Iranian Journal of Pharmaceutical Sciences
2022: 18 (2): 87-94
www.ijps.ir

Original Article

Effect of intraductal drug delivery of orexin receptor antagonists into rat mammary gland on milk triacylglycerol level: Possible molecular interaction with Gpat1 and *Gpat4* genes

Shima Jafari Zandieh[a], Homayoun Khazali[a,*]

[a] *Department of Animal Sciences and Marine Biology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran.*

Abstract

Although previous studies have demonstrated the importance of the orexin system in regulating enzymes involved in lipogenesis, its exact mechanism and the extent of this effect on different enzymes have remained unexplored. In this regard, this study is aimed at investigating the intra-duct injection of orexin receptor 1 and 2 antagonists (OX1RA and OX2RA) into the mammary glands. More specifically, the study probes the effect of this injection on glycerol 3-phosphate acyl transferase (*Gpat1* and *Gpat4)* genes as well as the milk's triacylglycerol (TAG) level in lactating Wistar rats. Twenty four lactating Wistar rats were randomly divided into different experimental groups including the control group, the group receiving OX1RA and OX2RA intraductally (at doses of 5, 10, and 20 µg/kg of B.W.). Six hours after injection, the collection of milk samples were done using rat milking device, and TAG measurements were carried out. Moreover, using real time PCR, we measured target genes by a specific primer for each gene. One-way ANOVA with Tukey's post hoc tests were used to analyze the results and the level of significance was considered $P<0.05$. The findings of the present study showed that the injection of orexin antagonists at a dose of 10 µg/kg resulted in a significant reduction in *Gpat1* and *Gpat4* gene expressions. In addition, the injection of antagonists with the same dose caused a significant decrease in TAG levels. Intra-ductal injection of orexin antagonists into the mammary gland decreases milk TAG levels and decreases the expression of *Gpat1* and *Gpat4* genes.

Keywords: Orexin antagonist, GPAT1, GPAT4, Triacylglycerol, Mammary gland, Rat.

1. Introduction

Milk is a quintessential component on which all the nutritional needs of the baby are dependent [1]. Milk contains a high degree of nutrients such as proteins, fatty acids, etc [2]. Milk fat plays an important role in determining the quality and energy of dairy products [3]. Mammalian epithelial cells of lactating animals are highly active in the synthesis of the triacylglycerol (TAG) center [4]. In mammals, there are 4 isoforms of the enzyme glycerol 3-phosphate acyl transferase (GPAT) and they are divided into two categories [5]: mitochondria, in the outer membrane of the mitochondria containing

Corresponding Author: Homayoun Khazali, Department of Animal Sciences and Marine Biology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran.
E-mail: h_khazali@sbu.ac.ir

Cite this article as: Jafari Zandieh Sh., Khazali H., Effect of intraductal drug delivery of orexin receptor antagonists into rat mammary gland on milk triacylglycerol level: Possible molecular interaction with Gpat1 and *Gpat4* genes, Iran.J.Pharm.Sci, 2022, 18 (2): 87-94.

GPAT1, and microsomal, in the endoplasmic reticulum containing GPAT4 [6, 7]. Transferase is converted to phosphatidic acid,phosphatidic acid is converted to diacylglycerol (DAG) and finally, DAG is converted by diglyceride acyltransferase (DGAT) to TAG [8]. GPAT4 enzyme is found in brown adipose tissue and rat's testicles with high expression, and in white adipose tissue and liver with moderate expression [9, 10]. During lactation, *Gpat*4 mRNA is highly expressed in the mammary gland of the rats [11]. GPAT4 plays a very important role in TAG synthesis and the maintenance of the energy balance in tissues during lactation [12]. It has been also found that by isolating adipocytes, oxytocin raises lipogenesis [13]. GPAT1 activity has a major effect on TAG regulation. In addition to the synthesis of glycerolipids, this enzyme is also involved in regulatory physiological function in adipose tissue and liver [14, 15].

Orexin, or hypocretin, is a neuropeptide produced in the lateral hypothalamus and is derived from prepro-orexin (PPO) 130 amino acid peptide in rats with a 33-amino acid peptide, orexin A (OXA), along with a 28-amino acid peptide, orexin B (OXB) [16]. These two peptides have two types of receptors called orexin-1 (OX1R) and arexin-2 (OX2R) bound to G-protein [17]. Orexin decreased oxytocin and the effect of a specific OX1RA receptor antagonist was reported to have antagonized oxytocin secretion and increased oxytocin [18]. The high distribution of orexinergic neurons and orexin receptors in various tissues and regions have shown its role in regulating neuroendocrine functions such as nutrition, metabolism, sleep and wakefulness, and controlling reproductive processes [19]. Orexins have different affinity for these two receptors; while OX1R binds to OXA with more specificity, OX2R shows the same inclination to both OXs. For this reason, the antagonists that have been designed and developed for OXRs to date also have such properties [20]. These antagonists include antagonists that act selectively like OX1R (such as SB-334867 compound) or OX2R (such as JNJ-10397049 compound) and those that act dually, non-selectively (such as ACT-078573 compound) [21]. Studies have shown that orexin reduces prolactin through dopamine [22]. The decline in the expression of orexin during pregnancy and lactation increases prolactin and after 48 to 72 h of starvation during lactation, the NPY mRNA levels decrease and MCH mRNA and PPO increase, resulting in increased appetite and decreased prolactin and lactation [23].

Given the importance of milk availability during lactation and proper nutrition of infants, as well as the prominent role of two enzymes, namely Gpat1 and Gpat4 in the milk lipid synthesis of the mammary gland, this study is aimed at investigating the effect of OX1R and OX2R antagonists on the milk levels of TAG as well as the expression of *Gpat1* and *Gpat4* genes in the mammary gland.

2. Materials and Methods

2.1. Animals

In this study, 24 lactating Wistar rats that were having their second pregnancy [24] and weighed 200 to 250 g were randomly selected and used. These rodents were maintained at a temperature of 22 ± 2 °C under twelve-hour dark/light period conditions with relative humidity of 50%. At this stage, the animals had free, unlimited access to water and food and were transferred to this place one week prior to the experiments to acclimate to the laboratory environment. The animals were treated according to the international standards of working with animals. Moreover, the ethics committee of Shahid Beheshti University supervised the whole process and approved it with the code IR.SBU.REC 1399.043.

2.2. Materials

The materials used in this study included SB-334867 antagonist (Orexin1 receptor antagonist, purchased from Tocris) and JNJ-10397049 (Orexin2 receptor antagonist, purchased from Tocris) which were diluted in a volume ratio of 1:1 (v/v).

2.3. General Procedure, Milking, and Surgical Methods

Rats were randomly divided into one control group and six groups receiving intraductal OX1R and OX2R antagonists at doses of 5, 10, and 20 µg/kg of B.W.. The doses were selected based on our previous experience and further optimized using pilot studies [25, 26]. In order to inject into the mammary gland, all the 12 pairs of mammary glands received injection [27]. Anesthesia control of the animal was performed using isoflurane by the inhalation of 2-4% of the concentration of this substance through the nasal passage. Meanwhile, their eyes were oily and closed; subcutaneously, meloxicam (5-10 mg/kg) was used to anesthetize the animals. The hair around the nipple was removed with a cream and the nipple was cleansed with alcohol. Using a pair of micro-dissecting tweezers, we lifted the skin around the nipple to open the nipple. Then, a 20 µL insulin syringe with a gauge of 31, containing OX1R and OX2R antagonists was gently injected into the nipple duct. After the injection, the animals were isolated from isoflurane to regain consciousness. Six hours after the injection, rat milking was performed with Rat milking device (WAT-2006, Tokyo, Japan) and fresh milk samples were collected and stored in -20 °C until TAG measurement [28]. Then the animals went to deep anesthesia and subsequently the mammary gland tissue was removed and kept at -80 °C for later analysis [29]. The rats were euthanized after the experiment using a chamber prefilled with carbon dioxide (CO_2) gas with a concentration of 70%, which is a common and safe method for euthanizing [30].

2.4. Measurement of TAG

Using Rat EnzyChrom Triglyceride Assay Kit (Cat# ETGA-200- BioAssay System, USA), according to company's instructions with ELISA Leader BioTek 800TS (USA). The TAG assay was run in three replicates for each sample and it was measured at 570 nm.

2.5. RNA Extraction and Gene Expression

RNA breast tissue was extracted using the Gene JET RNA Purification Kit (Thermo Scientific, USA). To remove possible DNA contamination, the whole RNA was treated with DNase (Thermo Scientific, USA) and then used for the cDNA synthesis using the Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific, USA). All steps were performed according to factory instructions. Real Time PCR was used to measure target genes using specific primers for each gene (Table 1).

Table 1. Primers used in this study (From left to right 5´ → 3´).

Gene	Forward	Reverse
β actin	GTGGGGCGCCCCAGGCACCA	GTCCTTAATGTCACGCACGATTTC
GPAT1	GTCCAAAGCCATCCAGAAAG	GAAACAAGAGCGGCAGATTC
GPAT4	ATGTTCCTGTTGCTGCCTTT	GGTTCTTCTCCTTGGCTC CT

β actin= beta actin, Gpat1= glycerol-3-phosphate acyltransferase1, Gpat4= glycerol-3-phosphate acyltransferase4.

Relative gene expression for each sample was performed by the SYBR Premix Ex TaqTM II kit (TakaRa, Japan) using the Corbett-RG 6000X device (Corbett Research, Australia). In each sample in the strips, the samples are duplicated and their volume is 20 µL. Cycle threshold (CT) then recognized the reaction and calculated the CT genes based on it. Finally, the target genes are calculated relative to the reference gene using the formula $2^{-\Delta\Delta CT}$ [25].

2.6. Data Analysis

Findings were analyzed using GraphpadPrism software, the results were analyzed as median (min, max) from the mean of the evaluation. The data normality condition was assessed by Shapiro Wilk test; then, the data were measured by one-way test of ANOVA and the Tukey post hoc. Data differences were considered significant at P <0.05 and graphs were plotted using GraphpadPrism 8 software.

3. Results and Discussion

The results of this study revealed that the injection of OX1RA at doses of 5, 10, and 20 µg/kg compared to the control group had a significant difference in terms of decreased TAG milk level (P <0.001, P <0.01, P <0.001, respectively) (Figure 1).

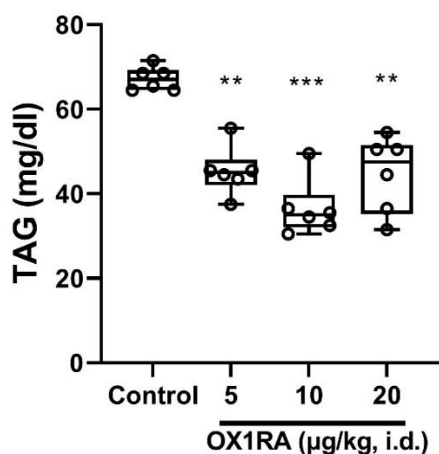

Figure 1. TAG level in the groups of control, OX1RA5, OX1RA10, and OX1RA20. ** P <0.01, *** P <0.01 compared to that of the control group. Data were presented as median (min, max) (n = 6).

The injection of OX2RA at a dose of 10 µg/kg compared to a dose of 20 µg/kg showed a significant difference in terms of decreased TAG milk level (P <0.002) (Figure 2).

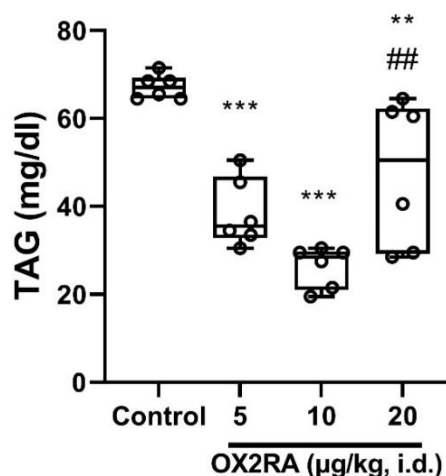

Figure 2. TAG level in the groups of control, OX2RA5, OX2RA10, and OX2RA20. ** P <0.01, *** P <0.01 compared to that of the control group, ## P <0.01 compared to OX2RA2. Data were presented as median (min, max) (n = 6).

The results of this study show that the injection of OX1RA at doses of 5 µg/kg and 10 µg/kg showed a significant difference in terms of reduced expression of *Gpat1* gene compared to the control group (P <0.001, P = 0.024) (Figure 3).

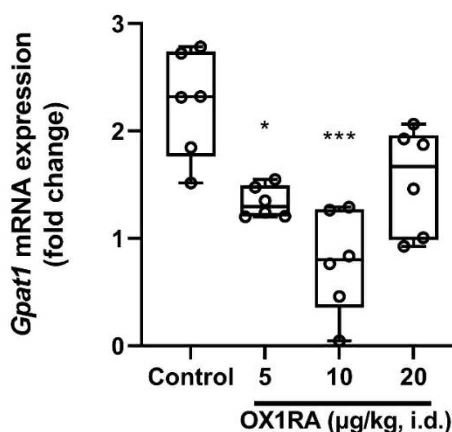

Figure 3. *Gpat1* gene expression in the groups of control, OX1RA5, OX1RA10, and OX1RA20. * P <0.05 compared with the control group, *** P <0.001 compared with the control group. Data were presented as median (min, max) (n = 6).

Injection of OX2RA at a dose of 10 µg/kg compared to the control group showed a significant difference in terms of decreased expression of *Gpat1* gene (P <0.001) (Figure 4).

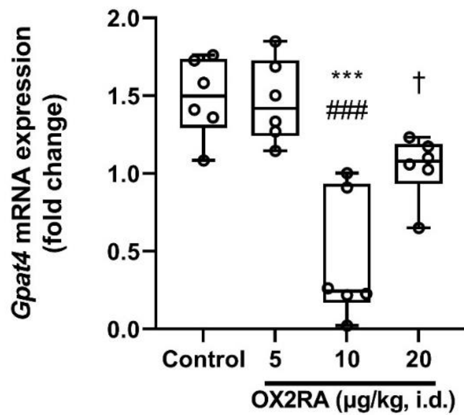

Figure 4. *Gpat1* gene expression in the groups of control, OX2RA5, OX2RA10, and OX2RA20. *** P <0.001 compared with the control group. Data were presented as median (min, max) (n = 6).

The results of this study show that the injection of OX1RA at a dose of 10 µg/kg compared to the control group showed a significant difference in terms of decreased expression of *Gpat4* gene (P <0.001). Moreover, injection of OX1RA with a dose of 5 µg/kg compared to a dose of 10 µg/kg and a dose of 10 µg/kg compared to a dose of 20 µg/kg showed a significant decrease of *Gpat4* gene (*P*<0.001) (Figure 5).

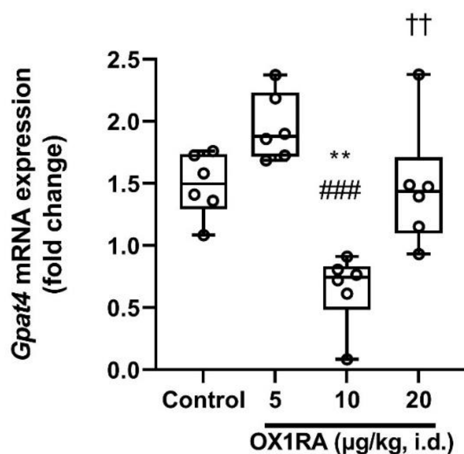

Figure 5. *Gpat4* gene expression the groups of control, OX1RA5, OX1RA10, and OX1RA20. ** P <0.01 compared to control group, ### P <0.001 compared to OX1RA5, †† P <0.01 compared to OX1RA10. Data were presented as median (min, max) (n = 6).

The injection of OX2RA at a dose of 10 µg/kg showed a significant difference in terms of decreased expression of *Gpat4* gene (P <0.001). Likewise, compared to the control group also, injection of OX2RA with a dose of 1µg/kg compared to a dose of 10 µg/kg and a dose of 10 µg/kg compared to a dose of 20 µg/kg resulted in a significant decrease expression of *Gpat4* gene (respectively P = 0.044, P <0.001) (Figure 6).

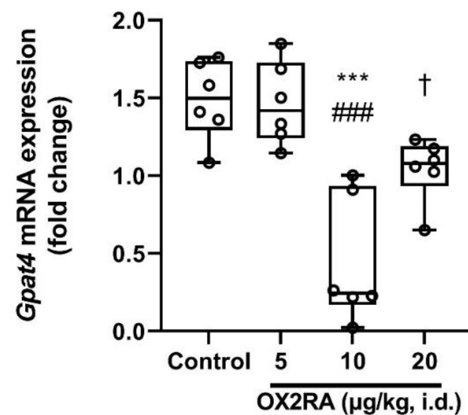

Figure 6. *Gpat4* gene expression in the groups of control, OX2RA5, OX2RA10, and OX2RA20. *** P <0.001 compared to control group, ### P <0.001 compared to OX2RA5, † P <0.05 compared to OX2RA10. Data were presented as median (min, max) (n = 6).

The present study was the first scrutiny in the literature on the intra-ductal injection of OX1RA and OX2RA into the mammary gland and the effects of their interaction on gene expression of some important enzymes in lipid synthesis such as GPAT1 and GPAT4. The impacts of this injection on TAG measurements were also discussed. The results of the current study reveal that these injections reduce the expression of *Gpat1* and *Gpat4* genes, and also decrease TAG.

In the present study, the effects of intra-ductal injection of OX1RA and OX2RA antagonists on some lipid synthesis enzymes were investigated and the results showed that these enzymes are reduced by injection. The paucity of research into the effect of OXS on lipid synthesis enzymes is witnessed in

the literature. Digby et al. demonstrated the role of orexin in fat metabolism by isolating adipocytes and treated with OXA and OXB [31]. Another study by Wortley et al. show that there is the relationship between orexin and fat metabolism and discovered that a high-fat diet with high triglycerides stimulates the expression of the orexin gene [32]. Skrzypski et al. showed that isolating rats' OXA fat cells increased TAG and lipogenesis and decreased lipolysis [33]. Pruszynska-Oszmalek et al. also observed that the isolation of pigs' OXA adipocytes, rather than OXB, is associated with the regulation of porcine lipid metabolism [34]. These resulting discrepancies regarding OXB effect can be interpreted as such that injecting OXA and OXB antagonists into rats' mammary glands bring about a different result in comparison to the time when the fat cells of the pigs' uterus becomes isolated.

In the present study, the effect of injections of OX1RA and OX2RA antagonists on *Gpat*4 gene expression was investigated and the results showed that these injections reduce *Gpat4* gene expression. Beigneux et al. demonstrated the isoforms of this enzyme and also showed that the enzyme is highly expressed in the mammary gland during lactation and plays an important role in the synthesis of triacylglycerol [11].

The effect of injections of OX1RA and OX2RA antagonists on *Gpat1* gene expression was investigated and the results showed that these injections reduce the expression of *Gpat1* gene. Collison et al. identified the isoform of the enzyme [35], and years later, Coleman and Lee contended that this enzyme is highly expressed in adipose tissue [15].

This is the first study to be conducted on the above mentioned gap and has a number of limitations including financial limitations. Further research avenues are warranted for testing more genes and paving the way for a more vivid picture, especially in the immunohistochemical areas.

4. Conclusion

Overall, our results exhibited that the intra-ductal injection of antagonists into the mammary gland decrease TAG levels. Moreover, the final result of the injection of OX1RA and OX2RA was found to decrease the expressions of *GPAT1* and *GPAT4* genes.

Acknowledgments

The authors wish to state that there was no conflict of interest in this study. All authors have read and confirmed the final article. This study is financially supported by Shahid Beheshti University under grant number [D/130/1/3172].

References

[1] Wilde CJ, Knight CH and Flint DJ. Control of milk secretion and apoptosis during mammary involution. *J. Mammary Gland Biol. Neoplasia* (1999) 4: 129-36.

[2] Grigor MR, Poczwa Z and Arthur PG. Milk lipid synthesis and secretion during milk stasis in the rat. *J. Nutr*. (1986) 116: 1789-97.

[3] Heesom KJ, Souza PF, Ilic V and Williamson DH. Chain-length dependency of interactions of medium-chain fatty acids with glucose metabolism in acini isolated from lactating rat mammary glands. A putative feed-back to control milk lipid synthesis from glucose. *Biochem. J*. (1992) 281: 273-8.

[4] Rohlfs EM, Louie DS and Zeisel SH. Lipid synthesis and secretion by primary cultures of rat mammary epithelial cells. *J. Cell Physiol*. (1993) 157: 469-80.

[5] Takeuchi K and Reue K. Biochemistry, physiology, and genetics of GPAT, AGPAT, and lipin enzymes in triglyceride synthesis. *Am. J. Physiol. Endocrinol. Metab*. (2009) 296: E1195-209.

[6] Ganesh Bhat B, Wang P, Kim JH, Black TM, Lewin TM, Fiedorek FT, Jr. and Coleman RA. Rat sn-

glycerol-3-phosphate acyltransferase: molecular cloning and characterization of the cDNA and expressed protein. *Biochim. Biophys. Acta* (1999) 1439: 415-23.

[7] Chen YQ, Kuo MS, Li S, Bui HH, Peake DA, Sanders PE, Thibodeaux SJ, Chu S, Qian YW, Zhao Y, Bredt DS, Moller DE, Konrad RJ, Beigneux AP, Young SG and Cao G. AGPAT6 is a novel microsomal glycerol-3-phosphate acyltransferase. *J. Biol. Chem*. (2008) 283: 10048-57.

[8] Hasegawa-Sasaki H and Ohno K. Extraction and partial purification of acyl-CoA:1-acyl-sn-glycero-3-phosphocholine acyltransferase from rat liver microsomes. *Biochim. Biophys. Acta* (1980) 617: 205-17.

[9] Wang H, Airola MV and Reue K. How lipid droplets "TAG" along: Glycerolipid synthetic enzymes and lipid storage. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* (2017) 1862: 1131-45.

[10] Cooper DE, Grevengoed TJ, Klett EL and Coleman RA. Glycerol-3-phosphate Acyltransferase Isoform-4 (GPAT4) Limits Oxidation of Exogenous Fatty Acids in Brown Adipocytes. *J. Biol. Chem*. (2015) 290: 15112-20.

[11] Beigneux AP, Vergnes L, Qiao X, Quatela S, Davis R, Watkins SM, Coleman RA, Walzem RL, Philips M, Reue K and Young SG. Agpat6--a novel lipid biosynthetic gene required for triacylglycerol production in mammary epithelium. *J. Lipid Res*. (2006) 47: 734-44.

[12] Nagle CA, Vergnes L, Dejong H, Wang S, Lewin TM, Reue K and Coleman RA. Identification of a novel sn-glycerol-3-phosphate acyltransferase isoform, GPAT4, as the enzyme deficient in Agpat6-/- mice. *J. Lipid Res*. (2008) 49: 823-31.

[13] Eriksson H, Björgell P, Akerlund M, Hauksson A and Melin P. Effects of a tocolytic oxytocin analogue on lipid and carbohydrate metabolism. *Gynecol. Obstet. Invest*. (1990) 29: 97-100.

[14] Kim SJ, Kim JE, Kim YW, Kim JY and Park SY. Nutritional regulation of renal lipogenic factor expression in mice: comparison to regulation in the liver and skeletal muscle. *Am. J. Physiol. Renal Physiol*. (2017) 313: 887-98.

[15] Coleman RA and Lee DP. Enzymes of triacylglycerol synthesis and their regulation. *Prog. Lipid Res*. (2004) 43: 134-76.

[16] Wang C, Wang Q, Ji B, Pan Y, Xu C, Cheng B, Bai B and Chen J. The Orexin/Receptor System: Molecular Mechanism and Therapeutic Potential for Neurological Diseases. *Front. Mol. Neurosci*. (2018) 11: 220.

[17] Scammell TE and Winrow CJ. Orexin receptors: pharmacology and therapeutic opportunities. *Annu. Rev. Pharmacol. Toxicol*. (2011) 51: 243-66.

[18] Ocskó T, Gálfi M, Radács M, Molnár Z, Kis GK, Rákosi K, Molnár AH, László F, László FA and Varga C. Effects of orexin-monoaminergic interactions on oxytocin secretion in rat neurohypophyseal cell cultures. *Regul. Pept*. (2012) 175: 43-8.

[19] Steiner MA, Sciarretta C, Brisbare-Roch C, Strasser DS, Studer R and Jenck F. Examining the role of endogenous orexins in hypothalamus-pituitary-adrenal axis endocrine function using transient dual orexin receptor antagonism in the rat. *Psychoneuroendocrinology* (2013) 38: 560-71.

[20] Goforth PB and Myers MG. Roles for Orexin/Hypocretin in the Control of Energy Balance and Metabolism. *Curr. Top. Behav. Neurosci*. (2017) 33: 137-56.

[21] Sakurai T. The role of orexin in motivated behaviours. *Nat. Rev. Neurosci*. (2014) 15: 719-31.

[22] Fujisawa S, Komatsubara M, Ogura-Ochi K, Tsukamoto-Yamauchi N, Toma K, Inagaki K, Wada J and Otsuka F. Orexin A modulates prolactin production by regulating BMP-4 activity in rat pituitary lactotorope cells. *Peptides* (2019) 113: 35-40.

[23] Hsueh YC, Cheng SM and Pan JT. Fasting stimulates tuberoinfundibular dopaminergic neuronal activity and inhibits prolactin secretion in oestrogen-primed ovariectomized rats: involvement of orexin A and neuropeptide Y. *J. Neuroendocrinol*. (2002) 14: 745-52.

[24] Sutherland RC, Aizlewood ES and Wakerley JB. Changing characteristics of the milk-ejection reflex during pregnancy, lactation and after weaning in the rat. *J. Reprod. Fertil*. (1986) 76: 123-30.

[25] Hosseini A and Khazali H. Central Orexin A Affects Reproductive Axis by Modulation of Hypothalamic Kisspeptin/Neurokinin B/Dynorphin Secreting Neurons in the Male Wistar Rats. *Neuromolecular Med*. (2018) 20: 525-36.

[26] Faedo S, Perdonà E, Antolini M, di Fabio R, Merlo Pich E and Corsi M. Functional and binding kinetic studies make a distinction between OX1 and OX2 orexin receptor antagonists. *Eur. J. Pharmacol*. (2012) 692: 1-9.

[27] Kenyon E, Westerhuis JJ, Volk M, Hix J, Chakravarty S, Claucherty E, Zaluzec E, Ramsey L, Madaj Z, Hostetter G, Eagleson B, Shapiro E, Moore A and Sempere LF. Ductal tree ablation by local delivery of ethanol prevents tumor formation in an aggressive mouse model of breast cancer. *Breast Cancer Res*. (2019) 21: 129.

[28] Okabayashi K and Watanabe T. Excretion of bisphenol A into rat milk. *Toxicol. Mech. Methods*. (2010) 20: 133-6.

[29] Krause S, Brock A and Ingber DE. Intraductal injection for localized drug delivery to the mouse mammary gland. *J. Vis. Exp*. (2013) 80: 50692.

[30] Conlee KM, Stephens ML, Rowan AN and King LA. Carbon dioxide for euthanasia: concerns regarding pain and distress, with special reference to mice and rats. *Lab Anim*. (2005) 39: 137-61.

[31] Digby JE, Chen J, Tang JY, Lehnert H, Matthews RN and Randeva HS. Orexin receptor expression in human adipose tissue: effects of orexin-A and orexin-B. *J. Endocrinol*. (2006) 191: 129-36.

[32] Wortley KE, Chang GQ, Davydova Z and Leibowitz SF. Peptides that regulate food intake: orexin gene expression is increased during states of hypertriglyceridemia. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. (2003) 284: 1454-65.

[33] Skrzypski M, T TL, Kaczmarek P, Pruszynska-Oszmalek E, Pietrzak P, Szczepankiewicz D, Kolodziejski PA, Sassek M, Arafat A, Wiedenmann B, Nowak KW and Strowski MZ. Orexin A stimulates glucose uptake, lipid accumulation and adiponectin secretion from 3T3-L1 adipocytes and isolated primary rat adipocytes. *Diabetologia* (2011) 54: 1841-52.

[34] Pruszynska-Oszmalek E, Kolodziejski PA, Kaczmarek P, Sassek M, Szczepankiewicz D, Mikula R and Nowak KW. Orexin A but not orexin B regulates lipid metabolism and leptin secretion in isolated porcine adipocytes. *Domest. Anim. Endocrinol*. (2018) 63: 59-68.

[35] Collison LW, Murphy EJ and Jolly CA. Glycerol-3-phosphate acyltransferase-1 regulates murine T-lymphocyte proliferation and cytokine production. *Am. J. Physiol. Cell Physiol*. (2008) 295: 1543-9.