

Up-regulation of Oxytocin Receptor Gene and Protein in the Sheep Anterior Pituitary by a Dopamine Derivative (Salsolinol)

KONRAD GÓRSKI^{1*}, MAŁGORZATA HASIEC¹, MARLENA ZIELIŃSKA-GÓRSKA², FERENC FÜLÖP³, TOMASZ MISZTAŁ¹

¹Department of Endocrinology, The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna, Poland

²Department of Neuroendocrinology, The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna, Poland

³Institute of Pharmaceutical Chemistry and Research Group for Stereochemistry, Hungarian Academy of Sciences, University of Szeged, Szeged, Hungary

*Corresponding author: k.gorski@ifzz.pl

ABSTRACT

Górski K., Hasiec M., Zielińska-Górska M., Fülöp F., Miształ T. (2017): **Up-regulation of oxytocin receptor gene and protein in the sheep anterior pituitary by a dopamine derivative (salsolinol)**. Czech J. Anim. Sci., 62, 150–156.

Specific oxytocin receptors (OTR) have been identified in the anterior pituitary (AP), and their expression has been shown to change in relation to the animal physiological stage, whereas salsolinol (a derivative of dopamine) has been shown to stimulate the synthesis and release of oxytocin (OT) in lactating sheep. In the present study, the expression of both *OTR* mRNA and OTR protein in the AP were examined by real-time quantitative PCR and enzyme-linked immunosorbent assay, either in anestrus or lactating sheep 48 h after weaning lambs. Moreover, the effect of salsolinol administered via an intracerebroventricular (i.c.v.) infusion was tested in additional sheep at the same physiological stages. The i.c.v. infusions of Ringer-Locke (control) and salsolinol solutions were carried out from 10:00 to 15:00 h in a serial manner, i.e. five 30-min infusions at 30-min intervals. We observed both *OTR* gene and OTR protein expression in the AP, in both anestrus and lactating sheep, but it was significantly ($P < 0.01$ and $P < 0.05$, respectively) higher in the AP of lactating animals compared to anestrus animals. Salsolinol i.c.v. treatment in anestrus sheep evoked a significant ($P < 0.05$) increase in both *OTR* gene and OTR protein expression compared to control animals. In contrast, salsolinol did not affect either *OTR* gene or OTR protein expression in lactating sheep. In conclusion, the expression of OTR in the sheep AP is upregulated by salsolinol. The effect of salsolinol was more pronounced in non-lactating sheep, with a reduced response due to ongoing OTR expression in lactating animals. Increased expression of OTR in the AP of lactating sheep may be related to the stimulation of pituitary lactotrophs by OT following the release of prolactin during suckling.

Keywords: oxytocin; sheep; lactation

The peptide oxytocin (OT) is synthesized in the magnocellular neurosecretory cells of the hypothalamic supraoptic (SON) and paraventricular (PVN)

nuclei, and is stored in the axon terminals located in the posterior pituitary (Gimpl and Fahrenholz 2001; Bealer et al. 2010; Kochman 2013). Activa-

Supported by the National Science Centre, Poland (grant No. N N311 517140) and by subsidy from the Ministry of Science and Higher Education, Poland.

doi: 10.17221/30/2016-CJAS

tion of these hypothalamic neurons leads to OT release into the circulation where it is transported to different peripheral tissues (Gimpl and Fahrenholz 2001). OT released from centrally projecting oxytocinergic neurons forms the active pool of the hormone in the brain (Sabatier et al. 2007). Its actions are mediated by a specific oxytocin receptor (OTR), which is a G protein-coupled receptor. OT has been extensively studied for its numerous peripheral and central functions; the most well-known are inducing uterine contractions during parturition and milk ejection during lactation, as well as its effects on sexual, feeding, maternal, and social behaviours. Indeed, released under the influence of suckling stimulus, OT is essential for the milk ejection reflex, as it acts on myoepithelial cells of the alveoli to induce the movement of milk from the place of storage to the nipple (Marnet et al. 1998).

Evidence exists that OTR are present in the anterior pituitary (AP), and their expression increases during gestation (Breton et al. 1995; Zingg and Laporte 2003). One of the best documented OT actions in this tissue is the stimulation of prolactin (PRL) release (Samson and Schell 1995; Kennett and McKee 2012). During lactation, the OT surge induced by suckling is accompanied by a PRL surge (Kennett and McKee 2012). Kennett et al. (2009) have shown that intravenous administration of an OT antagonist attenuates the PRL surge induced by the suckling stimulus.

In recent years, salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) has emerged as an important neural factor regulating the hormones involved in lactation. As a dopamine derivative, salsolinol has been generally associated with dysfunction of dopaminergic neurons, though presently it is also considered to be a physiological stimulator of PRL release in rodents (Toth et al. 2001) and ruminants (Hashizume et al. 2008; Misztal et al. 2008). In lactating sheep, the suckling stimulus increases the extracellular content of salsolinol in the infundibular nucleus-median eminence, simultaneously with PRL release into the peripheral circulation (Misztal et al. 2008). Intracerebroventricular (i.c.v.) infusion of salsolinol increases PRL release (Gorski et al. 2010), and stimulates *PRL* gene expression in the AP of lactating sheep (Hasić et al. 2012). Moreover, an i.c.v. infusion of a salsolinol analogue, 1-methyl-3,4-dihydroisoquinoline (1-MeDIQ) in sheep reduces basal

PRL release and attenuates the suckling-induced PRL surge (Gorski et al. 2010; Misztal et al. 2010). Our recent studies have shown that salsolinol may also be an OT stimulating factor during lactation. Salsolinol, when i.c.v. administered in lactating sheep, upregulated OT gene expression in the PVN and SON, increased OT peptide content in the posterior pituitary, and stimulated OT release into the peripheral circulation (Gorski et al. 2016).

To further extend our research on the relationship between salsolinol and OT, in the present study, we have determined the changes in *OTR* gene and protein expression in the AP by comparing anestrus and lactating sheep. Additionally, we have investigated the effect of salsolinol administered via i.c.v. infusion on OTR expression.

MATERIAL AND METHODS

Animal management. All experimental procedures were conducted in accordance with the Polish Guide for the Care and Use of Animals and approved by the Local Ethics Committee. The experiment was performed on two groups of Polish Longwool sheep: Group 1, anestrus sheep ($n = 12$), and Group 2, lactating sheep, 48 h after the weaning of 8-week-old lambs ($n = 12$). Sheep in Group 2 were mated naturally in September and lambled during the following February. All animals were maintained indoors in individual pens under natural lighting conditions (52°N, 21°E). They were fed twice a day a diet formulated for non-pregnant or pregnant and lactating sheep, according to the recommendations of the National Research Institute of Animal Production, Krakow, Poland (1993 norms), hay and water were available *ad libitum*. Sheep were well-adapted to the experimental conditions, and always had visual contact with neighbouring sheep, even during the experimental period, to prevent stress as a result of social isolation.

Brain surgery. Anestrus and pregnant (third month of pregnancy) sheep were implanted with a stainless steel guide cannula (outer diameter 1.4 mm) into the third ventricle of the brain. The implantation was performed under general anaesthesia (a mixture of xylazine 40 mg/kg body weight, and xylapan and ketamine 10–20 mg/kg body weight, administered intravenously; all Bio-wet, Poland), through a drill hole in the skull, in

accordance with the stereotactic coordinate system for the sheep hypothalamus (Welento et al. 1969), using the procedure described by Traczyk and Przekop (1963). The guide cannulae were fixed to the skull with stainless steel screws and dental cement Villacryl S (Zhermapol, Poland). The external opening of the cannula was closed with a stainless steel cap. The placement of the cannula was confirmed during surgery by the outflow of the cerebrospinal fluid, and postmortem by the injection of blue ink.

Drug treatment and experimental design. Salsolinol was synthesized and kindly provided by Prof. Ferenc Fülöp from the Institute of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Szeged, Hungary. It was dissolved in Ringer-Locke's solution (RL), then aliquoted and stored at -20°C . A new aliquot of the drug solution was used for each infusion to maintain the biological activity of the molecules during the experiment.

Both anestrus and lactating sheep were randomly divided into two groups: control ($2 \times n = 6$) and experimental ($2 \times n = 6$), and were given an i.c.v. infusion of RL or salsolinol solution, respectively. The infusions were carried out from 10:00 to 15:00 h in a serial manner (five 30-min infusions at 30-min intervals) using a BAS Bee™ microinjection pump and calibrated 1.0-ml gas-tight syringes (Bioanalytical Systems Inc., USA). The salsolinol dosage ($5 \times 15 \mu\text{g}/60 \mu\text{l}/30 \text{ min}$) was selected based on our previous studies (Gorski et al. 2010; Hasić et al. 2012). Immediately after the end of the experiment (15:00 h) sheep were deeply anaesthetized with sodium pentobarbital (30 mg/kg body weight; Biowet) and euthanized by decapitation. The AP was rapidly dissected, immediately flash frozen in liquid nitrogen, and stored at -80°C until assayed.

Gene expression assay. Tissue samples were homogenized using a TissueLyser LT bead mill (Qiagen, Germany). Total RNA was extracted and purified using a NucleoSpin RNA II kit (Macherey-Nagel GmbH & Co., Germany) with on-column DNase treatment, in accordance with the manufacturer's instructions. The total RNA concentration and purity were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA). Sample integrity was assessed electrophoretically using ethidium bromide-stained 1.5% agarose gels. Total RNA (1 g in a volume of 20 l) was reverse-transcribed into first-strand cDNA

using a Maxima First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) in accordance with the manufacturer's instructions.

Relative amounts of specific *OTR* transcripts were determined by real-time quantitative PCR (qPCR) analysis, using a Rotor-Gene Q cycler (Qiagen). *OTR* mRNA analysis was performed using the Luminaris HiGreen qPCR Master Mix (Thermo Fisher Scientific) and HPLC-grade oligonucleotide primers (Genomed, Poland). Specific primers for determining the expression of the gene of interest and endogenous control genes were designed with Primer3 software (Untergasser et al. 2012). The qPCR reactions were run in triplicate using the following protocol: initial denaturation and enzyme activation at 95°C for 15 min, followed by 35 cycles of denaturation at 95°C for 15 s, annealing at 56°C for 20 s, and extension at 72°C for 20 s. After the cycles, a final melting curve analysis with continuous fluorescence measurements was performed to confirm the specificity of the amplification. To confirm that single amplification products were produced, samples were stained with ethidium bromide and electrophoresed on an agarose gel before visualization under a UV light camera. The *OTR* mRNA levels were normalized to the endogenous control gene, *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)*. Initially, three housekeeping genes – *GAPDH*, β -*actin (ACTB)*, and *tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (YWHAZ)* – were tested. The BestKeeper software (Pfaffl et al. 2004) was used to determine the most stable housekeeping gene for normalizing the expression of genes of interest. All primers are listed in Table 1.

ELISA protein quantification. Frozen AP was homogenized in ice cold phosphate-buffered saline (pH 7.4) (Sigma-Aldrich, USA) using the TissueLyser LT bead mill. Afterwards, samples were frozen at -20°C and stored overnight. After two freeze-thaw cycles, the homogenates were centrifuged at 5000 g for 5 min at 4°C , then supernatant fluids were removed and assayed immediately. The concentration of *OTR* protein in the AP extracts was determined with a commercial ELISA kit (Sheep *OTR* ELISA Kit; Mybiosource, USA). The assay sensitivity was 0.094 ng/ml, and both intra- and inter-assay variability was 10%. The absorbance measurement at 450 nm was performed using a Spectramax M2 microplate reader (Molecular Devices LLC, USA). The obtained results were

doi: 10.17221/30/2016-CJAS

Table 1. Sequences of the primers used in the qPCR analysis

Gene	GenBank Acc. No.	Primer sequence
OTR	NM_001009752	F: TCAGCAACGTCAAGCTCATC
		R: TCCACATCTGCACGAAAAA
GAPDH	NM_001034034	F: AGAAGGCTGGGGCTCACT
		R: GGCATTGCTGACAATCTTGA
ACTB	NM_001009784	F: CTTCTTCTGGGCATGG
		R: GGGCAGTGATCTCTTTCTGC
YWHAZ	NM_001267887	F: AGACGGAAGGTGCTGAGAAA
		R: GAAGCGTTTGGGATCAAGAA

F = forward primer, R = reverse primer

normalized to the total protein concentration in samples, assayed with the Bradford reagent (Sigma-Aldrich).

Data analysis. RotorGene Q software (Qiagen) was used to generate and collate the qPCR data. The relative gene expression was determined using the Relative Expression Software Tool 2008 (Qiagen). The results are presented as the relative gene expression of the target gene compared with the endogenous control gene. The average relative quantity of gene expression in the control group was set to 1.0.

The non-parametric Mann-Whitney U test was used to assess the significance of the differences in *OTR* mRNA and *OTR* protein expression between the experimental and control groups. Calculations were performed using STATISTICA software (Version 10). All data are expressed as mean \pm SEM.

RESULTS

The analyses revealed the presence of specific *OTR* transcripts and *OTR* protein in the AP of both anestrus and lactating sheep. *OTR* mRNA expression was up-regulated ($P < 0.001$) in lactating sheep when compared to the AP of anestrus sheep (Figure 1A). Similarly, the *OTR* protein content in the AP of lactating sheep was increased ($P < 0.05$) compared to the levels in anestrus animals (Figure 1B).

Salsolinol infused into the third ventricle of the brain up-regulated ($P < 0.01$) *OTR* mRNA expression in the AP of anestrus sheep. However, in lactating sheep, treatment with salsolinol was ineffective (Figure 1A). Salsolinol administered via i.c.v. infusion also evoked a significant ($P < 0.05$) increase in the *OTR* protein content in the AP of

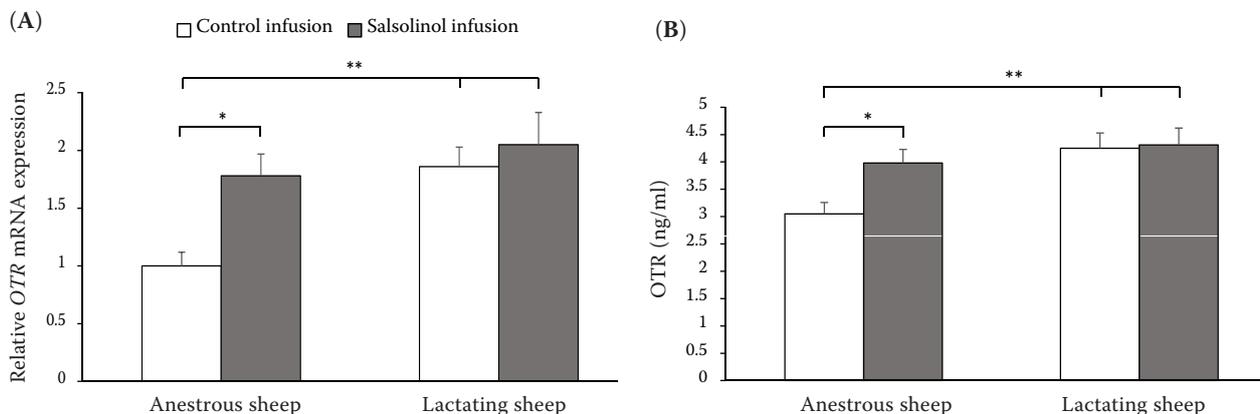


Figure 1. Relative oxytocin receptor (*OTR*) mRNA expression (A) and oxytocin receptor (*OTR*) polypeptide expression (B) in the anterior pituitary of anestrus sheep ($n = 12$) and lactating sheep ($n = 12$) infused with vehicle (control; white bars) or salsolinol (grey bars). Data are presented as mean \pm SEM

** $P < 0.01$, * $P < 0.05$ according to the non-parametric Mann-Whitney U test

anestrous sheep, while in lactating animals, OTR protein expression was not significantly changed in response to salsolinol (Figure 1B).

DISCUSSION

The present study demonstrates the variations in *OTR* gene expression and OTR protein concentrations in the sheep AP, which were higher during lactation than in seasonal anestrus. Moreover, the dopamine derivative salsolinol, which is a stimulatory agent for the release of both PRL (Gorski et al. 2010) and OT (Gorski et al. 2016) in lactating sheep, also up-regulated the OTR contents in the AP, which were higher during lactation than during seasonal anestrus.

The central sites of OT synthesis are localized in the hypothalamic PVN and SON. Although most of OT neurons project into the posterior pituitary, where the neurohormone is stored and released into the circulation, OT also reaches the anterior pituitary through the short portal vessels connecting these two parts (Gibbs 1984; Samson and Schell 1995). Additionally, magnocellular oxytocinergic neurons innervate the median eminence, where they can act on the anterior pituitary (Yang et al. 2013). OTR immunoreactivity was found in the rat AP (Adan et al. 1995), and early studies showed that most OTR are present in lactotrophic cells (Breton et al. 1995) where OT plays an important role in stimulating PRL secretion (Samson et al. 1986). In addition, OT is involved in stimulating ACTH release from rat pituitary cells (Schlosser et al. 1994), and the *OTR* gene has also been found in mouse AP corticotrophs (Nakamura et al. 2008). Recently, Gonzales-Iglesias et al. (2015) provided evidence that gonadotrophs and somatotrophs, in addition to lactotrophs, are sensitive to OT stimulation, and responded with transient elevations in the intracellular Ca^{2+} concentration and hormone release in a dose-dependent manner. These responses were also evoked by an OTR agonist, and abolished by an OTR antagonist, suggesting that the OT-induced effects are mediated by OTRs in all three cell types (Gonzalez-Iglesias et al. 2015). It has been suggested that OT may act directly on different AP cell types to coordinate hormone release.

The increased OTR expression in the AP during lactation seen in our study may mediate the

biochemical adaptations of the pituitary cells, especially lactotrophs, to facilitate an increase in the PRL synthesis and secretion. Several groups have identified the ability of OT to act directly on AP lactotrophs to release PRL (Samson and Schell 1995; McKee et al. 2007; Tabak et al. 2010). McKee et al. (2007) demonstrated that OT action at the lactotroph is required for PRL surges in cervically stimulated ovariectomized rats. According to Samson et al. (1986), the OT surge precedes that of PRL in response to suckling. Moreover, attenuation of OT action by the administration of a selective OT antagonist (desGly-NH₂-d(CH₂)₅[D-Tyr₂,Thr₄]OVT) is able to block the suckling-induced PRL surge (Kennett et al. 2009). These data further support the thesis that OT stimulates PRL release from AP lactotrophs, and underline the importance of this phenomenon for lactation.

The salsolinol stimulatory effect on both *OTR* mRNA and OTR protein expression in the sheep AP was shown for the first time in this study, and extends our research. Interestingly, *OTR* mRNA levels as well as protein contents reached similar values in anestrous sheep as those seen in lactating animals. Although salsolinol was not detected in seasonally anestrous sheep (Misztal et al. 2008), the increase in OTR in the pituitary cells as a result of salsolinol treatment may indicate their preparedness for the signals associated with lactation. In turn, salsolinol administered via i.c.v. infusion in lactating sheep only slightly enhanced *OTR* mRNA expression. We assume that the level of OTR expression during lactation is so high that additional stimulating factors cannot evoke a further increase in expression. In contrast, a significant upregulation of *PRL* mRNA expression in the AP after i.c.v. treatment with salsolinol was evident 48 h after lamb separation (Hasić et al. 2012).

Based on available data, the proper mechanism of salsolinol action on OTR expression is difficult to determine. Although a specific salsolinol receptor has not been identified, high-affinity binding sites for salsolinol have been detected in the median eminence, hypothalamus, and AP (Toth et al. 2001; Homicsko et al. 2003). It has also been shown that OTRs are highly upregulated during parturition and lactation. Gonadal steroids are thought to be the key factors mediating this regulation. In the AP, a marked increase in OTR expression was observed after estrogen treatment in ovariectomized female rats (Breton and Zingg 1997; Quinones-Jenab et

doi: 10.17221/30/2016-CJAS

al. 1997). The *OTR* promoter sequence contains elements of the estrogen response element (ERE); however, it is not a completely functional ERE (Gimpl and Fahrenholz 2001). Estrogen modulation probably involves additional factors, and might be mediated via other functional promoter elements (Zingg and Laporte 2003). The sequence analysis of the rat *OTR* gene revealed the presence of cAMP response elements (CRE). Some data suggest that cAMP may induce the up-regulation of *OTR* gene expression. Treatment with forskolin, a compound that elevates the intracellular cAMP concentration, increases OT binding and *OTR* mRNA expression in rabbit amnion cells *in vitro* (Jeng and Soloff 2009). Additionally, dibutyryl-cAMP has been shown to increase *OTR* gene expression *in vitro* (Ivell et al. 2001). In turn, salsolinol has been shown to be a stimulator of cAMP accumulation in the AP (Radnai et al. 2005). Regarding these data, it is assumed that salsolinol may induce *OTR* gene expression in pituitary cells via the activation of intracellular cAMP. Further studies are required, especially concerning the identification of specific salsolinol receptors and the intracellular mechanism of its action.

In conclusion, the expression of *OTR* in the sheep AP is upregulated by salsolinol. The effect of salsolinol may be more pronounced in non-lactating sheep, with a less robust response observed with ongoing *OTR* expression in lactating animals. The increased *OTR* expression in the AP of lactating sheep may be related to the stimulation of pituitary lactotrophs by OT and the release of PRL during suckling.

Acknowledgement. The authors would like to thank Prof. Ferenc Fülöp (Szeged University, Hungary) for preparing and providing salsolinol, and veterinary surgeon J. Rutkowski for help with brain surgery.

REFERENCES

- Adan R.A., Van Leeuwen F.W., Sonnemans M.A., Brouns M., Hoffman G., Verbalis J.G., Burbach J.P. (1995): Rat oxytocin receptor in brain, pituitary, mammary gland, and uterus: partial sequence and immunocytochemical localization. *Endocrinology*, 136, 4022–4028.
- Bealer S.L., Armstrong W.E., Crowley W.R. (2010): Oxytocin release in magnocellular nuclei: neurochemical mediators and functional significance during gestation. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 299, 452–458.
- Breton C., Zingg H.H. (1997): Expression and region-specific regulation of the oxytocin receptor gene in rat brain. *Endocrinology*, 138, 1857–1862.
- Breton C., Pechoux C., Morel G., Zingg H.H. (1995): Oxytocin receptor messenger ribonucleic acid: characterization, regulation, and cellular localization in the rat pituitary gland. *Endocrinology*, 136, 2928–2936.
- Gibbs D.M. (1984): High concentrations of oxytocin in hypophysial portal plasma. *Endocrinology*, 114, 1216–1218.
- Gimpl G., Fahrenholz F. (2001): The oxytocin receptor system: structure, function, and regulation. *Physiological Reviews*, 81, 629–683.
- Gonzalez-Iglesias A.E., Fletcher P.A., Arias-Cristancho J.A., Cristancho-Gordo R., Helena C.V., Bertram R., Tabak J. (2015): Direct stimulatory effects of oxytocin in female rat gonadotrophs and somatotrophs *in vitro*: comparison with lactotrophs. *Endocrinology*, 156, 600–612.
- Gorski K., Romanowicz K., Herman A., Molik E., Gajewska A., Tomaszewska-Zaremba D., Misztal T. (2010): The possible involvement of salsolinol and hypothalamic prolactin in the central regulatory processes in ewes during lactation. *Reproduction in Domestic Animals*, 45, e54–e60.
- Gorski K., Marciniak E., Zielinska-Gorska M., Misztal T. (2016): Salsolinol up-regulates oxytocin expression and release during lactation in sheep. *Journal of Neuroendocrinology*, 28, 1–9.
- Hashizume T., Shida R., Suzuki S., Nonaka S., Yonezawa C., Yamashita T., Kasuya E., Sutoh M., Olah M., Szekacs D., Nagy G.M. (2008): Salsolinol is present in the bovine posterior pituitary gland and stimulates the release of prolactin both *in vivo* and *in vitro* in ruminants. *Domestic Animal Endocrinology*, 34, 146–152.
- Hasiec M., Herman A.P., Molik E., Dobek E., Romanowicz K., Fulop F., Misztal T. (2012): The stimulatory effect of salsolinol on prolactin gene expression within the anterior pituitary of lactating sheep: *in vivo* and *in vitro* study. *Small Ruminant Research*, 102, 202–207.
- Homicsko K.G., Kertesz I., Radnai B., Toth B.E., Toth G., Fulop F., Fekete M.I.K., Nagy G.M. (2003): Binding site of salsolinol: its properties in different regions of the brain and the pituitary gland of the rat. *Neurochemistry International*, 42, 19–26.
- Ivell R., Kimura T., Muller D., Augustin K., Abend N., Bathgate R., Telgmann R., Balvers M., Tillmann G., Fuchs R. (2001): The structure and regulation of the oxytocin receptor. *Experimental Physiology*, 86, 289–296.
- Jeng Y.J., Soloff M.S. (2009): Characterization of the cyclic adenosine monophosphate target site in the oxytocin

- receptor gene in rabbit amnion. *Biology of Reproduction*, 81, 473–479.
- Kennett J.E., McKee D.T. (2012): Oxytocin: an emerging regulator of prolactin secretion in the female rat. *Journal of Neuroendocrinology*, 24, 403–412.
- Kennett J.E., Poletini M.O., Fitch C.A., Freeman M.E. (2009): Antagonism of oxytocin prevents suckling- and estradiol-induced, but not progesterone-induced, secretion of prolactin. *Endocrinology*, 150, 2292–2299.
- Kochman K. (2013): Neurohormones: oxytocin, vasopressin and related peptides – structure, genes, receptors, and evolution. *Journal of Animal and Feed Sciences*, 22, 283–294.
- Marnet P.G., Negro J.A., Labussiere J. (1998): Oxytocin release and milk ejection parameters during milking of dairy ewes in and out of natural season of lactation. *Small Ruminant Research*, 28, 183–191.
- McKee D.T., Poletini M.O., Bertram R., Freeman M.E. (2007): Oxytocin action at the lactotroph is required for prolactin surges in cervically stimulated ovariectomized rats. *Endocrinology*, 148, 4649–4657.
- Misztal T., Gorski K., Tomaszewska-Zaremba D., Molik E., Romanowicz K. (2008): Identification of salsolinol in the mediobasal hypothalamus of lactating ewes and its relation to suckling-induced prolactin and GH release. *Journal of Endocrinology*, 198, 83–89.
- Misztal T., Gorski K., Tomaszewska-Zaremba D., Fulop F., Romanowicz K. (2010): Effects of a structural analogue of salsolinol, 1-MeDIQ, on pituitary prolactin release and dopaminergic activity in the mediobasal hypothalamus in nursing sheep. *Brain Research*, 1307, 72–77.
- Nakamura K., Fujiwara Y., Mizutani R., Sanbe A., Miyauchi N., Hiroshima M., Yamauchi J., Yamashita T., Nakamura S., Mori T., Tsujimoto G., Tanoue A. (2008): Effects of vasopressin V1b receptor deficiency on adrenocorticotropin release from anterior pituitary cells in response to oxytocin stimulation. *Endocrinology*, 149, 4883–4891.
- Pfaffl M.W., Tichopad A., Prgomet C., Neuvians T.P. (2004): Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper – Excel-based tool using pair-wise correlations. *Biotechniques Letters*, 26, 509–515.
- Quinones-Jenab V., Jenab S., Ogawa S., Adan R.A.M., Burbach J.P., Pfaff D.W. (1997): Effects of estrogen on oxytocin receptor messenger ribonucleic acid expression in the uterus, pituitary, and forebrain of the female rat. *Neuroendocrinology*, 65, 9–17.
- Radnai B., Kandar Z., Somogyvari-Vigh A., Mergl Z., Olah M., Fulop F., Vecsernyes M., Nagy G.M. (2005): Salsolinol induces a decrease in cyclic AMP at the median eminence and an increase at the adenohypophysis in lactating rats. *Brain Research Bulletin*, 65, 105–110.
- Sabatier N., Rowe I., Leng G. (2007): Central release of oxytocin and the ventromedial hypothalamus. *Biochemical Society Transactions*, 35, 1247–1251.
- Samson W.K., Schell D.A. (1995): Oxytocin and the anterior pituitary gland. *Advances in Experimental Medicine and Biology*, 395, 355–364.
- Samson W.K., Lumpkin M.D., McCann S.M. (1986): Evidence for a physiological role for oxytocin in the control of prolactin secretion. *Endocrinology*, 119, 554–560.
- Schlosser S.F., Almeida O.F., Patchev V.K., Yassouridis A., Elands J. (1994): Oxytocin-stimulated release of adrenocorticotropin from the rat pituitary is mediated by arginine vasopressin receptors of the V1b type. *Endocrinology*, 135, 2058–2063.
- Tabak J., Gonzalez-Iglesias A.E., Toporikova N., Bertram R., Freeman M.E. (2010): Variations in the response of pituitary lactotrophs to oxytocin during the rat estrous cycle. *Endocrinology*, 151, 1806–1813.
- Toth B.E., Homicsko K., Radnai B., Maruyama W., DeMaria J.E., Vecsernyes M., Fekete M.I.K., Fulop F., Naoi M., Freeman M.E., Nagy G.M. (2001): Salsolinol is a putative endogenous neuro-intermediate lobe prolactin-releasing factor. *Journal of Neuroendocrinology*, 13, 1042–1050.
- Traczyk W., Przekop F. (1963): A method for testing the function of the hypothalamus and pituitary in the sheep in chronic experiments. *Acta Physiologica Polonica*, 14, 227–236.
- Untergasser A., Cutcutache I., Koressaar T., Ye J., Faircloth B.C., Remm M., Rozen S.G. (2012): Primer3 – new capabilities and interfaces. *Nucleic Acids Research*, 40, 115–127.
- Welento J., Sztejn S., Milart Z. (1969): Observations on the stereotaxic configuration of the hypothalamus nuclei in the sheep. *Anatomischer Anzeiger*, 124, 1–27.
- Yang H.P., Wang L., Han L., Wang S.C. (2013): Nonsocial functions of hypothalamic oxytocin. *ISRN Neuroscience*, 2013, Article ID 179272.
- Zingg H.H., Laporte S.A. (2003): The oxytocin receptor. *Trends in Endocrinology and Metabolism*, 14, 222–227.

Received: 2016–04–06

Accepted after corrections: 2016–10–06