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A role for LH in the regulation of expression of mRNAs encoding components of the insulin-like growth factor (IGF) system in the ovine corpus luteum

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Abstract

Evidence suggests the insulin-like growth factor (IGF) system may be involved in luteal maintenance and regression. However, previous studies have only investigated a few components of the system, primarily in bovine and non-ruminant species. The present study investigated gene expression for the components of the IGF system in ovine corpora lutea (CL) at various key stages of the oestrous cycle (Experiment 1), and the possible regulatory effects of LH on IGF gene expression in ovine CL using a GnRH antagonist model system (Experiment 2). Experiment 1 revealed that IGF-I ($P < 0.001$), type I ($P = 0.008$) and II ($P = 0.005$) IGF-Rs and IGFBP-5 ($P < 0.05$) mRNA levels were significantly elevated in early regressing CL. In contrast, IGF-II levels were high in CL but did not vary throughout the oestrous cycle, while IGFBP-2, -3, -4 and -6 mRNA levels were highest throughout the luteal phase but lower in regressing CL ($P < 0.05$). IGFBP-1 mRNA could not be detected in any CL. Abrogation of LH action following GnRH antagonist administration (Experiment 2) resulted in a significant increase in expression for IGF-I ($P < 0.001$), type II IGF-R ($P = 0.004$) and IGFBP-5 ($P < 0.05$) after only 12 h, but these increases were transient. IGF-II, type I IGF-R and IGFBP-2, -3, -4 and -6 mRNA levels remained unaffected by GnRH antagonist treatment. These data highlight the role that LH plays in regulating IGF-I gene expression and lends further support that IGF-I may be a key luteotrophic factor in sheep.

Keywords: Corpus luteum; LH; Growth factors; IGF; GnRH antagonist

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1. Introduction

The corpus luteum (CL) is a continuation of follicular maturation where it develops from the cells of the ovulatory follicle into a transient, yet dynamic, endocrine organ that secretes progesterone. It has long been recognised that pituitary-derived LH is the major luteotrophic hormone in ruminants (reviewed by Niswender et al., 2000). However, in recent years it has become apparent that a multitude of intra-ovarian growth factors (Redmer et al., 1996; Jablonka-Shariff et al., 1997) appear to be implicated in luteal development.

The components of the insulin-like growth factor (IGF) system are also thought to play a role in luteal function. The mammalian IGF system is replete with two ligands (IGF-I and IGF-II), two cell-surface receptor types (types I and II IGF-R) (Froesch et al., 1985), which show affinity for either ligand and mediate their effects, and at least six IGF binding proteins (IGFBP-1 to -6 (Hwa et al., 1999)). IGF-I appears to stimulate key components in the steroidogenic pathway, leading to increased progesterone secretion in ruminants (Khan-Dawood et al., 1994; Liebermann et al., 1996), humans (Villavicencio et al., 2002) and pigs (Miller et al., 2003). In addition, IGF-II has also been shown to stimulate progesterone synthesis in vitro (Savion et al., 1981; McArdle and Holtorf, 1989; Monniaux and Pisselet, 1992; Sauerwein et al., 1992). The IGFBPs are thought to be involved in luteal function by modulating the effects of the IGFs, in particular IGF-I (Brown and Braden, 2001). However, the effects of all of the binding proteins on IGF action in the ovine CL have not been fully established.

It can be hypothesized that the components of the IGF system may play a role in the development and maintenance of the CL. In addition, it is conceivable that the regulation of the IGF components may play a role in luteal regression in sheep. However, the patterns of expression for the different components of the IGF system in the CL throughout the various key stages of the oestrous cycle, and following the initiation of luteolysis, have not been fully demonstrated in sheep. A key candidate for the regulation of IGF gene expression following luteolysis is the gonadotrophin, LH, given that abrogation of LH action, via decreases in LH receptor expression (Guy et al., 1995; Smith et al., 1996), is a classical indicator of luteolysis.

The purpose of this study was therefore to (i) investigate the expression of mRNAs encoding all of the components of the IGF system in the ovine CL throughout the oestrous cycle and (ii) examine the effects of LH on IGF gene expression in vivo by using a modification of the GnRH antagonist model (Campbell et al., 1990) to disrupt the normal pattern of LH secretion, without affecting endogenous FSH secretion patterns.

2. Materials and methods

All chemicals and reagents were purchased from Sigma Chemical Company, UK, unless otherwise stated and were of molecular biology grade where possible. All experiments were carried out with the approval of the University of Wales, Aberystwyth Ethical Review Committee, in accordance with the Animals (Scientific Procedures) Act 1986.

2.1. Animals, treatments and tissue collection

2.1.1. Experiment 1

During the breeding season, oestrus was synchronised in 30 mature Scottish Blackface ewes by the administration of two injections of Estrumate® (125 μg, i.m.; Schering-Plough) given 9 days apart. Ewes ($n = 5$) were sacrificed by the administration of Somulose™ (1 ml 10 kg^{-1}