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Effects of peripheral oxytocin administration on body weight, food intake, adipocytes, and biochemical parameters in peri- and postmenopausal female rats

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Abstract. Recent studies have revealed that the administration of oxytocin has beneficial effects on the regulation of body weight, food intake, and metabolic functions, especially in obese individuals. Obesity is common in women after the menopause and drives many components of metabolic syndrome. Weight gain in menopausal women has been frequently reported. Although obesity and associated metabolic disorders are frequently observed in peri- and postmenopausal women, there are few medical interventions for these conditions. In this study, we evaluated the effects of chronic oxytocin administration on appetite, body weight, and fat mass in peri- and postmenopausal female rats. Sixteen naturally premenopausal or menopausal rats were intraperitoneally injected with oxytocin (1,000 μg/day) for 12 days. The daily changes in their body weight and food intake were measured at the same time as the oxytocin and vehicle injections. Intraperitoneally administering oxytocin for 12 days significantly reduced food intake, body weight, and visceral adipocyte size. In addition, oxytocin administration caused reductions in serum triglyceride and low-density lipoprotein-cholesterol levels, while it did not disturb hepatic or renal functions or locomotor activity. This is the first study to show the effects of oxytocin on the metabolic and feeding functions of peri- and postmenopausal female rats. Oxytocin might be a useful treatment for metabolic disorders caused by the menopause or aging.

Key words: Menopause, Oxytocin, Body weight, Food intake, Adipocyte

OXYTOCIN, a 9-amino acid neuropeptide, which was first demonstrated to be biologically active and synthesized in 1953 [1], is mainly secreted by (1) the parvocellular neurons of the paraventricular nucleus (PVN) and (2) the magnocellular neurons within the supraoptic nucleus (SON) and a subregion of the PVN [2]. The physiological role of oxytocin in female reproduction is well established; *i.e.*, it has been demonstrated to be involved in the maintenance of labor and lactation. In addition, oxytocin contributes to the processes that regulate social memory [3]. Moreover, it has been suggested to play a role in anxiety, and the intravenous administration of oxytocin was effective at reducing repetitive behaviors in adults that had been diagnosed with autism [4].

Recently, it has been reported that oxytocin was effective against obesity, and it was demonstrated that centrally secreted oxytocin was effective at aiding the regulation of body weight, metabolism, and appetite [5]. It was suggested that the oxytocin receptor might be involved in regulating energy metabolism, including food intake and energy expenditure. Takayanagi Y, *et al.* reported that oxytocin receptor-deficient mice developed late-onset obesity [6]. In addition, an intracerebroventricular oxytocin infusion significantly reduced body weight gain and affected adipocyte fatty acid β-oxidation [7]. The effects of oxytocin on food intake have been documented in many species, such as mice, rats, monkeys, and humans [8, 9]. According to these studies, the central or peripheral administration of oxytocin caused reductions in food intake and/or body weight gain [10-13]. In addition, these studies suggested that the administration of oxytocin led to a reduction in fat mass by promoting lipolysis in adipose tissue and decreasing the formation of new adipocytes [7, 14-16]. Interestingly, these effects of oxytocin on food intake and fat mass were more noticeable in diet-induced and genetically obese rodents

[7, 17, 18], diet-induced obese prairie voles [19], obese rhesus monkeys [9], and obese humans [20-22].

Weight gain is common in women after the menopause, which is the permanent process by which menses ends. While hormone replacement treatment helps to prevent osteoporosis, coronary heart disease, dementia, and obesity [23], long-term hormone replacement therapy might increase the risk of breast cancer, thromboemboli, and strokes [24-26].

As mentioned above, oxytocin was found to efficiently reduce body weight and food intake. In our previous study, we demonstrated that the administration of oxytocin had beneficial effects on body weight, food intake, and fat mass in ovariectomized obese rats [27]. Other previous studies also used ovariectomized rats as a menopause model to evaluate the effects of oxytocin and other drugs [28, 29]. However, it is possible that the responses to oxytocin seen in perimenopausal rats differ from those observed in rats in which the menopause was surgically induced. Therefore, the present study was conducted to evaluate the effects of oxytocin on body weight and food intake in naturally peri- and postmenopausal rats.

Materials and Methods

Animals

Female Wistar rats were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan), and were housed in the animal laboratory of Tokushima University for a year before the experiments. A total of 24 rats were used in this study (16 of the 24 rats were divided into two groups and used to evaluate the effects of chronic oxytocin administration on appetite, body weight, fat mass, biochemical analysis, and quantitative polymerase chain reaction. Another 8 of 24 rats were divided into two groups and used to evaluate locomotor activity). The animals were maintained under a 12-hour light/dark cycle (lights turned on at 08:00 and turned off at 20:00) and controlled temperature (24°C) conditions. They were given free access to food and water. All 16 rats were divided into oxytocin (8 rats) and control (8 rats) group which injected oxytocin or vehicle for 12 days. The food intake and body weight were measured every day. After 12 days of injection, tissue (brain, blood, visceral fat, liver, and uterus) were collected at same time. The tissue sampling procedures were carried out under sodium pentobarbital (60–80 mg/kg, intraperitoneally administered)-induced anesthesia. All the experimental and animal care procedures were performed according to the ethical standards of the animal care and use committee of Tokushima University.

Vaginal cytology for the staging of the estrous cycle using stained vaginal smears

For 8 days, vaginal epithelial smears were collected and used to evaluate the estrous cycle. All of the rats were brought into the laboratory, a glass pipette filled with sterilized water was inserted into the vaginal orifice to a depth of 5 mm, and the vagina was flushed two or three times. Then, a small sample of the collected fluid was dropped onto a slide and dried in air. All of the slides were stained with Giemsa stain. Then, cytological examinations were performed, and the stages of the estrous cycle (proestrus, estrus, metestrus, and diestrus) were analyzed based on cell type and the relative numbers of each cell type in the vaginal smears [30].

Daily oxytocin injections and measurement of food intake and body weight

One-year-old 16 rats were randomly divided into oxytocin and control groups. The rats (374.64–516.13 g, and average body weight 432 g) in the oxytocin group were intraperitoneally injected with oxytocin (1,000 μg, dissolved in 0.3 mL saline), and the control rats (359.05–472 g, and average body weight 413.3 g) were injected with saline 2 hours before the dark phase for 12 days. The dose of oxytocin was set according to the dose recommended in a previous study [27], which was chosen as a dose that would affect the regulation of body weight and food intake. Daily changes in body weight and food intake were measured before the injection of oxytocin or vehicle. Food intake was determined to the nearest 0.1 g by weighing food before the injection of oxytocin or vehicle.

Tissue collection and processing

The brain, blood, visceral fat (the parametrial, perirenal, and mesenteric deposits), liver, and uterus were collected at 24 hours after the final injection of oxytocin/saline. The visceral fat and liver tissue samples were dissected and placed into two tubes, before being used for the histological analysis or polymerase chain reaction (PCR) experiments. Each sample was around 300–400 mm^3 in size, and the histological tissue samples were fixed in 4% paraformaldehyde, embedded in paraffin, and stained with hematoxylin and eosin. The visceral fat was peeled away from the surrounding tissue and weighed immediately. Whole blood was centrifuged at 3,000 rpm for 20 minutes at 4°C, and the serum was removed and stored at –80°C, before being used for the subsequent analyses.

Measurement of locomotor activity

Pre-calibrated radio telemetry transmitters (TA11TA-F10; Data Sciences International, New Brighton, MN, USA) were surgically implanted into another 8 perime-

nopausal rats and used to measure locomotor activity. All 8 rats were maintained under a 12-hour light/dark cycle (lights turned on at 08:00 and turned off at 20:00) and controlled temperature (24°C) condition for one week after transmitters were implanted. They were given free access to food and water. After one week, rats were divided into oxytocin and control groups ($n = 4$ per group). Oxytocin (1,000 µg/day) or saline was intraperitoneally injected at 2 hours before the dark phase. Twenty-four-hour measurements of locomotor activity were obtained before and after each injection. These frequency data were converted using the DATAQUEST software (Data Sciences) and total activity of twenty-four-hours after injection of oxytocin or saline was analyzed by the Student's t-test.

Histology

Fixed visceral fat samples were embedded in paraffin and then sliced into sections. Serial 4-µm-thick sections were stained with hematoxylin and eosin. The Zeiss Imager M2 microscope and the AxioVision (version 4.8) acquisition software (Zeiss) were used to capture the histological images. We captured one image from each rat. In each captured image, the mean area of 50 randomly selected adipocytes per specimen was measured using the Image J software.

Biochemical analysis

The serum levels of total protein (TP), albumin (ALB), blood urea nitrogen (BUN), creatinine (CRE), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), gamma-glutamyl transferase (γ-GT), total cholesterol (T-CHO), free cholesterol (F-CHO), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and total bilirubin (T-BIL) and the urinary albumin (UA) level were measured by a commercial laboratory (Oriental Yeast Co., Ltd).

Quantitative polymerase chain reaction

The whole hypothalamus was dissected from each frozen brain, and then total RNA was isolated from it. The target region of the brain was dissected out *via* an anterior coronal cut at the posterior 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 [31]. To isolate total RNA, a TRIzol reagent kit (Invitrogen Co., Carlsbad, CA, USA) and an RNeasy® mini kit (Qiagen GmbH, Hilden, Germany) were used according to the manufacturers' instructions. Then, cDNA was synthesized with oligo (deoxythymidine) primers at 50°C using the SuperScript III first-strand synthesis

system for the 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 neuropeptide Y (NPY), pro-opiomelanocortin (POMC), oxytocin, and the oxytocin receptor were quantified. The obtained data were normalized to the expression level of the housekeeping gene GAPDH. The PCR conditions were as follows: the initial denaturation and enzyme activation were performed at 95°C for 20 sec, followed by 45 cycles of denaturation at 95°C for 3 sec and annealing and extension for 30 sec.

Statistical analysis

All results are expressed as mean ± standard error of the mean (SEM) values. Comparisons between the oxytocin and control groups were conducted using two-way repeated-measures analysis of variance (ANOVA) or the Student's t-test. The Student's t-test was used for comparisons of visceral weight, size of visceral adipocytes, locomotor activity for 24 hours after oxytocin injection, mRNA expression, and serological data between the oxytocin and control groups. Two-way repeated-measures ANOVA was used for comparisons of the overall changes in body weight and food intake between the oxytocin and control groups. p-values of <0.05 were considered to indicate significant differences. *Cohen's d* (small effect: 0.2, medium effect: 0.5, large effect: 0.8) value was reported for analyses conducted using the Student's t-test and ANOVA.

Results

Effects of oxytocin on food intake and body weight

Vaginal smear examinations confirmed that the 16 rats used in this experiment were in a peri- or postmenopausal state. A peri- or postmenopausal state was diagnosed based on the detection of irregular or continuous diestrus stages on vaginal cytology.

Injecting oxytocin for 12 days significantly affected the body weights of the rats (two-way ANOVA; treatment: $f = 6.0$, $p < 0.001$; time: $f = 0.20$, $p = 0.99$; interaction: $f = 0.01$, $p = 0.99$); however, there was no significant difference in mean body weight between the oxytocin and control groups on any day (Fig. 1A). Similarly, the chronic injection of oxytocin significantly affected the body weight changes of the rats (two-way ANOVA; treatment: $f = 10.37$, $p < 0.0015$; time: $f = 7.66$, $p < 0.001$; interaction: $f = 0.55$, $p = 0.87$); however, there were no significant differences in the body weight changes observed on each day between the oxytocin and control groups (Fig. 1B).

The injection of oxytocin for 12 days significantly

affected cumulative food intake (two-way ANOVA; treatment: $f = 67.411$, $p < 0.000$; time: $f = 170.713$, $p < 0.000$; interaction: $f = 2.631$, $p = 0.004$) and cumulative food intake relative to body weight (two-way ANOVA; treatment: $f = 51.879$, $p < 0.001$; time: $f = 129.9$, $p < 0.001$; interaction: $f = 2.11$, $p = 0.01$), cumulative food intake and cumulative food intake relative to body weight from days 4 to 12 was significantly lower in the oxytocin group than in the control group (Student's t test; $p < 0.05$) (Fig. 1C, 1D).

Effects of oxytocin on visceral fat weight

In the histological examinations of visceral adipocytes, the mean visceral adipocyte area was significantly smaller than in the control group (Student's t-test; $t = 10.66$, $p < 0.000$) (Fig. 2A, 2B). On the other hand, visceral fat weight did not differ significantly between the oxytocin and control groups (Fig. 2C).

Effects of oxytocin on biochemical factors

In the biochemical analysis, the oxytocin group exhibited significantly lower serum AST levels than the control group (Student's t-test; $t(13) = 2.86$, $p < 0.05$). The serum levels of ALT and LDH tended to be lower in the oxytocin group than in the control group, but these differences were not significant. The oxytocin group demonstrated significantly higher CRE levels than the control group (Student's t-test; $t(13) = -2.98$, $p < 0.05$). On the other hand, the oxytocin group displayed significantly lower LDL-C and HDL-C levels than the control group (Student's t-test; $t(13) = 2.82$, $p < 0.05$, and $t(13) = 2.44$, $p < 0.05$, respectively). Furthermore, the oxytocin group demonstrated significantly lower TG levels than the control group (Student's t-test; $t(13) = 4.03$, $p < 0.01$). In addition, the oxytocin group exhibited significantly lower T-BIL levels than the control group (Student's t-test; $t(13) = 3.52$, $p < 0.01$). No significant changes in the serum levels of other factors were found between the oxytocin and control groups (Fig. 3).

Effects of oxytocin on hypothalamic factors

The mRNA expression levels of oxytocin tended to be higher in the oxytocin group than in the control group, but these differences were not significant. The mRNA
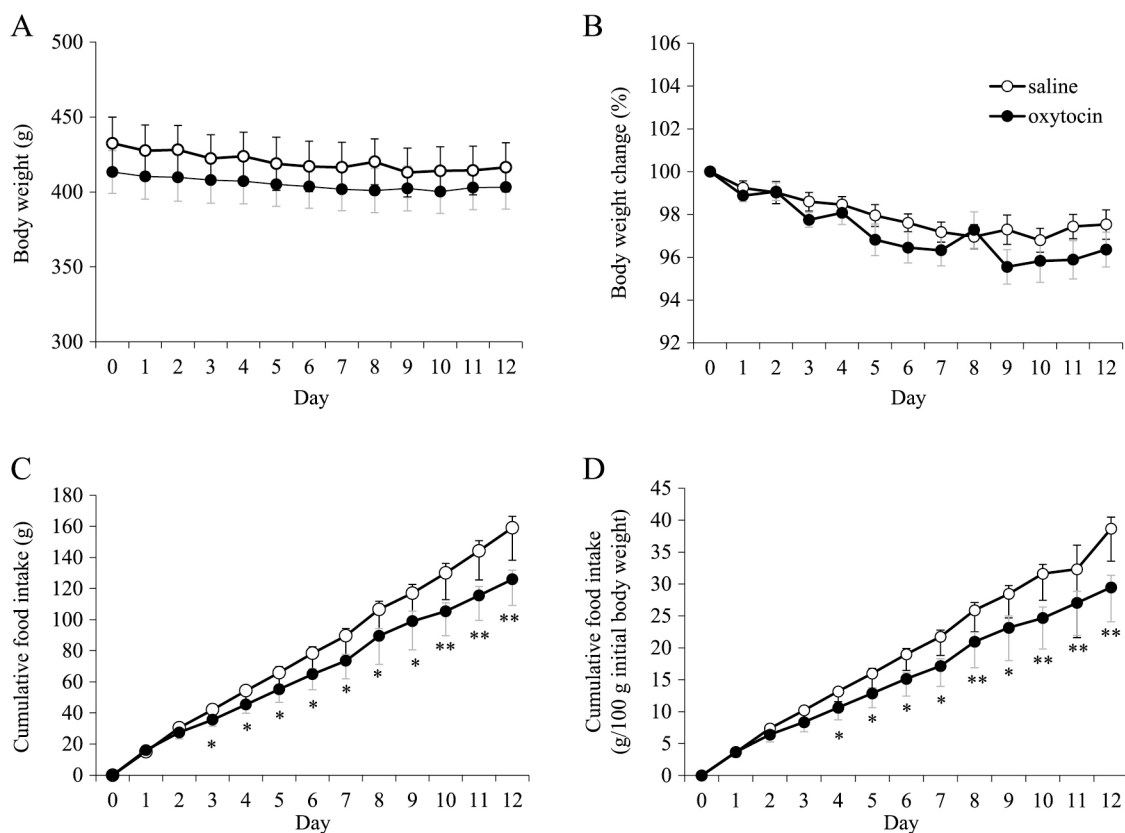
Fig. 1 (A) Body weight, (B) body weight change (% of initial body weight), (C) cumulative food intake, and (D) cumulative food intake relative to body weight in the control and oxytocin groups

Saline was intraperitoneally injected in the control group, and oxytocin (1,000 μg/day) was intraperitoneally injected in the oxytocin group at 2 hours before the dark phase. Data are expressed as mean ± SEM values. ($n = 8$ in each group); * $p < 0.05$, ** $p < 0.01$ *vs.* control

expression levels of the oxytocin receptor did not differ between the control and oxytocin groups. Furthermore, there were no significant differences in the mRNA levels of POMC between the two groups. However, the oxytocin group displayed significantly higher mRNA expression levels of NPY than the control group (Student's t-test; $t(14) = -2.55$, $p < 0.05$) (Fig. 4).

Effects of oxytocin on locomotor activity

In both groups, locomotor activity exhibited circadian rhythms during the measurement period. The total locomotor activity for 24 hours after injection procedure tended to lower in the oxytocin group, but it did not differ significantly between the control and oxytocin groups. The injection of oxytocin did not affect the pattern of locomotor activity in either group (Fig. 5).

Discussion

Obesity, which drives many components of metabolic syndrome, is commonly encountered in women after the menopause [32, 33]. However, the relationships among obesity, weight gain, and menopausal transition are not fully understood. Numerous studies have suggested that follicular dysfunction and alterations in the central nervous system-based regulation of hormonal levels might contribute to obesity during the menopausal transition period [34, 35]. Furthermore, it has been reported that weight gain during the menopausal transition period is caused by reductions in energy expenditure and fat oxidation, and increases in energy intake and the consumption of protein, polyunsaturated fat, and dietary fiber [36]. Although estrogen replacement might be an effective treatment for these conditions, the long-term use of this treatment might increase the risk of adverse events, such as thromboemboli and breast cancer.

Oxytocin is already used to induce labor in the clinical setting. Recently, there have been some important discoveries about novel oxytocin functions, particularly its effects on food intake and body weight regulation [15]. Interestingly, as mentioned above, oxytocin was shown to have more marked effects on appetite, body weight, and fat mass in obese animals and humans than in their non-obese counterparts [7, 9, 10, 12, 15, 16, 19]. However, in a cross-sectional study that evaluated the interactions among circulating oxytocin, the menopause, and obesity in pre- and postmenopausal women, it was reported that circulating oxytocin levels were significantly lower in postmenopausal women, especially in obese postmenopausal women, and that circulating oxytocin exhibits significant negative associations with the menopause and body weight [37]. Moreover, the mRNA expression levels of the oxytocin receptor in the PVN and SON of the hypothalamus were positively correlated with the serum estradiol concentration [38]. In line with these findings, the circulating levels of oxytocin and its receptor are sensitive to hormones and age. Thus, oxytocin might have useful effects in menopausal women with decreased estrogen levels. Therefore, in this study, we aimed to evaluate the effects of oxytocin administration on body weight and food intake in peri- and postmenopausal female rats.

In the present study, compared with that seen in the control group food intake was markedly decreased from the 3rd day onwards in the oxytocin group. This finding was similar to those obtained in previous studies, in which the administration of oxytocin *via* the nasal, intraperitoneal, or central route caused reductions in food intake and the energy balance in animals [10, 13, 39]. Interestingly, a previous study suggested that the anorectic effects of estrogen might be partially mediated by oxytocinergic pathways [40]. Specifically, estradiol-treated rats exhibited increased mRNA and protein levels of oxytocin in their brains, and the anorectic effects of
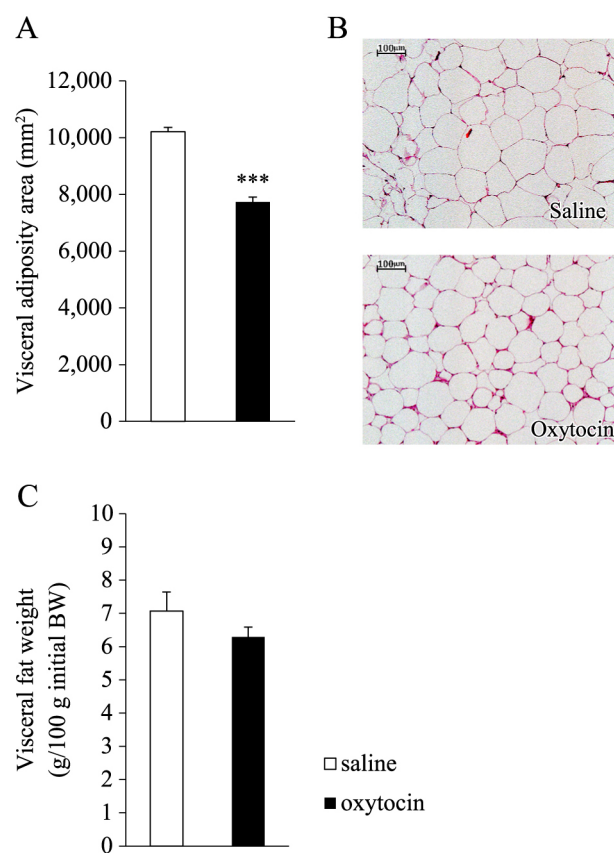

Fig. 2 (A) Adipocyte cell area of visceral fat, (B) Representative microphotographs of the control and oxytocin-injected rats, (C) Visceral fat weight

The black bar indicates 100 μm. Data are expressed as mean ± SEM values. ($n = 8$ in each group); *** $p < 0.001$
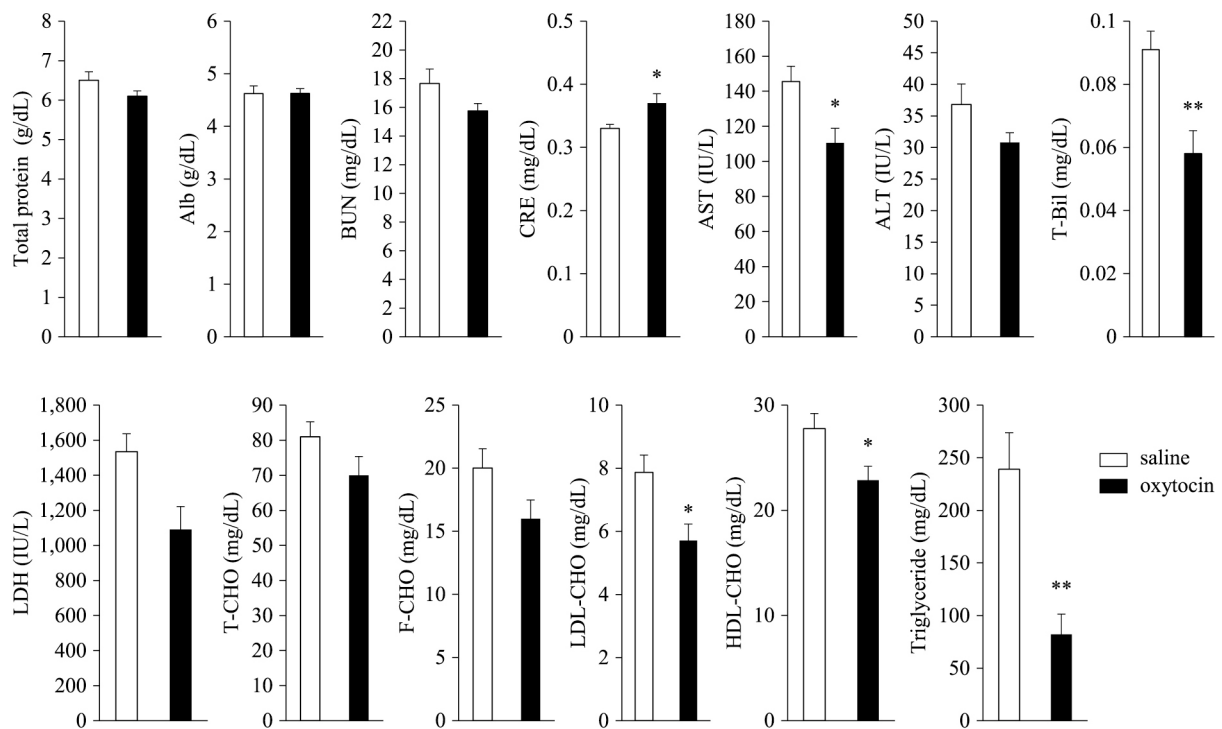
Fig. 3   Blood biochemistry analysis of total protein, albumin (ALB), blood urea nitrogen (BUN), creatinine (CRE), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), total bilirubin (T-BIL), total cholesterol (T-CHO), free cholesterol (F-CHO), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), the LDL-C to HDL-C ratio (LDL/HDL), triglycerides, and the serum glucose level in the control and oxytocin groups

Data are expressed as mean ± SEM values. ($n = 8$ in each group); * $p < 0.05$, ** $p < 0.01$
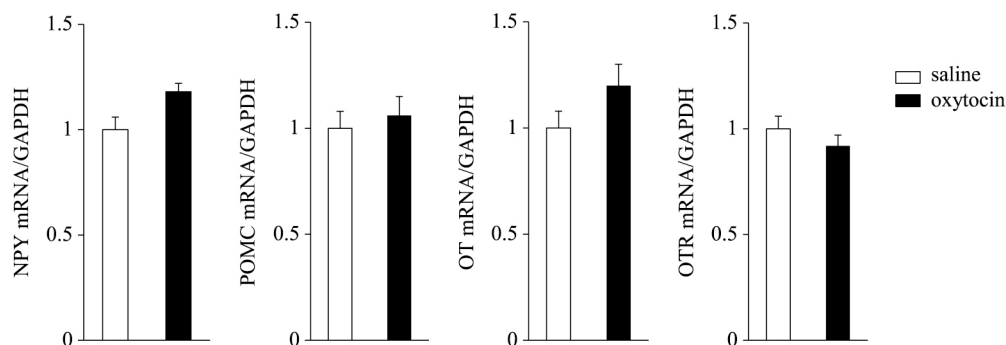

Fig. 4   Hypothalamic mRNA expression levels of neuropeptide Y (NPY), proopiomelanocortin (POMC), oxytocin (OT), and the oxytocin receptor (OTR) in the control and oxytocin groups

The mRNA expression levels of each gene were normalized to those of GAPDH, and the values for the control rats were defined as 1.0. Data are expressed as mean ± SEM values. ($n = 8$ in each group); * $p < 0.05$

estradiol were reversed by an oxytocin antagonist [40]. These findings indicate that oxytocinergic pathways might act downstream of estradiol. If this were true, oxytocin might be an ideal drug candidate for maintaining appropriate metabolic conditions in postmenopausal women.

In the current study, the chronic intraperitoneal administration of oxytocin caused reductions in body weight and adipocyte size compared with those seen in the control group. Previous studies have reported that oxytocin is directly and indirectly involved in lipid metabolism in adipose tissue [7, 10, 12]. Oxytocin receptors are expressed in adipocytes [41], and oxytocin directly regulates lipid metabolism *via* the oxytocin receptor in adipocytes. Similarly, *in vitro* experiments have shown that a higher dose of oxytocin induced lipolysis in fully differentiated 3T3-L1 adipocytes [42], and that administering oxytocin to epididymal adipose
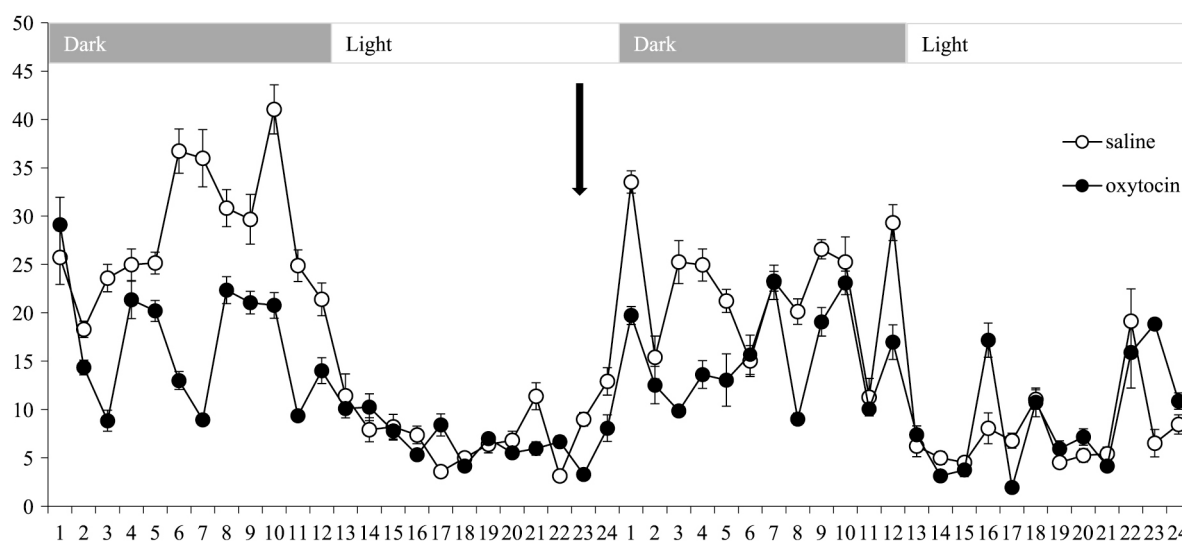
Fig. 5 Locomotor activity seen in every one-hour period during the 24 hours before and after the intraperitoneal administration of 1,000 μg/day oxytocin (*n* = 4) or saline (*n* = 4)

The arrow indicates the time at which each substance was administered. Data are expressed as mean values.

tissue caused the glycerol level of the culture medium to increase [7]. In our previous study, we demonstrated that the administration of oxytocin caused reductions in adipocyte size as well as body weight and food intake in surgically ovariectomized female rats [27]. Although the current study did not demonstrate the role of oxytocin in lipid metabolism or the underlying regulatory mechanism, the findings of this study provide further evidence that the administration of oxytocin induces lipolysis in naturally menopausal rats. In addition to metabolic disturbances, osteoporosis is another major problem experienced by menopausal women [43], while it has been reported that oxytocin reverses osteopenia in ovariectomized mice and regulates body composition [14]. Thus, oxytocin might be useful as a supplement that targets aging-related obesity and the associated pathologies, such as metabolic disorders and osteoporosis.

In the present study, the levels of LDL-C, HDL-C, and TG were reduced by the administration of oxytocin for 12 days. Obviously, the menopause plays a very important role in age-specific metabolic changes. For example, the risk of hyperlipidemia is often increased in menopausal and postmenopausal women [33]. Our results indicate that the administration of oxytocin might partially ameliorate menopause-induced hyperlipidemia. Moreover, oxytocin might help to prevent cardiovascular diseases, which are closely linked to hyperlipidemia. Hence, further studies involving more detailed analyses, such as metabolomic analyses, are needed to clarify the role oxytocin plays in the regulation of lipid metabolism.

On the other hand, the serum levels of ALT and LDH were not affected by chronic oxytocin administration;

however, the levels of AST and T-BIL were reduced. In addition, other parameters (the TP, ALB, and BUN levels) were not affected by such treatment. These results indicate that the chronic administration of oxytocin might not adversely affect hepatic functions. The CRE level was increased in the oxytocin group; however, some studies have reported that oxytocin had a protective effect against nephrotoxicity and oxidative renal injuries [44, 45]. In addition, the administration of oxytocin did not affect locomotor activity in our study, indicating that it did not induce behavioral abnormalities. These findings are consistent with those of previous studies, in which it was shown that peripheral oxytocin administration did not promote locomotor activity [10, 14, 39]. However, it has been reported that peripheral oxytocin administration reduced locomotor activity in male mice [13]. The discrepancies between these findings and those of the present study might have been caused by differences in species, sex, and/or age (including whether the animals were menopausal) between the previous study and the current study.

Furthermore, we did not yet observe any histological changes in liver tissue (data not shown). On the other hand, it has been reported that in high fat diet-fed obese mice liver weight and the amount of fat in hepatocytes were reduced by oxytocin treatment [10]. In present study, the serum level of AST was decreased in oxytocin group and there were no histological changes, however, we could not explain the reason this time. Regarding the specific reasons for the discrepancies between the findings of the latter study and the present study, differences in species, age, and/or the experimental method (such as

whether metabolic syndrome was induced) might have contributed to them. Our previous study, which evaluated the effects of short-term oxytocin administration on ovariectomized rats [27], obtained similar results to those seen in the current study. Thus, these two studies indicate that the chronic use of oxytocin does not have any obvious adverse effects.

It has been reported that POMC neurons in the hypothalamus are innervated and activated by oxytocin neurons, leading to reductions in food intake [15]. Similarly, POMC neurons in the arcuate nucleus of the hypothalamus are in contact with oxytocin neurons and are regulated by oxytocin *via* oxytocin receptors [46]. In addition, NPY/AgRP neurons have been reported to inhibit oxytocin neurons [15]. Thus, in the current study, we evaluated the hypothalamic mRNA expression levels of NPY, POMC, oxytocin, and the oxytocin receptor after administering oxytocin for 12 days. As a result, we found that the intraperitoneal administration of oxytocin did not alter the mRNA expression level of POMC, oxytocin, or the oxytocin receptor, whereas the hypothalamic mRNA expression level of NPY was elevated in the oxytocin group.

Previous studies have indicated that peripherally administered oxytocin reduces food intake and body mass [9, 13, 15, 21, 27]; however, there is still insufficient information about the mechanisms responsible for the central and peripheral effects of oxytocin. Oxytocin is one of the few hormones that acts *via* a positive feedback loop to stimulate its own release [47]. In addition, the fact that the intraperitoneal injection of oxytocin enhanced oxytocin release in the PVN [48] indicates that central oxytocin activity is stimulated by circulating oxytocin. In spite of the fact that some studies have shown that the peripheral injection of oxytocin activated hypothalamic oxytocin neurons and the intraperitoneal injection of oxytocin increased c-Fos immunoreactivity in oxytocin-synthesizing neurons [13, 48, 49], the hypothalamic oxytocin mRNA expression level was not affected by the administration of oxytocin in the current study. Intriguingly, the hypothalamic mRNA level of NPY, which is a potent orexigenic factor [50], was increased by the administration of oxytocin in the present study. In fact, arcuate nucleus NPY neuronal synthesis reduces with age, and the level of the NPY receptor in the brain was decreased in aged rats [51, 52]. NPY might regulate age-related changes in energy metabolism and contribute to age-related alterations in food intake. In the present study, the hypothalamic mRNA level of NPY was increased. It is possible that the reduction in food intake induced by chronic oxytocin administration increases hypothalamic NPY activity in order to prevent excessive reductions in body weight and food intake. In our previous study, the short-term (for 6 days) administration of oxytocin did not alter the expression level of NPY [27], indicating that counter-regulation of NPY expression might be induced by longer-term oxytocin administration. However, these findings do not definitively demonstrate whether NPY expression is stimulated by oxytocin. Therefore, further studies are needed to examine this issue.

This is the first study to evaluate the effects of oxytocin administration on metabolic functions in naturally peri- or postmenopausal rats. The main limitation of previous studies into the effects of oxytocin was that only ovariectomized rats; *i.e.*, rats in a non-physiological condition, were used [27]. The present study indicates that oxytocin might have beneficial effects, even in physiologically estrogen-deficient rats. For example, it showed that oxytocin reduces food intake and might reduce body weight; visceral adipocyte size; and TG, LDL-C, and HDL-C levels. However, the safety of oxytocin treatment should be clarified before it is used clinically because oxytocin was only administered for 12 days in this study.

Conclusion

In conclusion, the administration of oxytocin for 12 days reduced food intake, body weight, and adipocyte size in peri- and postmenopausal rats. Blood tests and physiological examinations indicated that the administration of oxytocin did not cause any changes in renal or liver functions or have any effects on locomotor activity. This is the first study to examine the effects of oxytocin on metabolic and feeding functions in naturally peri- or postmenopausal rats. Our study indicates that oxytocin might be effective at preventing the metabolic disorders induced by the menopause or aging.

Conflicts of Interest

The authors have no conflicts of interest to disclose.

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