

Protein synthesis in mammary epithelial cells harvested from cows treated with growth hormone or atropine*

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ABSTRACT

Mammary epithelial cells, harvested from either mammary tissue or milk from cows treated with growth hormone or atropine, were cultured *in vitro*. Protein synthesis in these cells was determined by measuring the incorporation of [3,4,5]-³H-leucine into protein secreted into the cell culture medium. Incorporation was measured between 0 and 11 h after addition of labelled leucine to the cell culture medium. No difference in incorporation was observed between epithelial cells isolated from milk compared to those from mammary tissue, nor was there any difference in incorporation in epithelial cells derived from the milk of control, growth hormone or atropine-treated cows.

KEY WORDS: mammary epithelial cells, growth hormone, atropine, protein synthesis

INTRODUCTION

Administration of exogenous growth hormone (GH) markedly improves milk production (including protein yield) in lactating cows (Bauman, 1992). In contrast, the alkaloid atropine causes a reduction in milk production and protein concentration (Auldust et al., 2003). These galactopoetic factors may be useful models for investigating the underlying mechanisms of milk protein synthesis.

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Our study tests two hypotheses. Firstly, that mammary epithelial cells derived from milk behave in a similar manner to those derived from mammary tissue, and are therefore a viable, non-invasive alternative as an *in vitro* model of mammary function. Secondly, that the altered protein production observed in the GH (McCoard et al., 2004, companion paper) and atropine-treated (McCoard et al., 2004) is due to changes in the protein synthesis within the mammary gland. Clusters of mammary epithelial cells derived from both milk and mammary gland were used as a short-term primary culture that accurately reproduces mammary function *in vitro* (Wheeler et al., 1995).

MATERIAL AND METHODS

Twelve Jersey cows were either administered with a subcutaneous injection of GH (n=4), an 8-h infusion of atropine (n=4) or control saline (n=4) as described by McCoard et al. (2004 and companion paper). Milk samples were collected prior to slaughter and mammary tissue was removed immediately post mortem, and kept at 37°C until isolation of epithelial cells. Epithelial cells were isolated from bovine mammary tissue using collagenase digestion, and all cells were cultured as previously described (Johnston et al., 2004). Then 0.2 GBq [3,4,5]-³H-leucine (110 Ci/mmol; American Radiolabelled Chemicals Ltd., St.Louis, MO, USA) was added to each assay well and media sampled between 0 and 11 h after the addition of the labelled leucine. For analysis, 100 µL of unlabelled leucine (76 mM) and 25 µL of bovine skim milk were added to the media samples and proteins precipitated in 15% trichloroacetic acid (300 µL) and the washed pellet resuspended in 250 µL 35 mmol/L SDS in 0.3 mol/L NaOH. The entire protein pellet was mixed with 2 mL of Starscint scintillation liquid and analysed for total radioactivity as a measure of protein synthesis. The significance of treatment effects was determined by analysis of variance using Genstat Seventh Edition (Version - 7.1.0.206 © 2003 Lawes Agricultural Trust).

RESULTS

There was no difference in total incorporation of labelled leucine in protein secreted by the mammary epithelial cells isolated from either mammary tissue or milk at any of the times studied (Figure 1). There was also no difference between the control, atropine or GH treatment groups with respect to incorporation of labelled leucine in the epithelial cells derived from milk (Figure 2).

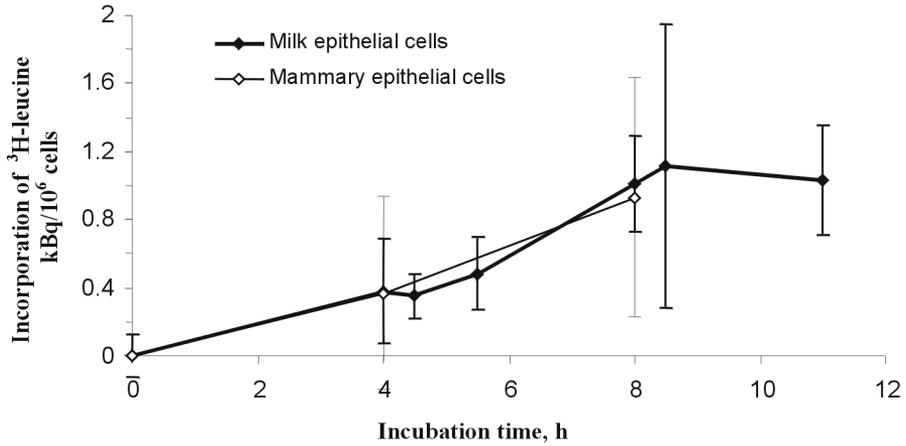


Figure 1. Comparison of *in vitro* protein synthesis in mammary epithelial cells derived from bovine milk or mammary tissue. Each point represents the mean \pm SD for four cows in triplicate. There was no significant difference between the two groups at any time point ($P>0.1$)

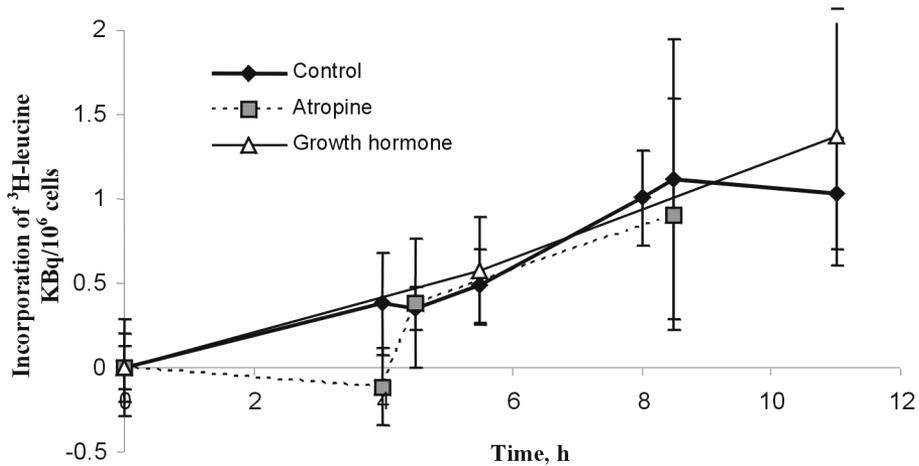


Figure 2. The effect of the infusion of growth hormone and atropine on *in vitro* protein synthesis in mammary epithelial cells derived from bovine milk. Each point represents the mean \pm SD for four cows in triplicate. There was no significant difference between the three treatment groups at any time point ($P>0.1$)

DISCUSSION

Our study shows that mammary epithelial cells obtained from mammary tissue or milk appear to synthesise proteins at the same rate, suggesting that the use of mammary epithelial cells derived from milk may be a suitable *in vitro* model to study mammary function.

Protein synthesis in mammary epithelial cells isolated from atropine or GH treated cows was not different from the control cows. This was in spite of significant differences in milk production and protein yield between these groups (McCoard et al., 2004 and companion paper). Our *in vitro* model using milk epithelial cells therefore shows no evidence that these observed differences are due to changes in protein synthesis per se. Further studies such as pulse-chase experiments are required to clarify if total incorporation of labelled amino acid in protein is an accurate measurement of protein synthesis in this model.

CONCLUSIONS

Our study shows that mammary epithelial cells derived from milk may be suitable as an *in vitro* model of mammary function. However, further investigation is required to determine if this model accurately reflects the synthesis of secreted proteins by the mammary gland in response to galactopoetic factors.

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