

OPIOID MECHANISMS SUPPRESS THE EXPRESSION OF MILK PROTEINS IN PRE-PARTURIENT MAMMARY EXPLANT TISSUE

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The process of mammogenesis involves the proliferation of mammary alveoli and their constituent epithelial cells which acquire the capacity for the synthesis of milk and its proteins. The lactational potential of a cow is not attained if these mechanisms are not fully functional at the time of peak lactation. We have identified a key period of 30 to 40 days pre-calving during which milk protein gene expression is low but inducible in mammary tissue explants *in vitro* with the appropriate lactogenic hormones, insulin (I), cortisol (F) and prolactin (P) (Sheehy *et al.* 1997). The identification of factors that limit gene induction during this period will be of commercial importance to the dairy industry.

In this study we have investigated the impact of the stress-related hormone β endorphin (β Ep) on the ability of I, F and P to induce β and κ casein gene expression in mammary explants from biopsies collected 30 days pre-calving. Mammary explants (each of 1mg; 20 per well) were floated in cell culture medium (M199) for 4 days in the presence of I (5 μ g/mL) and F (500ng/mL). P (500ng/mL) was then added together with increasing concentrations of β Ep (50, 500 and 5000pg/mL) for a further 2 days. Total RNA was analysed for milk protein mRNA expression by incubating membranes from slot blots with specific cDNA probes and quantitating the signal by densitometry.

The addition of P induced β and κ casein gene expression. Co-incubation with β Ep (50pg/mL) reduced this expression (Figure 1). To assess if β Ep was operating through the μ opioid receptor we substituted the specific μ agonist D-Ala-Met-enkephalin-Gly-ol (DAMGO; 5, 50, 250, 2500 pg/mL) for β Ep, which also suppressed this gene expression (Figure 2). To confirm the specificity of the response tissue maintained with I, F and P was incubated with DAMGO together with the specific μ receptor antagonist D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH₂ (CTAP), which binds only to the μ receptor. This antagonist prevented the DAMGO-induced suppression of gene expression.

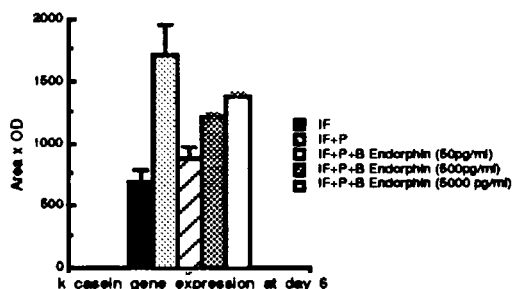


Figure 1. The influence of β endorphin on κ casein gene expression.

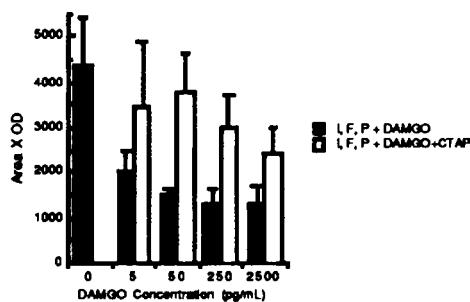


Figure 2. The influence of DAMGO and CTAP on κ casein gene expression.

The perception of stress resulting in β endorphin release in cows 30 to 40 days pre-calving may suppress milk protein synthesis. We speculate that the timing of this stress may be important in determining its impact on milk protein synthesis for the entire lactation.

SHEEHY, P.A., NICHOLAS, K.R., GOODEN, J.M. and WYNN, P.C. (1997) In 'Recent Advances in Animal Nutrition in Australia', pp. 176-184. (University of New England: Armidale NSW).