker effects of OT in perimenopausal cases than in postmenopausal cases. However, we did not examine the conditions of endogenous OT in middle-aged

Table 1.

Primer	Sequence	Annealing T (℃)
OT forward	GAA CAC CAA CGC CAT GGC CTG CCC	62
OT reverse	TCG GTG CGG CAG CCA TCC GGG CTA	
OTR forward	CGA TTG CTG GGC GGT CTT	67
OTR reverse	CCG CCG CTG CCG TCT TGA	
GAPDH forward	ATG GCA CAG TCA AGG CTG AGA	64
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	

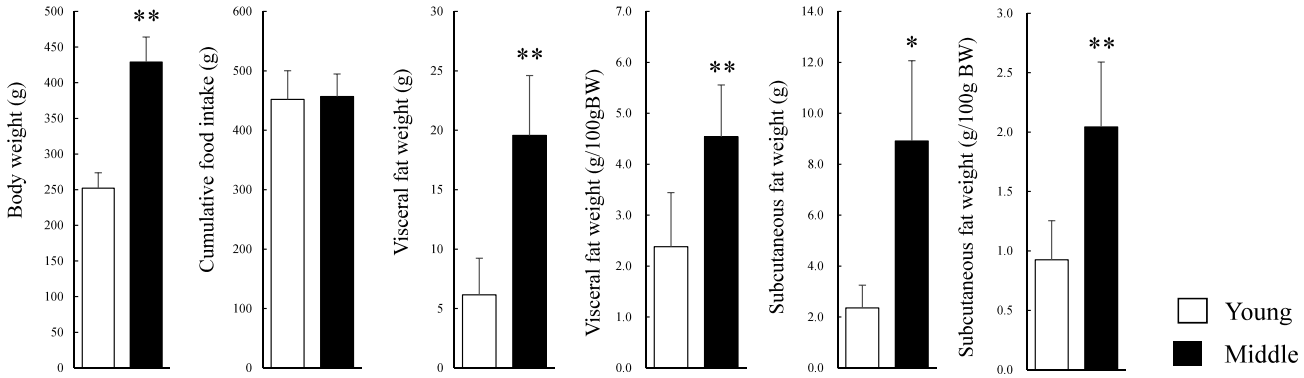

Figure 1. Body weight, cumulative food intake, and visceral and subcutaneous fat weights in young (Young) and middle-aged (Middle) rats. Data are expressed as means + SD values. * $P<0.05$, ** $P<0.01$.

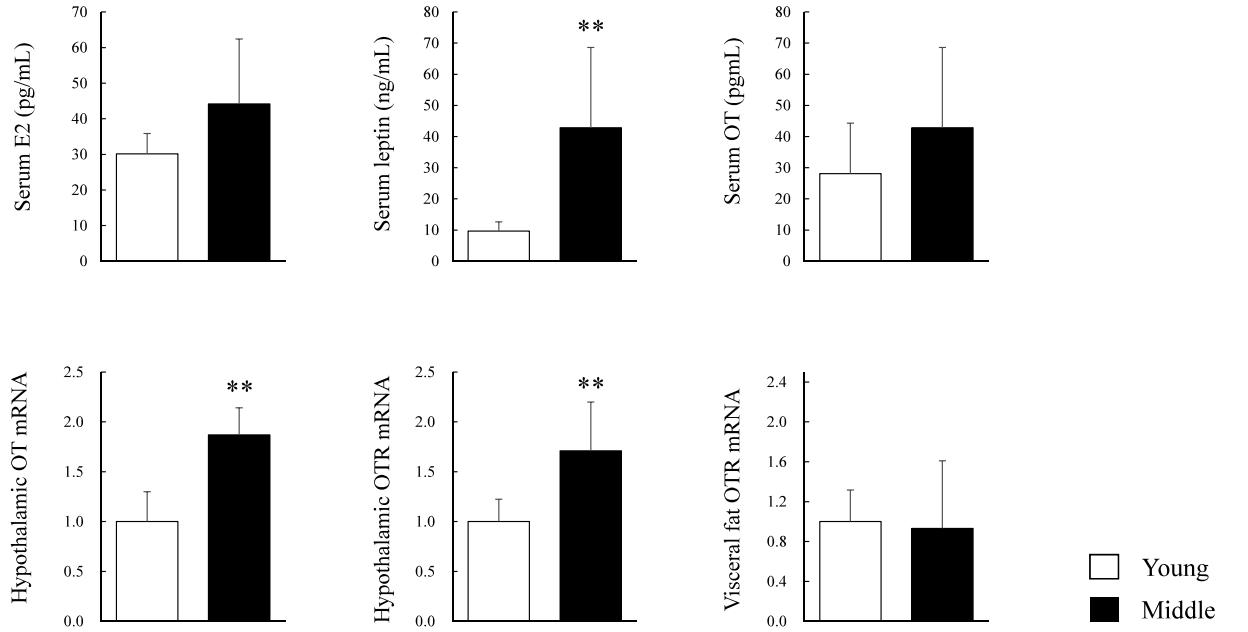

Figure 2. Serum levels of estradiol (E2), leptin, and oxytocin (OT), hypothalamic mRNA levels of OT and OT receptor (OTR), and visceral fat mRNA levels of OTR in young (Young) and middle-aged (Middle) rats. Data are expressed as means + SD values. ** $P<0.01$.

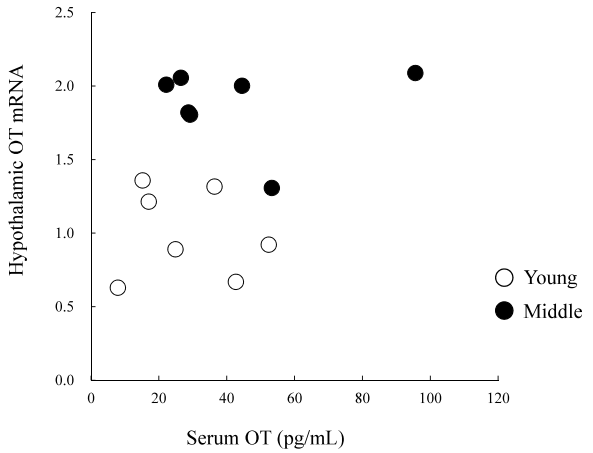

Figure 3. Correlation between serum oxytocin (OT) and hypothalamic OT mRNA levels.

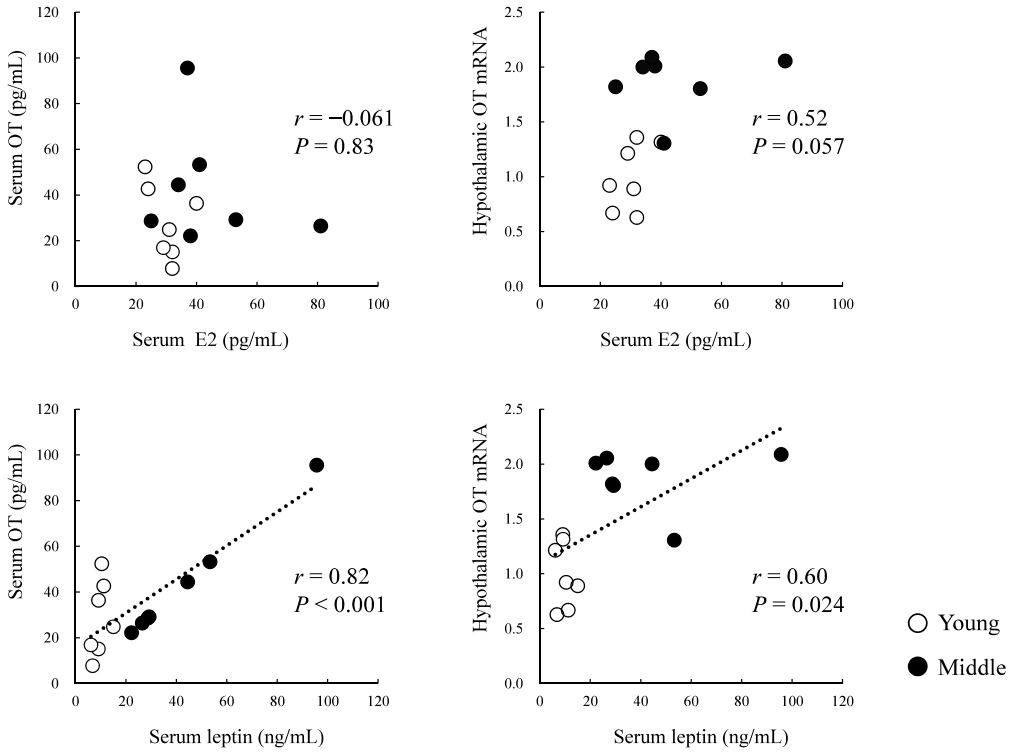

Figure 4. Correlation between serum estradiol (E2) and oxytocin (OT) levels, serum E2 and hypothalamic OT mRNA levels, serum leptin and OT levels, and serum leptin and hypothalamic OT mRNA levels.

perimenopausal rats in our previous study, but measured serum and hypothalamic OT levels in rats in the present study. The results obtained showed that serum OT levels were similar in middle-aged rats and young rats, while hypothalamic OT and OTR mRNA levels were higher in middle-aged rats than in young rats. Therefore, in contrast to OVX rats, the effects of hypothalamic OT may be enhanced in middle-aged rats. Nevertheless, body and fat weights were higher in middle-aged rats than in young rats. We speculate that increases in body and fat weights in middle-aged perimenopausal rats were caused by factors other than OT, and that elevated hypothalamic OT and OTR mRNA levels may be a counter-regulation to attenuate body weight gain and prevent obesity. The present results also revealed that hypothalamic OT mRNA levels did not correlate with serum OT levels and, thus, central and peripheral OT may be independently regulated. Previous studies reported the peripheral effects of OT (i.e., OT directly promoted lipolysis in adipose tissue through OTR). However, OTR mRNA levels in visceral fat did not significantly differ between middle-aged and young rats in the present study. Therefore, changes in the direct effects of OT may not be related to the accumulation of fat in middle-aged rats (4).

We previously reported reductions in the hypothalamic mRNA levels of OT and OTR and serum levels of OT in OVX rats, and these decreases were restored by supplementation with E2 (17). Furthermore, the administration of E2 increased serum OT levels in postmenopausal women, particularly those with obesity (24), and the co-administration of OT antagonists reduced the anorectic effects of the exogenous E2 supplementation in OVX rats (25). A correlation was also observed between hypothalamic OTR mRNA levels and serum E2 concentrations in aging female rats (26). Therefore, the effects of endogenous OT and/or OTR appear to be regulated by E2, while the anorectic effects of E2 may be mediated, at least partly, by endogenous OT. The results obtained herein revealed no significant differences in serum E2 levels between middle-aged and young rats and serum E2 levels did not correlate with serum OT levels or hypothalamic OT mRNA levels. Therefore, E2 maintains serum OT and hypothalamic OT/OTR mRNA levels, and other factors may increase hypothalamic OT/OTR mRNA levels in middle-aged perimenopausal rats. As described above, the effects of exogenous OT on body weight did not significantly differ between middle-aged rats and OVX rats in our previous study. Since the endogenous effects of OT are already enhanced under perimenopausal conditions, any additive effects by exogenous OT may be limited. The administration of exogenous leptin, an anorectic factor, activates hypothalamic OT (19). In the present study, serum leptin levels were higher in middle-aged rats than in young rats, and serum leptin levels correlated with serum OT levels and hypothalamic OT mRNA levels. Therefore, endogenous leptin may be associated with endogenous OT levels, at least in the rats examined in this study.

In summary, endogenous serum OT and E2 levels were not lower in perimenopausal rats than in younger rats, and in contrast to hypothalamic and serum OT levels, E2 levels did not correlate with leptin levels. Moreover, body and fat weight gain in perimenopausal cases may be induced by factors other than OT. Therefore, the administration of OT alone may not be sufficient to prevent metabolic disorders induced by the perimenopausal status.

CONFLICTS OF INTEREST

No conflicts of interest exist.

ACKNOWLEDGMENT

None

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