

## ABSTRACT

Lactation is the last stage of reproductive cycle in female mammal. Among many physiological adaptations of nursing mothers, there is an inhibition of reproductive activity that allows the body to regenerate after pregnancy and parturition. In this period, the factors such as endogenous opioid peptides, prolactin, dopamine (DA) and kisspeptin play an important role in the process of regulating the secretion of hypothalamic gonadotropin-releasing hormone (GnRH) and luteinizing pituitary hormone (LH). Salsolinol, a DA derivative, is another compound that during lactation may be involved in the regulation of the secretion activity of the GnRH/LH axis. This may be confirmed by the increase of salsolinol concentration in the hypothalamus, especially during suckling, as well as the participation of this compound during the regulation of other pituitary hormones release. The hypothesis of the present doctoral thesis is that salsolinol regulates secretory activity of the hypothalamic-pituitary GnRH/LH axis in sheep during lactation. To verify this hypothesis four experiments were conducted.

The aim of the first experiment, performed on sheep ( $n = 8$ ) in the fifth week of lactation, was to investigate the effect of structural analogue of salsolinol (1-MeDIQ) infusion into the third brain ventricle (IIIv) on GnRH release to the median eminence and LH release to the peripheral blood. GnRH concentration was assayed in perfusates obtained by the push/pull method from the infundibular nucleus/median eminence (IN/ME) and LH concentration was determined in blood samples collected from the jugular vein every 10 min. A significant decrease in GnRH concentration in the collected perfusates was observed in response to 1-MeDIQ infusion, as compared with the control infusion. The analysis of mean GnRH concentrations in 30-min periods revealed that GnRH concentrations decreased significantly from the first 1-MeDIQ infusion. The significant decrease of the mean LH concentration in blood samples was also estimated. There were no significant changes in the frequency and amplitude of LH pulses between the studied groups.

The aim of the second experiment was to study the effect of salsolinol, infused into the IIIv of sheep ( $n = 8$ ), 48 hours after weaning 8-week-old lambs, on plasma LH concentration. After the last (fourth) series of 30-min infusions, sheep were slaughtered to measure the expression of LH $\beta$  subunit mRNA in the anterior pituitary (AP). It was found, that salsolinol infused into the IIIv significantly increased plasma LH concentration, which was due to the increased amplitude of LH pulses. The frequency of LH pulses did not differ significantly between the both groups. The infusion of salsolinol also did not significantly affect LH $\beta$  mRNA level.

The aim of the third experiment was to study the effect of 1-MeDIQ infused into the IN/ME on GnRH release to the cerebrospinal fluid (CSF) and on plasma LH concentration in sheep in the fifth week of lactation. CSF samples were collected from IIIv, simultaneously with 1-MeDIQ or control infusion. No significant differences in mean GnRH concentration in CSF were found between the studied groups. However, the analysis of the parameters of the pulsatile LH release showed a significant increase in LH pulse frequency in the sheep after 1-MeDIQ infusion, which was connected with statistically significant increase in mean LH concentration in this group in comparison with the control.

The fourth experiment was performed *in vitro* to study the effect of salsolinol on LH release from AP to the medium and the expression of LH $\beta$  subunit messenger RNA in the AP tissue. AP explants were dissected from 9 sheep that were euthanized and decapitated 48 h after weaning 8-week-old lambs, and then cut along the longitudinal fissure into 2 halves. Explants were incubated for 135 min (include 15-min preincubation). The AP halves were incubated in Parker's medium with saline (NaCl, control, n=6) and in Parker's medium with saline containing two doses of salsolinol: 20  $\mu\text{g/ml}$  (n=6) or 100  $\mu\text{g/ml}$  (n=6). During the following incubation, the medium was exchanged every 15 min and replenished fresh. It was showed that both doses of salsolinol caused a significant decrease in the medium LH concentration as compared with the control. There were no significant differences in the levels of LH $\beta$  subunit gene expression between the studied groups.

The presented results can lead to the conclusion that salsolinol is able to modulate secretory activity of the GnRH/LH axis in sheep during lactation. The modulating action of salsolinol on GnRH/ LH secretion concerns the release of both hormones and can occur directly (inhibitory effect) or indirectly (stimulatory effect). Also, under the presented experimental conditions (lactation anestrus/seasonal anestrus), salsolinol did not affect the LH $\beta$  subunit messenger RNA expression in the pituitary gland.