Exp. Anim. 66(2), 159–166, 2017

—Original—

Effects of 17β-estradiol on leptin signaling in anterior pituitary of ovariectomized rats

Chunhua YIN[1)]*, Lumei KANG[2)]*, Cong LAI[3)]*, Jing ZHOU[3)], Bin SHI[3)], Lei ZHANG[3)], and Hongping CHEN[3,4)]

[1)]*Department of Gynecology and Obstetric, First Affiliated Hospital of Nanchang University, Nanchang, Jiangxi 330006, P.R. China*
[2)]*Department of Animal Science, Medical College, Nanchang University, Nanchang, Jiangxi 330006, P.R. China*
[3)]*Department of Histology and Embryology, Medical College, Nanchang University, Nanchang, Jiangxi 330006, P.R. China*
[4)]*Jiangxi Province Key Laboratory of Tumor Pathogen's and Molecular Pathology, Nanchang 330006, P.R. China*

Abstract: Leptin is secreted predominantly by adipocytes and exerts its role mainly by interaction with the long form of leptin receptor (LEPR_V2). It has been identified that LEPR_V2 is widely distributed in various tissues, including the anterior pituitary. Cross-talk between leptin and estrogens has been indentified. Estrogen is known to modulate the tissue-specific expression of LEPR_V2 and leptin in ovariectomized (OVX) rats, a model of postmenopausal condition. Our previous data showed that 17β-estradiol (E_2) up-regulated the expression of LEPR_V2 protein and mRNA in rat dorsal root ganglion (DRG) in an estrogen receptor alpha (ERα)-dependent manner. But it is still unclear whether estrogen can regulate leptin signalling in the pituitary of OVX rats. In the present study, we found that ovariectomy decreased the expressions of LEPR_V2. Administration of E_2 increased the expressions of LEPR_V2 in a dose-dependent manner. In addition, E_2 improved LEPR_V2, STAT3, and SOCS3 protein levels in OVX rats. The effects of exogenous E_2 were attenuated by ICI 182,780, a specific estrogen receptors antagonist. However, E_2 did not change the *Lepr_v1*, a type of short form of leptin receptor (LEPR), or *leptin* mRNA levels. Thus, E_2 plays a crucial role in regulating pituitary sensitivity to leptin in OVX rats. Our findings implied that exogenous E_2 had potential roles in modification of the function of pituitary in postmenopausal women.
Key words: 17β-estradiol, anterior pituitary, leptin, leptin receptor, ovariectomized rat

Introduction

Menopause, as the culmination of reproductive aging, is characterized by an altered hormonal status including estrogens. The progressive loss of estrogens is associated with an increased risk of developing obesity, diabetes, and cardiovascular disease [3, 17, 20]. Furthermore, manopause also causes pituitary dysfunction [21].

Estrogen is an important class of hormones which has vital effects on pituitary. Estradiol (E_2) is able to exert a rapid apoptotic action in anterior pituitary cells, especially in lactotropes and somatotrophs, through binding the estrogen receptors [38]. Administration of E_2 stimulates the increase of mammotrophs in a dose-dependent manner [37]. In gonadectomized rats, estradiol increases adrenocorticotropic hormone (ACTH) in the plasma

(Received 2 October 2016 / Accepted 29 November 2016 / Published online in J-STAGE 26 December 2016)
Address corresponding: H. Chen, Department of Histology and Embryology, Medical College, Nanchang University, Nanchang, Jiangxi 330006, P.R. China
**Chunhua YIN, Lumei KANG and Cong LAI contributed equally to this work.*
Supplementary Figures: refer to J-STAGE: https://www.jstage.jst.go.jp/browse/expanim

[24], and ovariectomy decreases the ACTH response to corticotropin-releasing factor [36]. However, to the best of our knowledge, the effects of estrogen on leptin signaling in pituitary of OVX rats have not been fully elucidated.

Leptin is a 16-kDa polypeptide hormone, which is initially reported to be secreted by adipose tissue [39]. Leptin plays an important role in regulating food intake, energy expenditure, body weight homeostasis [9], cardiovascular system [11], reproduction function [6] and neuroendocrine response [2]. Furthermore, leptin is capable of modification the functinon of pituitary [13, 30].

The leptin receptor (LEPR) belongs to the family of cytokine-like receptors with a single transmembrane domain [33]. The cytoplasmic sequence of the receptor contains a signal transducer and activator of transcription (STAT) activating motif. Stimulation of JAK-STAT pathway that mediates at least some action of leptin [8]. Six isoforms of the LEPR have been characterized that differ in the truncation of the cytoplasmic sequence. The long isoform of LEPR (LEPR_V2) is the active long intracellular form of the receptor and can activate both JAK and STAT proteins [31]. The short form of the LEPR (LEPR-V1) is a transporter molecule for leptin that promotes leptin stability [32]. LEPR_V2 is detected mainly in the peripheral tissues including the testes, pituitary, hypothalamus and ovary. The specific site of expression of LEPR within these tissues has important implications with regard to the function of leptin [14]. Our previous research has demonstrated that LEPR_V2 is expressed in the rat dorsal root ganglion (DRG) [7], and chronic constriction sciatic nerve injury-induced neuropathic pain is alleviated by intrathecal leptin through inhibition the expression of $P2X_{2/3}$ receptors [19].

Ovariectomized rats were considered a very useful model of postmenopausal condition. This model was useful to investigate the effects of hypoestrogenism and hormonal replacement on pituitary. The present study was designed to elucidate the effects of estrogen therapy on leptin signalling in pituitary of OVX rats. In addition, the mechanisms of E_2 in regulation of leptin signalling were also investigated. It is hoped that our novel finding will help to understand the roles of hormone replacement therapy in the regulation of pituitary function in postmenopausal women.

Materials and Methods

Animals

Nine to ten weeks old Sprague-Dawley rats (235.9 ± 5.0 g) were provided by the Center of Laboratory Animal Science of Nanchang University. The rats were fed a commercial diet (Murine bred diet; Slacom, China). The rats were maintained on a 12 L:12 D cycle (0800–2000 h) at an ambient temperature of 22 ± 2°C and a humidity of 45–55% under the conventional condition in the center. All animal procedures were approved by the Animal Care Committee of the Medical College of Nanchang University and were performed in accordance with the principles outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. During all the experiments, efforts were made to minimize both animal suffering and the number of animals used. Female rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and ovariectomized via two small dorsal incisions. Two weeks after surgery the rats were used.

Experimental protocols

Two experiments were conducted. In experiment 1, the female rats were divided into five groups with 6 rats in each group: 1) sham-operated control+vehicle (Sham group); 2) OVX+vehicle (OVX group); 3) OVX+E_2 (17β-estradiol, 2 μg/kg per day; OVX+E_2 2 group); 4) OVX+E_2 (10 μg/kg per day; OVX+E_2 10 group); and 5) OVX+E_2 (25 μg/kg per day; OVX+E_2 25 group). The Sham animals were subjected to the same general surgical procedure as the OVX group, except that ovraries were not excised. E_2 was dissolved in the ethanol and then diluted with sesame oil. The rats were given all injections subcutaneously and all injections were given daily for 14 days.

In experimental 2, rats were divided into four groups with 8 rats in each group: 1) OVX+vehicle (OVX group); 2) OVX+E_2 (25 μg/kg per day; E_2 group); 3) OVX+ICI182,780 (500 μg/kg per day; ICI group); and 4) OVX+17β-estradiol+ICI182,780 (E_2, 25 μg/kg per day; ICI182,780, 500 μg/kg per day; E2+ICI group). E_2 and ICI182,780 were dissolved in the ethanol and then diluted with sesame oil. The rats were given all injections subcutaneously and all injections were given daily for 14 days. ICI182,780 administration 1 h prior to E_2 in each injection of E_2+ICI Group.

Tissue collection

Rats were sacrificed 24 h after the last drugs injection. They were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The anterior pituitaries were carefully detached. Then these tissues were immediately frozen in liquid nitrogen and stored at −80°C. Each anterior pituitary wrapped with aluminum foil was froze in the liquid nitrogen for 10 s, the pituitary was then hammered into powder quickly. On the dry ice, the powder was separated for total RNA (One quarter powder) and protein (Three quarters powder) isolation. Blood was collected by cardiac puncture before the animals were sacrificed. Blood samples were centrifuged and the serum was stored at −80°C prior to being analyzed.

Enzyme-linked immunosorben assays (ELISA)

A competitive ELISA kit (Cayman Chemimcal, USA) was used to determine the serum concentration of E_2 and leptin according to the procedure. The intra-assay coefficients of variance were 6% and<10%, respectively.

Real-time PCR quantification

Total RNA from the anterior pituitary of rats was isolated using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. 1,000 ng total RNA was used as a template for reverse transcription using the Applied Biosystems Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Real-time quantitative PCR for *leptin*, *Lepr_v1*, and *Lepr_v2* was performed using ABI7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). The expression levels of *leptin*, *Lepr_v1*, and *Lepr_v2* were normalized to GAPDH. Probes were purchased from Applied Biosystems. All assays were performed in triplicate.

Western blot

The anterior pituitaries from ovariectomized rats were lysed with RIPA buffer (150 mM sodium chloride, 1.0% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0), supplementing with protease inhibitor cocktail. Protein concentration was determined using Bradford protein assay reagent with bovine serum albumin (BSA) as a standard. Samples of 30 μg of total protein were separated by 8% (w/v) sodium dodecyl sulphate–polyacrylamide gel electrophoresis and transferred onto a polyvinlylidene difluoride membrane (Bio-Rad Laboratories, Hercules, CA, USA). The membrane was blocked with 5% (w/v) BSA in phosphate buffer saline (pH 7.4) for 3 h at room temperature. After incubation with primary antibody of LEPR polyclonal antibody (Santa Cruz Biotechnology, CA, USA ; dilution 1:350), phospo-STAT3 (1:1,000; Abcam, USA), STAT3 (1:1,000; Abcam, USA), or SOCS3 (1:1,000; Abcam, USA) overnight at 4°C, the membrane was then incubated with peroxidase conjugated secondary antibodies (Cell SignalingTechnology, Danvers, MA, USA) for 1h at room temperature. Immunodetection was completed using Pierce-enhanced chemiluminescence substrate (Thermo Scientific, USA) and then exposed to X-ray film. GAPDH (1:10,000; ABclonal, USA) was used as a loading control.

Statistical analysis

Data were showed as the mean ± S.D. Comparisons of means between two groups were carried out using a *t*-test. Statistical comparisons were performed by analysis of variance (ANOVA) with Dunnett's test for multiple comparisons. A value of $P<0.05$ was considered to be significant.

Results

Body weight, serum E_2 and leptin levels

At the endpoint of the experiment 1, the body weight, Serum E_2 and leptin levels were evaluated. As expected, ovariectomy lead to a significant increase body weight and a decrease in serum E_2 levels when compared with the sham group. E_2 suppress the OVX-induced body weight increase (Fig. 1A). Serum E_2 levels were higher ($P<0.01$) in the OVX+E_2 10 group (1.68-fold) and OVX+E_2 25 group (2.25-fold) when compared to the OVX group (Fig. 1B). The serum leptin level also increased in the OVX rats, and administration of E_2 (10 μg/kg and 25 μg/kg) for 14 days decreased the serum leptin level when compared to the OVX group (Fig. 1C).

E_2 increased the Lepr-v2 mRNA expression in the anterior pituitary of OVX rats

Real-time PCR was performed to detected the expression of *leptin, Lepr-v1*, and *Lepr-v2* mRNA in the anterior pituitary of all the animal groups. Ovariectomy led to a significant decrease in *Lepr-v2* mRNA levels as compared with the sham group. Administration of E_2 (10 μg/kg and 25 μg/kg) for 14 days suppressed the ovariectomy-induced decrease in expression of *Lepr-v2* mRNA (Fig. 2). Thus, E_2 increased the *Lepr-v2* mRNA in a dose-dependent manner. Furthermore, the effects

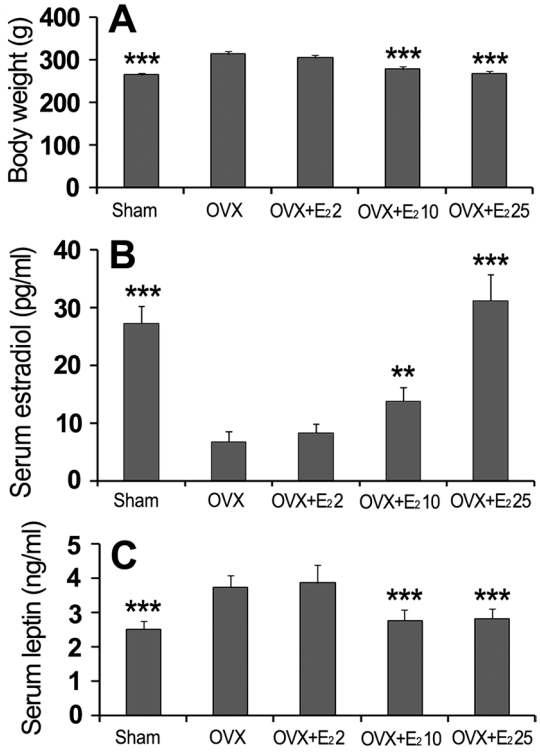

Fig. 1. Effects of exogenous E_2 on the body weight (A), serum estradiol (B) and leptin (C) levels of OVX rats. Administration of 10 μg/kg (OVX+E_2 10 group) and 25 μg/kg (OVX+E_2 25 group) E_2 for 14 days significantly increased the serum estradiol levels of OVX rats. The same volume of vehicle was injected in the sham and OVX groups. Data were showed as mean ± S.D., n=6. Comparisons of means between two groups were carried out using a *t*-test and multiple comparisons were performed by ANOVA with Dunnett's test. ***P*<0.01 and ****P*<0.001 vs. OVX group.

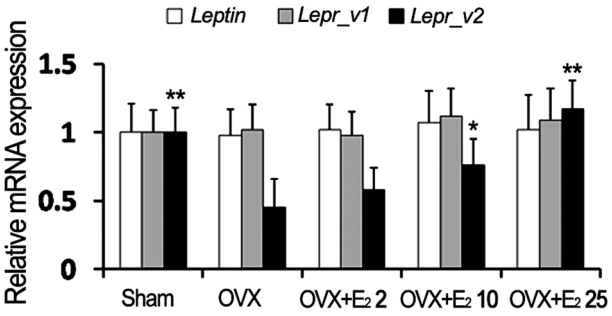

Fig. 2. Effects of exogenous E_2 on *leptin*, *Lepr_v1*, and *Lepr_v2* mRNA expressions in the anterior pituitary of OVX rats. Administration of 10 μg/kg (OVX+E_2 10 group) and 25 μg/kg (OVX+E_2 25 group) E_2 for 14 days significantly stimulated *Lepr_v2* mRNA expression. When compared to the OVX group, the *Lepr_v2* mRNA level in the OVX+E_2 10 and OVX+E_2 25 groups was 0.76 and 1.17 folds, respectively. E_2 did not change *leptin* or *Lepr_v1* mRNA expressions. Data were showed as mean ± S.D., n=6. Comparisons of means between two groups were carried out using a *t*-test and multiple comparisons were performed by ANOVA with Dunnett's test. **P*<0.05 and ***P*<0.01 vs. OVX group.

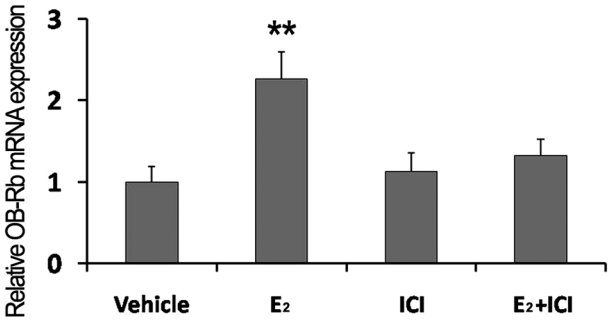

Fig. 3. Effects of exogenous E_2 and ICI 182,780 (ICI) on *Lepr_v2* mRNA expression in the anterior pituitary of OVX rats. Administration of 25 μg/kg E_2 for 14 days significantly increased *Lepr_v2* mRNA expression. The effect of E_2 was blocked by ICI 182,780 (500 μg/kg). When compared to the vehicle group, the *Lepr_v2* mRNA level in the E_2 and E_2+ICI groups was 2.26 and 1.32 folds, respectively. Data were showed as mean ± S.D., n=6. Statistical comparisons were performed by ANOVA with Dunnett's test. ***P*<0.01 vs. Vehicle group.

were efficiently attenuated by ICI 182,780 (Fig. 3).

E_2 increased the LEPR_V2, p-STAT3 and SOCS3 protein expression in the anterior pituitary of OVX rats

To investigate the potential mechanism of E_2 in reversing of ovariectomy-induced decrease of leptin signaling at protein levels, the LEPR_V2, p-STAT3 and SOCS3 proteins were measured by Western blot. The results indicated that ovariectomy led to a significant decrease in LEPR_V2 protein when compared with the sham group. E_2 suppressed the ovariectomy-induced decrease in expression of LEPR_V2 protein in a dose-dependent manner (Fig. 4). In addition, ICI 182,780 attenuated the E_2-induced increase of LEPR_V2, p-STAT3, and SOCS3 at protein levels (Fig. 5). The results suggested that E_2 activated the leptin signalling in an estrogen receptor-dependent manner.

Discussion

The progressive loss of estrogens induced by menopause leads to typical menopausal symptoms which may seriously compromise the quality of life. Hormone replacement therapy (HRT) with estrogens used to be standard therapy for treatment of menopausal symptoms such as hot flushes and depression [29]. Estrogens is also

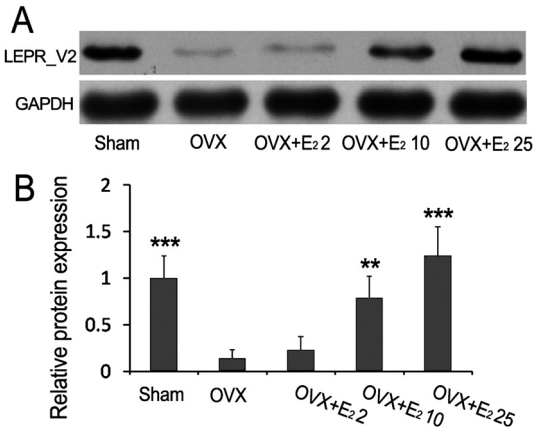

Fig. 4. Effects of exogenous E_2 on LEPR_V2 protein expression in the anterior pituitary of OVX rats. Administration of 10 μg/kg (OVX+E_2 10 group) and 25 μg/kg (OVX+E_2 25 group) E_2 for 14 days significantly increased LEPR_V2 protein expression. When compared to the sham group, the LEPR_V2 protein level in the OVX+E_2 10 and OVX+E_2 25 groups is 0.79 and 1.24 folds, respectively. The same volume of vehicle was injected in the sham and OVX groups. Data were showed as mean ± S.D., n=6. Comparisons of means between two groups were carried out using a *t*-test and multiple comparisons were performed by ANOVA with Dunnett's test ***P*<0.01 and ****P*<0.001 vs. OVX group.

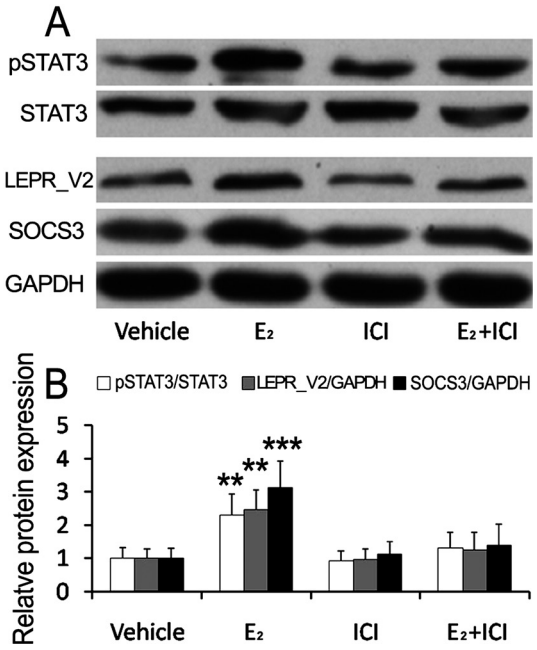

Fig. 5. Effects of exogenous E_2 and ICI 182,780 (ICI) on pSTAT3, LEPR_V2, and SOCS3 protein expressions in the anterior pituitary of OVX rats. (A): LEPR_V2, pSTAT3, and SOCS3 protein levels in the vehicle, E_2, ICI, and E_2+ICI groups were analyzed by Western Blot. The OVX rats were administrated E_2 (25 μg/kg), ICI (500 μg/kg), and E_2 (25 μg/kg)+ICI (500 μg/kg) for 14 days. The same volume of vehicle was injected in the OVX group. (B): The densitometry analysis showed the fold change of protein levels. E_2 significantly increased the levels of pSTAT3 (2.30 folds), LEPR_V2 (2.46 folds), and SOCS3 (3.12 folds) protein when compared to the vehicle group. The effect of E_2 was blocked by ICI 182,780 (500 μg/kg). When compared to the vehicle group, the protein level of pSTAT3, LEPR_V2, and SOCS3 in the E_2+ICI group was 1.32, 1.26, and 1.40 folds, respectively. The Data were showed as mean ± S.D., n=3. Statistical comparisons were performed by ANOVA with Dunnett's test. ***P*<0.01 and ****P*<0.001 vs. vehicle group.

involved in the regulation of the neuroendocrine response. Estradiol short-term therapy modulates the neuroendocrine control of the hypothalamus-pituitary unit and induces the recovery of both gonadotropins synthesis and secretion in hypogonadotropic patients with functional hypothalamic amenorrhea [10]. Pituitary dysfunction is associated with estrogen-deficiency [21].

Leptin is a powerful stimulator of PRL release and its actions occur in part through stimulation of extracellular signal-regulated kinase [34]. Leptin exerts a stimulatory effect on growth hormone releasing hormone (GHRH)-induced GH secretion in GH-secreting pituitary adenomas [12]. In addition, leptin stimulates the expression of GH in primary pig pituitary cells and bovine pituitary tissues [1]. It has been postulated that leptin might increase thyroid-stimulating hormone (TSH) secretion, since there is a synchronicity between leptin and TSH secretion in normal humans. And the pulsatile and circadian rhythm was disorganized in leptin-deficient patients [23]. By contrast, leptin is identified to inhibited rat pituitary TSH release [27]. Leptin induces pituitary cells to produce and secrete both LH and FSH [26]. It is also showed that the influences of leptin on FSH secretion are biphasic [18]. The actions of leptin are mediated by LEPR which is widely distributed in many tissues including hypothalamus and pituitary [15, 16]. The factors of influence leptin or LEPR expression in pituitary may lead to the physiological function abnormal.

Administration of E_2 for short term improves the serum leptin level in both normal and OVX rats [5, 28]. In addition, E_2 stimulate, *in vivo* and *in vitro*, the expression of leptin in adipocytes [5, 22]. OVX induced up-regulation of body weight and fat mass, which is associated with the increase of serum leptin level. Treatment of E_2 for long term causes the body weight and fat mass down-regulation and lead to the serum leptin decrease in the

OVX rats [25]. In the present study, OVX caused the serum leptin level up-regulation with the body weight increase. And administration of E_2 for 14 days reduced the serum leptin level with the body weight decrease as compared to the OVX rats.

Estrogen can also modulate leptin actions by changing LEPR expression [25, 28]. In the previous report, we identify that estrogen can improve the expression of LEPR in rat DRG [7]. In addition, estrogen regulates the expression of LEPR in growth plate chondrocytes via estrogen receptor and activation of ERK1/2 signaling pathways [35]. However, the effect of estrogen on leptin signaling in pituitary of OVX rat remains unclear.

In the present study, we investigated the effects of estrogen on expressions of leptin, LEPR_V1 and LEPR_V2 in anterior pituitary of OVX rats. The results indicated that E_2 significantly increase *Lepr_v2* mRNA and protein in a dose-dependent manner in the anterior pituitary. Similar to our findings, ovariectomy decrease the LEPR_V2 expression and administration of E_2 reversed the ovariectomy-induced down-regulation of LEPR_V2 in the hypothalamus, DRG and skeletal muscle [4, 7, 25]. By contrast, ovariectomy increase the LEPR_V2 expression in the adipose tissue, and E_2 administration reverse the ovariectomy-induced up-regulation of LEPR_V2 [25]. Thus, estrogen regulated LEPR_V2 expression in a tissue-specific way. Our results indicated that the actions of estrogen in promotion LEPR_V2 expression was prevented by ICI182,780, a specific estrogen recepors antagonist. However, E_2 could not modulate the expressions of leptin and LEPR_V1. To further investigate the role of E_2 on leptin signalling in the pituitary, we examined the downstream effectors of LEPR_V2 activation, STAT3 and SOCS3. Our results indicated that ovariectomy caused the decrease of STAT3 and SOCS3. E_2 reversed the ovariectomy-induced down-regulation of STAT3 and SOCS3. These results indicated that estrogen increased the LEPR_V2 expression in the pituitary of OVX rats in an estrogen receptor-dependent manner. Estrogen regulates the LEPR_V2 expression in a tissue-specific manner.

In conclusion, our study was the first to investigate the effects of estrogen on the leptin signalling in the pituitary of ovariectomized rat. Our data showed that estrogen could not modulate the *leptin* or *Lepr_v1* expression. But, estrogen stimulated LEPR_V2, STAT3, and SOCS3 expression in an estrogen receptor-dependent way. These results suggested that E_2 activated LEPR_V2 and its downstream effectors, STAT3, and SOCS3 in the pituitary of OVX rats, and thereby might play a role in regulating pituitary sensitivity to leptin. However, the exact mechanism of estrogen in modulation leptin signaling in OVX pituitary is still not well indentified and need to be further investigated.

Acknowledgment

This study was supported by the National Natural Science Foundation of China (No. 81260318). Educational Department Foundation of Jiangxi Province (No. GJJ14162). Foundation of Health and Family Planning Commission of Jiangxi Providence (No. 20143198).

References

1. Accorsi, P.A., Munno, A., Gamberoni, M., Viggiani, R., De Ambrogi, M., Tamanini, C., and Seren, E. 2007. Role of leptin on growth hormone and prolactin secretion by bovine pituitary explants. *J. Dairy Sci.* 90: 1683–1691. [Medline] [CrossRef]
2. Ahima, R.S., Saper, C.B., Flier, J.S., and Elmquist, J.K. 2000. Leptin regulation of neuroendocrine systems. *Front. Neuroendocrinol.* 21: 263–307. [Medline] [CrossRef]
3. Alhurani, R.E., Chahal, C.A., Ahmed, A.T., Mohamed, E.A., and Miller, V.M. 2016. Sex hormone therapy and progression of cardiovascular disease in menopausal women. *Clin. Sci.* 130: 1065–1074. [Medline] [CrossRef]
4. Alonso, A., Fernández, R., Moreno, M., Ordóñez, P., Díaz, F., and González, C. 2007. Leptin and its receptor are controlled by 17β-estradiol in peripheral tissues of ovariectomized rats. *Exp. Biol. Med. (Maywood)* 232: 542–549. [Medline]
5. Brann, D.W., De Sevilla, L., Zamorano, P.L., and Mahesh, V.B. 1999. Regulation of leptin gene expression and secretion by steroid hormones. *Steroids* 64: 659–663. [Medline] [CrossRef]
6. Chehab, F.F. 2014. 20 years of leptin: leptin and reproduction: past milestones, present undertakings, and future endeavors. *J. Endocrinol.* 223: T37–T48. [Medline] [CrossRef]
7. Chen, H.P., Fan, J., and Cui, S. 2006. Detection and estrogen regulation of leptin receptor expression in rat dorsal root ganglion. *Histochem. Cell Biol.* 126: 363–369. [Medline] [CrossRef]
8. Darnell, J.E. Jr. 1996. Reflections on STAT3, STAT5, and STAT6 as fat STATs. *Proc. Natl. Acad. Sci. USA* 93: 6221–6224. [Medline] [CrossRef]
9. Farooqi, I.S. and O'Rahilly, S. 2014. 20 years of leptin: human disorders of leptin action. *J. Endocrinol.* 223: T63–T70. [Medline] [CrossRef]
10. Genazzani, A.D., Podfigurna-Stopa, A., Czyzyk, A., Katulski, K., Prati, A., Despini, G., Angioni, S., Simoncini, T., and Meczekalski, B. 2016. Short-term estriol administration modulates hypothalamo-pituitary function in patients with

functional hypothalamic amenorrhea (FHA). *Gynecol. Endocrinol.* 32: 253–257. [Medline] [CrossRef]
11. Ghantous, C.M., Azrak, Z., Hanache, S., Abou-Kheir, W., and Zeidan, A. 2015. Differential role of leptin and adiponectin in cardiovascular system. *Int. J. Endocrinol.* 2015: 534320. [Medline] [CrossRef]
12. Giusti, M., Bocca, L., Florio, T., Corsaro, A., Spaziante, R., Schettini, G., and Minuto, F. 2002. *In vitro* effect of human recombinant leptin and expression of leptin receptors on growth hormone-secreting human pituitary adenomas. *Clin. Endocrinol. (Oxf.)* 57: 449–455. [Medline] [CrossRef]
13. Hanew, K. 2003. The effects of human leptin fragment(126-140) on pituitary functions in man. *Eur. J. Endocrinol.* 149: 407–412. [Medline] [CrossRef]
14. Hoggard, N., Mercer, J.G., Rayner, D.V., Moar, K., Trayhurn, P., and Williams, L.M. 1997. Localization of leptin receptor mRNA splice variants in murine peripheral tissues by RT-PCR and in situ hybridization. *Biochem. Biophys. Res. Commun.* 232: 383–387. [Medline] [CrossRef]
15. Jin, L., Burguera, B.G., Couce, M.E., Scheithauer, B.W., Lamsan, J., Eberhardt, N.L., Kulig, E., and Lloyd, R.V. 1999. Leptin and leptin receptor expression in normal and neoplastic human pituitary: evidence of a regulatory role for leptin on pituitary cell proliferation. *J. Clin. Endocrinol. Metab.* 84: 2903–2911. [Medline]
16. Jin, L., Zhang, S., Burguera, B.G., Couce, M.E., Osamura, R.Y., Kulig, E., and Lloyd, R.V. 2000. Leptin and leptin receptor expression in rat and mouse pituitary cells. *Endocrinology* 141: 333–339. [Medline]
17. Karvonen-Gutierrez, C.A., Park, S.K., and Kim, C. 2016. Diabetes and Menopause. *Curr. Diab. Rep.* 16: 20. [Medline] [CrossRef]
18. Kosior-Korzecka, U. and Bobowiec, R. 2006. Leptin effect on nitric oxide and GnRH-induced FSH secretion from ovine pituitary cells *in vitro*. *J. Physiol. Pharmacol.* 57: 637–647. [Medline]
19. Li, X., Kang, L., Li, G., Zeng, H., Zhang, L., Ling, X., Dong, H., Liang, S., and Chen, H. 2013. Intrathecal leptin inhibits expression of the P2X2/3 receptors and alleviates neuropathic pain induced by chronic constriction sciatic nerve injury. *Mol. Pain* 9: 65. [Medline] [CrossRef]
20. Lizcano, F. and Guzmán, G. 2014. Estrogen Deficiency and the Origin of Obesity during Menopause. *BioMed Res. Int.* 2014: 757461. [Medline] [CrossRef]
21. Lyons, A., Ng, C., Fielding, K., Hosking, D., Selby, C., Lawson, N., Nottingham EPIC Study Group 1997. Pituitary dysfunction in recently post-menopausal women. *Clin. Endocrinol. (Oxf.)* 47: 431–438. [Medline] [CrossRef]
22. Machinal, F., Dieudonne, M.N., Leneveu, M.C., Pecquery, R., and Giudicelli, Y. 1999. *In vivo* and *in vitro* ob gene expression and leptin secretion in rat adipocytes: evidence for a regional specific regulation by sex steroid hormones. *Endocrinology* 140: 1567–1574. [Medline] [CrossRef]
23. Mantzoros, C.S., Ozata, M., Negrao, A.B., Suchard, M.A., Ziotopoulou, M., Caglayan, S., Elashoff, R.M., Cogswell, R.J., Negro, P., Liberty, V., Wong, M.L., Veldhuis, J., Ozdemir, I.C., Gold, P.W., Flier, J.S., and Licinio, J. 2001. Synchronicity of frequently sampled thyrotropin (TSH) and leptin concentrations in healthy adults and leptin-deficient subjects: evidence for possible partial TSH regulation by leptin in humans. *J. Clin. Endocrinol. Metab.* 86: 3284–3291. [Medline] [CrossRef]
24. McCormick, C.M., Linkroum, W., Sallinen, B.J., and Miller, N.W. 2002. Peripheral and central sex steroids have differential effects on the HPA axis of male and female rats. *Stress* 5: 235–247. [Medline] [CrossRef]
25. Meli, R., Pacilio, M., Raso, G.M., Esposito, E., Coppola, A., Nasti, A., Di Carlo, C., Nappi, C., and Di Carlo, R. 2004. Estrogen and raloxifene modulate leptin and its receptor in hypothalamus and adipose tissue from ovariectomized rats. *Endocrinology* 145: 3115–3121. [Medline] [CrossRef]
26. Ogura, K., Irahara, M., Kiyokawa, M., Tezuka, M., Matsuzaki, T., Yasui, T., Kamada, M., and Aono, T. 2001. Effects of leptin on secretion of LH and FSH from primary cultured female rat pituitary cells. *Eur. J. Endocrinol.* 144: 653–658. [Medline] [CrossRef]
27. Ortiga-Carvalho, T.M., Oliveira, K.J., Soares, B.A., and Pazos-Moura, C.C. 2002. The role of leptin in the regulation of TSH secretion in the fed state: *in vivo* and *in vitro* studies. *J. Endocrinol.* 174: 121–125. [Medline] [CrossRef]
28. Rocha, M., Bing, C., Williams, G., and Puerta, M. 2004. Physiologic estradiol levels enhance hypothalamic expression of the long form of the leptin receptor in intact rats. *J. Nutr. Biochem.* 15: 328–334. [Medline] [CrossRef]
29. Santoro, N., Epperson, C.N., and Mathews, S.B. 2015. Menopausal Symptoms and Their Management. *Endocrinol. Metab. Clin. North Am.* 44: 497–515. [Medline] [CrossRef]
30. Sone, M. and Osamura, R.Y. 2001. Leptin and the pituitary. *Pituitary* 4: 15–23. [Medline] [CrossRef]
31. Stahl, N., Farruggella, T.J., Boulton, T.G., Zhong, Z., Darnell, J.E. Jr., and Yancopoulos, G.D. 1995. Choice of STATs and other substrates specified by modular tyrosine-based motifs in cytokine receptors. *Science* 267: 1349–1353. [Medline] [CrossRef]
32. Stephens, T.W. and Caro, J.F. 1998. To be lean or not to be lean. Is leptin the answer? *Exp. Clin. Endocrinol. Diabetes* 106: 1–15. [Medline] [CrossRef]
33. Tartaglia, L.A., Dembski, M., Weng, X., Deng, N., Culpepper, J., Devos, R., Richards, G.J., Campfield, L.A., Clark, F.T., Deeds, J., Muir, C., Sanker, S., Moriarty, A., Moore, K.J., Smutko, J.S., Mays, G.G., Wool, E.A., Monroe, C.A., and Tepper, R.I. 1995. Identification and expression cloning of a leptin receptor, OB-R. *Cell* 83: 1263–1271. [Medline] [CrossRef]
34. Thorn, S.R., Meyer, M.J., Van Amburgh, M.E., and Boisclair, Y.R. 2007. Effect of estrogen on leptin and expression of leptin receptor transcripts in prepubertal dairy heifers. *J. Dairy Sci.* 90: 3742–3750. [Medline] [CrossRef]
35. Wang, S.J., Li, X.F., Jiang, L.S., and Dai, L.Y. 2012. Estrogen stimulates leptin receptor expression in ATDC5 cells via the estrogen receptor and extracellular signal-regulated kinase pathways. *J. Endocrinol.* 213: 163–172. [Medline] [CrossRef]
36. Wilson, M.E., Mook, D., Graves, F., Felger, J., Bielsky, I.F., and Wallen, K. 2003. Tamoxifen is an estrogen antagonist on gonadotropin secretion and responsiveness of the hypotha-

lamic-pituitary- adrenal axis in female monkeys. *Endocrine* 22: 305–315. [Medline] [CrossRef]

37. Yamamuro, Y., Aoki, T., and Sensui, N. 2001. Effect of estradiol and FBS on PRL cells, GH cells, and PRL/GH cells in primary cultures of pituitary cells from prenatal rats. *Exp. Biol. Med. (Maywood)* 226: 140–143. [Medline]
38. Zárate, S., Jaita, G., Zaldivar, V., Radl, D.B., Eijo, G., Ferraris, J., Pisera, D., and Seilicovich, A. 2009. Estrogens exert a rapid apoptotic action in anterior pituitary cells. *Am. J. Physiol. Endocrinol. Metab.* 296: E664–E671. [Medline] [CrossRef]
39. Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J.M. 1994. Positional cloning of the mouse obese gene and its human homologue. *Nature* 372: 425–432. [Medline] [CrossRef]