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Expression of the GnRH and GnRH receptor (GnRH-R) genes in the hypothalamus and of the GnRH-R gene in the anterior pituitary gland of anestrous and luteal phase ewes

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Abstract

Data exists showing that seasonal changes in the innervations of GnRH cells in the hypothalamus and functions of some neural systems affecting GnRH neurons are associated with GnRH release in ewes. Consequently, we put the question as to how the expression of GnRH gene and GnRH-R gene in the hypothalamus and GnRH-R gene in the anterior pituitary gland is reflected with LH secretion in anestrous and luteal phase ewes. Analysis of GnRH gene expression by RT-PCR in anestrous ewes indicated comparable levels of GnRH mRNA in the preoptic area, anterior and ventromedial hypothalamus. GnRH-R mRNA at different concentrations was found throughout the preoptic area, anterior and ventromedial hypothalamus, stalk/median eminence and in the anterior pituitary gland. The highest GnRH-R mRNA levels were detected in the stalk/median eminence and in the anterior pituitary gland.

During the luteal phase of the estrous cycle in ewes, the levels of GnRH mRNA and GnRH-R mRNA in all structures were significantly higher than in anestrous ewes. Also LH concentrations in blood plasma of luteal phase ewes were significantly higher than those of anestrous ewes.

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In conclusion, results from this study suggest that low expression of the GnRH and GnRH-R genes in the hypothalamus and of the GnRH-R gene in the anterior pituitary gland, amongst others, may be responsible for a decrease in LH secretion and the anovulatory state in ewes during the long photoperiod.

Keywords: Ewe; GnRH mRNA; GnRH-R mRNA; LH

1. Introduction

There is a general agreement that the annual reproductive cycle of sheep and other seasonal breeders is primarily due to changes in the activity of several neuro-endocrine systems and in particular in relation to GnRH release. Numerous studies have indicated that annual alteration of photoperiod plays a major role in the modulation of neural systems controlling GnRH release. Indeed substantial evidence implicates β-endorphin in suppression of GnRH/LH release during the sexually active phase in sheep (Domański et al., 1991; Curlewis et al., 1991). Melatonin affects GnRH/LH release in a different manner in ewes during the breeding (Misztal and Romanowicz, 2005) and non-breeding seasons (Viguie et al., 1995). Dopamine has a predominantly suppressive influence on GnRH release in anestrous ewes (Chomicka et al., 1994; Havern et al., 1994; Tortonese, 1999). The noradrenergic system activates GnRH secretion from the mediobasal hypothalamus–nucleus infundibularis region of ewes just prior to a preovulatory LH surge (Domański et al., 1991; Clarke et al., 1999); its role in this aspect in the anestrous ewes is rather inhibitory (Goodman et al., 1995; Tomaszewska-Zaremba and Przekop, 2005). The suppressive influence of GABA on GnRH neurons is transmitted through $GABA_A$ receptors in ewes during the breeding and non-breeding seasons (Scott and Clarke, 1993a,b; Tomaszewska-Zaremba et al., 2003). On the other hand, stimulation of $GABA_B$ receptors in the hypothalamus of sheep increases GnRH/LH release during the non-breeding (Jackson et al., 2000; Tomaszewska-Zaremba and Przekop, 2005) but not in the breeding season. Recent literature reports have shown that at least some neural systems in the hypothalamus of rats that participate in the control of GnRH release may also be involved in regulation of GnRH gene (Kim et al., 1994; Kang et al., 1995) and GnRH-R gene expression (Seong et al., 1995). It is still unclear whether these are common neural mechanisms for GnRH biosynthesis and GnRH release. At the pituitary level, the pattern of GnRH pulse signals and estradiol plays a crucial role in GnRH-R gene expression (Turzillo et al., 1998; Cheng et al., 2000) so transcriptional activity of the GnRH-R gene in the hypothalamus and the anterior pituitary gland may vary during different physiological states of animals. In the light of these results we posed the question as to what extent the secretory pattern of GnRH/LH release in anestrous and luteal phase ewes (Barrell et al., 1992) is associated with the expression of GnRH gene and GnRH-R gene in the hypothalamus and GnRH-R gene in the anterior pituitary gland, in order to establish whether the anovulatory state in the non-breeding season in ewes is associated not only with the secretory pattern of GnRH/LH release, but also with a decrease in the cellular content of GnRH mRNA and GnRH-R mRNA.

2. Material and methods

2.1. Animals

The studies were performed on 12, three-to-four-year-old Polish Merino ewes in two groups: anestrous ewes during mid non-breeding season (May) and ewes during the luteal phase