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Prolactin receptors are highly expressed in wool follicle dermal papillae.

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ABSTRACT

Seasonal variation in wool growth has major impacts on production and fibre characteristics. These changes in wool follicle output are driven, at least in part, by circulating prolactin. We therefore localised prolactin receptors (PRLR) in skin from Wiltshire sheep over a wool follicle cycle induced by prolactin. PRLR expression was visualised by in situ hybridisation, immunocytochemistry and radioligand binding. The three approaches showed concordant distributions of PRLR and its mRNA. PRLR mRNA was most abundant in the dermal papilla, but also evident in the outer root sheath, hair germ, skin glands and epidermis. Expression in the hair germ was up-regulated in telogen. Location of PRLR in the dermal papilla and outer root sheath indicates action of prolactin directly on the growth controlling cells within wool follicles. These spatial and cycle-related patterns of PRLR expression contribute to the growing knowledge of the control of wool growth and its application in the wool industry.

Keywords: dermal papilla; hair follicle; immunocytochemistry; in situ hybridisation; prolactin; prolactin receptor; seasonality; wool growth.

INTRODUCTION

The pronounced circannual rhythm of wool growth in the sheep breeds that predominate in New Zealand is manifested in a two to three fold seasonal variation in clean wool production (Bigham et al., 1978; Sumner et al., 1998). Corresponding effects on fibre diameter and tensile strength are seen. This is a vestige of ancestral hair cycles, and feed intake has much less influence in winter (when fibre growth ceases in primitive sheep) than when wool growth is maximal (Hawker and Crosbie, 1985). The primary environmental cue entraining cyclic wool growth is photoperiod (Morris, 1961; Nagorcka, 1979), and humoral transmission is by way of the pituitary hormone prolactin (Pearson et al., 1996; Rougeot et al., 1984). Skin is a known target tissue for prolactin (Choy et al., 1995; Ouhtit et al., 1993) and fibre growth can be altered by either local (Thomas, 1994) or systemic prolactin injection (Pearson et al., 1997). Therefore, the sites of action and regulation of prolactin receptors (PRLR) are crucial to understanding and ameliorating seasonal variation in wool growth.

The PRLR is a cell membrane spanning protein which belongs to the growth hormone/cytokine receptor superfamily (Kelly et al., 1993). Multiple isoforms of PRLR result from alternative splicing of a single gene. In sheep and cattle, two variants have been described: one full length and the other with a truncated cytoplasmic domain (Anthony et al., 1995; Bignon et al., 1997; Schuler et al., 1997). Both forms are expressed in sheep skin (Choy et al., 1997) but it is as yet unclear how they contribute to hormonal control of wool growth.

We describe here the localisation of PRLR and PRLR mRNA in wool follicles at different stages of growth. Examination of both active (anagen) and quiescent (telogen) follicle cycle phases was made possible by using New Zealand Wiltshire sheep, a highly seasonal breed in which complete follicle shut-down occurs (Parry et al., 1995). We sought to determine the levels of expression amongst the functionally distinct follicle cell populations, and how these might change in relation to prolactin stimulus and follicle growth status.

MATERIALS AND METHODS

Wool follicle growth cycles were induced in New Zealand Wiltshire sheep by release from suppression of prolactin secretion, as previously described (Parry et al., 1995; Pearson et al., 1993). Analyses reported here derive from sheep skin samples collected from two trials. In the first, prolactin was suppressed in 10 ewes by bromocriptine treatment and then elevated following bromocriptine pump removal (Nixon et al., 1997). In the second trial, a similar effect was achieved by maintaining 14 rams in short day length (L8:D16) for 13 weeks, then transferring them to long day length conditions (L16:D8). In both cases, there was abolition of the normal spring rise in prolactin, followed by elevation in mid summer, resulting in a synchronised induction of follicle regression and interruption of wool growth. Untreated control animals exposed to normal photoperiod underwent normal seasonal changes in circulating prolactin and grew wool continuously while treated sheep shed their fleeces. The sheep were serially slaughtered over the course of the wool growth cycle lasting 20 to 40 days. Mid side skin samples were frozen to -70°C or fixed in phosphate buffered 10% formalin, and the follicle cycle stages were determined by histology (Nixon, 1993).

In situ hybridisation was used to localise PRLR mRNA in skin sections according to a standard method (Parry et al., 1995). 35S-UTP labelled antisense and sense (control) cRNA probes corresponding to 610 bp of ovine PRLR cDNA (Anthony et al., 1995) were generated by in vitro transcription. Fixed skin samples from 18 sheep were
processed to paraffin wax and 7 µm sections mounted on silanised glass slides. Sections were then dewaxed, hydrated and exposed to serial pre-hybridisation treatments with 0.2M HCl, 1 µg/ml proteinase K, and acetic anhydride. Probes were diluted to 50,000 cpm/µl in buffer (150 mM NaCl, 50% formamide, 2xSSC, 0.2 mg/ml tRNA, 1.0 mg/ml herring sperm DNA, 10% dextran sulphate, 0.4 mg/ml BSA, 10 mM dithiothreitol) and the sections hybridised overnight at 55°C. Post-hybridisation treatments included washing at 57°C in 2xSSC/50% formamide and RNase digestion. Dried slides were coated with photographic emulsion and developed after 40 days exposure, then counterstained with haematoxylin and eosin. Control sections were treated before hybridisation with RNase or DNase.

**FIGURE 1:** Distribution of prolactin receptor mRNA in longitudinal sections of wool follicles as shown by *in situ* hybridisation. A: late anagen follicle bulb probed with sense PRLR cRNA (negative control showing absence of signal). B: adjacent section probed with antisense PRLR cRNA. C: section B as seen by dark field microscopy to show autoradiographic labelling. D: telogen follicle probed with sense PRLR cRNA; E: adjacent section probed with antisense PRLR cRNA. F: section E as seen by dark field microscopy. Bars indicate 50 µm. Haematoxylin and eosin counterstain. Prolactin receptor is most highly expressed in the dermal papilla (DP).
Immunocytochemistry was performed according to Choy et al. (1997). Sectioned skin samples from 12 sheep were prepared as above. Sections were blocked in 0.1% bovine serum albumin (BSA)/5% sheep serum then incubated with 15 µg/ml U5 monoclonal anti-PRLR at 4°C overnight. This antibody recognises a region in the extracellular domain of rat PRLR separate from the ligand binding site and highly conserved across species (Okamura et al., 1989). After washing, bound antibody was detected with colloidal gold-coupled goat anti-mouse IgG (Auroprobe LM, Amersham) and silver enhancement kit (IntenSE, Amersham). Sections were lightly counterstained with eosin.

Radioligand-binding was conducted as described by Choy et al. (1995). Prolactin binding sites in sheep skin were localised using iodinated human growth hormone (125I-hGH) and ovine prolactin (125I-oPRL). Frozen skin samples from untreated sheep were prepared as 10 µm cryosections, and incubated at room temperature for 18 h with either radiolabelled hormone alone (50 Kcpm/50 µl buffer), or radiolabelled hormone with excess unlabelled competitor (8 µg/ml oPRL, hGH or oGH) to determine specificity. After washing, dried slides were autoradiographed and counterstained with eosin as for in situ hybridisation.

RESULTS

Sites of PRLR gene expression were demonstrated by in situ hybridisation. PRLR antisense probe bound to cells of both epidermal and dermal derivation (Figure 1C). Sense strand PRLR cRNA (control probe) showed extremely low, evenly distributed signal (Figure 1A). In anagen follicles, the most densely labelled cell types were dermal papilla, outer root sheath (ORS), and lower inner root sheath (IRS). The germinal matrix (epithelial cells that give rise to keratinocytes) was very weakly labelled or unlabelled, as were connective tissue sheath, dermis, and differentiated keratinocytes. Radiolabelling was more variable, and generally weaker during anagen than at other stages of the wool growth cycle. In telogen follicles, PRLR mRNA was abundant in the secondary hair germ (a plate of cells that gives rise to the germinal matrix), epithelial strand and ORS, and most highly expressed in the dermal papilla (Figure 1C).

The pattern of immunoreactivity with the U5 antibody indicates the distribution of the PRLR protein. Anagen follicles from bromocriptine treated and untreated sheep showed similar patterns. Densest precipitation of silver occurred in dermal papilla, upper ORS, lower IRS (Figure 2A). Immunoreactivity in these tissues was dependent on differentiation state and varied along the follicle. Bulb cells immediately surrounding the dermal papilla were unstained but, in contrast to the mRNA pattern, more distant prekeratinocytes were immunoreactive. During telogen, the dermal papilla, secondary hair germ, epithelial strand, and ORS were all uniformly labelled. There were less site dependent differences as compared with anagen follicles.

Radioligand-binding experiments demonstrated high specific binding of 125I-hGH and 125I-oPRL to the dermal papilla, ORS and IRS cells of wool follicles (Figure 2C). Addition of unlabelled competitor (hGH or oPRL) reduced signal, demonstrating specificity (Figure 2D). PRLR localised by this and the immunocytochemical method were in the cytoplasm or at the cell surface.

All three methods gave similar, distinctive localisation patterns (Figures 1 and 2). Table 1 summarises assessments of relative labelling intensity obtained by each
method for different cell types present in the skin and wool follicle. Telogen follicles showed higher PRLR concentration than anagen follicles. Throughout the cycle, specific labelling was consistently observed in various extrafollicular skin structures, particularly sebaceous (grease) glands, sweat (suint) glands, and interfollicular epidermis.

**DISCUSSION**

For a cell membrane bound receptor such as PRLR, the site of expression indicates the target cells for hormone action. The distribution of PRLR shown in wool follicles therefore reveals potential sites that are involved in seasonal modulation of keratinocyte production. Concordant localisation results have been achieved with three methodologically unrelated techniques. These showed that, in all phases of the follicle cycle, PRLR is most concentrated in the dermal papilla, epithelial cells in the vicinity of the follicle bulb, and the ORS further up the follicle.

The dermal papilla is of particular interest because this cluster of specialised fibroblasts plays a key role in inducing follicle formation and regulating fibre growth from the germinal matrix (Oliveir and Jahoda, 1989; Watson et al., 1994). The high abundance of PRLR would thus permit incoming hormonal signals to act directly on the cells that dictate keratinocyte proliferation by as yet unspecified paracrine interactions. The ORS too has been implicated in interactions with other follicle cell populations, including the IRS and dermal papilla (Cotsarelis et al., 1992). This layer also contains proliferating cells and is a zone of differential cell migration. Although the significance of PRLR in this multifunctional tissue is less readily discernible, it seems likely that, as in the dermal papilla, it is involved in the control of follicle growth.

The germinative zone of anagen follicles is largely devoid of PRLR. However, with the shut down of proliferation and entry into telogen, these germ cells show marked increases in both PRLR mRNA and immunoreactivity. Receptor regulation in these cells could be important in altering follicle responsiveness to prolactin at different cycle stages. Differences in PRLR levels might also explain different effects of prolactin at different stages of the growth cycle (Pearson et al., 1997).

It is widely held that cyclic growth is an intrinsic property of skin follicles (Messenger, 1993), and it is the timing of cycles that is regulated by extrinsic signals such as photoperiod (Rougeot et al., 1984). Prolactin may well play such a role by acting directly within responsive follicles to initiate a complex growth cycle mechanism. Greater understanding of the local interactions within the follicle is required to further comprehend the effects of endocrine inputs (Paus, 1996).

The demonstration of PRLR in the key cells controlling the follicle is an important step in understanding the physiological and molecular mechanisms underlying seasonal wool growth. Further studies are required to determine whether responses to prolactin can differ amongst the various cell types possessing PRLR or at different stages of the follicle cycle, perhaps through regulation of long and short PRLR isoforms.

**ACKNOWLEDGEMENTS**

We thank Murray Ashby, Janet Wildermoth, and Tony Craven for assistance with animal trials. Dr Russ Anthony (Colorado State University) kindly provided ovine PRLR cDNA, and Dr Paul Kelly (INSERM, Paris) the U5 antibody. This work was funded by the New Zealand Foundation for Research, Science and Technology.

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