Summary

Physiological regulation of gonadotropin-releasing hormone/luteinising hormone (GnRH/LH) secretion in mammals is associated with complex interplay between excitatory and inhibitory neurotransmitter, neurohormone and neuropeptide systems within the hypothalamus that mediate oestrogen signals to GnRH cells. However, in ewes, as a seasonally breeding species, the pattern of these actions differs between the anoestrous and oestrous periods. Though there is substantial evidence that also GnRH itself may serve as a part of a local network that facilitates communication among GnRH neurons in rodents, this finding has not been fully explained in sheep.

In contrast to the fairly well-known neuroendocrine mechanisms of GnRH/LH release, the molecular processes governing the secretion of GnRH/LH still remain poorly understood. Literature reports have suggested that some hypothalamic neurotransmitters and neuromodulators which participate in the oestrogen-mediated control of GnRH release may also be involved in the regulation of *GnRH* and *GnRH* receptor (GnRHR) genes expression.

The principal goal of this study was to determine selected neuronal mechanisms controlling the GnRH/LH release at the level of *GnRH* and *GnRHR* genes expression in the hypothalamic-pituitary unit of anoestrous and follicular-phase sheep. For this purpose, the study included the following experimental approaches: first, we investigated the effect of dopamine D2 receptor antagonist (sulpiride) on the expression of *GnRH* mRNA and *GnRHR* mRNA in the hypothalamic-pituitary unit of anoestrous sheep; second, we examined the same aspect of action of small doses of GnRH in anestrous period. We also analysed the effect of $GABA_A$ receptor agonist (muscimol) or antagonist (bicuculline) on the transcript level for both *GnRH* and *GnRHR* in follicular-phase sheep.

The experiments were performed on 48, 3 to 4-year-old Polish Merino ewes during anoestrous period or during the oestrous time. All the drugs were infused into the third cerebral ventricle of sheep in a pulsatile manner. Using Real-time PCR technique, the levels of *GnRH* mRNA and *GnRHR* mRNA were measured in the preoptic area (POA), anterior (AH) and ventromedial (VMH) hypothalamus and GnRHR, besides of these hypothalamic areas, in the stalk/median eminence and in the anterior pituitary gland (AP). This analysis was supplemented with a double-antibody radioimmunoassay method to estimate plasma LH concentration.

The study demonstrates, for the first time, that infusion of sulpiride decreased the level of *GnRH* mRNA in the VMH of sheep but has no significant effect on transcript level in the POA and in the AH, despite the demonstrated downward trend. However, the analysis of *GnRHR* mRNA levels showed its tendency to structure-dependent variations; an increase of *GnRHR* mRNA levels in the POA, AH and AP, and the decrease of its quantity in the VMH and in the SME. The upregulation of LH release induced by sulpiride is mainly due to an increase in LH pulse frequency, because the amplitude of pulses did not differ significantly from the control group. Infusion of GnRH resulted in a significant increase in transcript level for *GnRH* in the VMH and for *GnRHR* in all analysed hypothalamic areas and in the AP. The upregulation of LH release induced by GnRH is mainly due to an increase in LH pulse amplitude, because the frequency of pulses did not differ significantly between control and GnRH-treated groups. Administration of muscimol or bicuculline decreased or increased the expression of mRNAs encoding both *GnRH* and *GnRHR*, respectively, and affected LH secretion in the same way.

In conclusion, this study indicates that hypothalamic neurotransmitters and neuropeptides i.e., dopamine, GnRH and GABA that participate in the control of GnRH/LH release may also be involved in the modulation of *GnRH* and *GnRHR* genes expression.

Summary

This suggests that regulation of gonadotropins secretion in sheep may occur at the level of molecular and cellular processes leading to GnRH and GnRHR biosynthesis. It must be emphasised that *GnRH* mRNA levels in various parts of the hypothalamus result not only from the transcriptional activity of the *GnRH* gene, but also from the stability of the *GnRH* transcript and its utilisation in GnRH biosynthesis.

keywords: sheep, hypothalamus, pituitary, mRNA GnRH, mRNA GnRH-R, LH, dopamine, GABA