SPECIFIC LOCALISATION OF OPIOID PEPTIDE IMMUNOREACTIVITY IN THE SKIN OF FINE-WOOLLED MERINO SHEEP

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The quality of a significant proportion of the Australian wool clip is compromised by a decrease in staple strength resulting from the induction of a weak point in fibres in response to a variety of environmental, disease and psychological stressors (Doyle *et al.* 1993). Although undernutrition is likely to contribute to the formation of tender wool, supplementary feeding does not always prevent the problem, and it is likely that hormones released in response to these stressors, such as adrenocorticotrophin (ACTH) and cortisol, have a direct effect on the wool follicle independent of nutritional status, since their systemic administration results in the formation of wool breaks (Lindner and Ferguson 1956). In this study we have determined if the hormones beta-endorphin (β-EP) and alpha-melanocyte stimulating hormone (α-MSH), both derived from the same precursor molecule as ACTH, are localised to specific wool follicle structures. These hormones, cleaved from the 241 amino acid protein proopiomelanocortin (POMC), are co-secreted with ACTH in response to stress and are associated with the provision of analgesia and the regulation of skin pigmentation respectively.

Mature Merino (21 μm) ewes (n = 20), immunised on 7 occasions over a period of 28 months, comprising 13 control animals (Freunds adjuvant only) and 7 ACTH-immune animals (Freunds adjuvant + ACTH:Ovalbumin(1:2) conjugate; 0.5 mg/injection) were given a booster injection 2 weeks prior to skin sampling. Blood samples were taken before the boost, 1 week after the boost and on the day of skin sampling. The plasma was assayed for ACTH specific antibodies by ELISA and for cortisol and β-EP by radioimmunoassay.

ACTH specific antibody titres ($1/\text{diln} \times 10^3$) increased significantly ($P < 0.03$) from the pre-boost level of 79.3 ± 15.0 to the post-boost titre of 250.6 ± 66.0 in ACTH-immune animals. At skin sampling, cortisol values were significantly higher ($P < 0.001$) for control sheep (18.0 ± 2.0 ng/mL) than ACTH-immune animals (1.7 ± 0.4 ng/mL), while circulating levels of β-EP were significantly increased ($P < 0.005$) in ACTH-immune (S2S.6 ± 154.6 pg/mL) when compared to control sheep (234.6 ± 97.2 pg/mL).

A total of S animals, 4 from each treatment group were randomly selected for examination of skin tissue sections. Skin biopsies (1 cm'> were removed by trephine, frozen in isopentane in dry ice and stored at -80°C before processing. Sections (S μm) were cut at -18°C and thaw mounted onto gelatin coated glass slides and desiccated at -20°C until assayed. β-EP and cc-MSH were detected by immunohistochemistry using a rabbit polyclonal primary antibody which was specifically located by a monoclonal antibody conjugated to alkaline phosphatase.

β-EP and cc-MSH were specifically co-localised in the epidermis, pilary canal, sebaceous gland and the outer root sheath when compared with sections incubated with pre-immune serum. In ACTH-immune animals, staining was localised to the same skin structures as for control animals, but was much more intense.

These results demonstrate that POMC peptides either accumulate or are synthesized in ovine skin, although the specific co-localisation of the 2 peptides would suggest the latter. The association with the outer root sheath suggests that these peptides regulate either the rate of cell division or keratin synthesis. Localisation in the sebaceous glands and associated structures suggests that these peptides are secreted onto the skin surface in sebum. Thus they may have a role in the maintenance of the skin's immune system and/or in the wound healing process.

This work was supported by the Wool Research and Development Corporation.

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