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Oestrogenic effects on *in vitro* co-culture of mammary explants and epithelial cells

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ABSTRACT

Mechanisms of oestrogen stimulation of mammary development were examined using ewe lambs treated with oestradiol (0.1mg/kg BW/day for 7 days; n=5) or controls (n=5). Mammary explants were incubated with [^{3}H]-thymidine to determine effects of oestrogen on epithelial cell proliferation. Also, explants of mammary stroma and parenchyma were co-cultured with a mammary epithelial cell line (MAC-T) to determine the ability of the explants to stimulate MAC-T growth. Oestrogen increased proliferation of ewe mammary epithelial cells ($P < 0.02$). In co-cultures, addition of mammary explants increased growth of MAC-T cells ($P < 0.01$), but there was no effect or interaction of tissue type (stroma vs. parenchyma) or oestrogen ($P > 0.10$). Proliferation of mammary explants was negatively correlated with growth of MAC-T cells in co-cultures ($r = -0.41$; $P < 0.07$). These results confirm the importance of oestrogen and mammary stroma in regulating mammary epithelial proliferation but fail to show a requirement for mammary stroma to mediate oestrogenic effects on epithelial cells.

Keywords: Mammary; co-culture; oestrogen.

INTRODUCTION

Oestrogens are potent stimulators of mammary development, but the specific nature of their effects is not clearly defined. Oestrogen promotes ductal elongation and cell proliferation in the mammary gland of mice (Lyons, 1958), and heifers (Woodward *et al.*, 1993). However, isolated mammary epithelial cells do not respond to oestrogen *in vitro* (Woodward *et al.*, 1994). In some cases, addition of mammary fibroblasts to epithelial cultures has been shown to restore oestrogen responsiveness (Haslam, 1988). This suggests that interactions between the stromal and parenchymal portions of the gland may be required for oestrogen responsiveness but the nature of such interactions is not fully understood. Oestrogen has been shown to affect the expression of stroma-derived, locally acting growth factors (Bates *et al.*, 1988). It has also been suggested that oestrogen acts via systemic intermediary molecules, i.e., estromedins (Sirbasku and Benson, 1980). More recently, local effects of oestrogen were clearly demonstrated by implanting antioestrogen-containing pellets in the murine mammary gland (Silberstein *et al.*, 1994). However, this study did not distinguish whether the primary site of oestrogen action was on epithelial or stromal cells. The purpose of our study was to clarify how oestrogen exerts its effects within the ovine mammary gland. The ability of explanted tissues to stimulate the proliferation of MAC-T cells was used as a bioassay to compare the stimulatory potential of stromal versus parenchymal tissues and to evaluate the effect of *in vivo* oestrogen treatment on this potential. MAC-T cells have been shown to respond in a dose-dependent manner to various mitogens but do not respond directly to oestrogen (McFadden and Cockrell, 1994; Woodward *et al.*, 1994).

MATERIALS AND METHODS

Three week old ewe lambs ($^1/_2$ Dorset, $^1/_4$ Rambouillet, $^1/_4$ Finnsheep) received seven daily s.c. injections of either oestradiol-17β (0.1 mg / kg BW in 20% benzyl benzoate: corn oil, n = 5) or vehicle (n = 5). At slaughter, mammary glands were removed and transported to the laboratory. Explants of mammary parenchymal tissue were collected under sterile conditions and incubated for 2 hrs in DMEM (GIBCO, Gaithersburg, MD, USA) with 1μCi [^{3}H]-thymidine/ml to label cells in the S-phase of the cell cycle. Labelled explants were fixed in Karnovsky's fixative (0.1M NaH_2PO_4, 1.2% glutaraldehyde, 0.4% paraformaldehyde, pH 7.2) and processed for histoautoradiography as described (Smith *et al.*, 1989). In addition, explants from parenchymal and stromal regions of the glands (average = 12.5 ± 0.4 mg each) were placed into separate 1 ml chambers of a NUNC® chamber slide (NUNC, Naperville, IL, USA) and co-cultured with MAC-T bovine mammary epithelial cells (Huynh *et al.*, 1991). MAC-T cells were plated at 2.5 x 10^4 cells / chamber and serum starved for 48 hrs in unsupplemented DMEM before explants and fresh DMEM were added. Co-cultures were maintained in a 37°C, 5% CO_2 incubator for 48 hrs. During the final 4 hrs of culture, 1μCi [^{3}H]-thymidine was added to each culture well. Cells were then fixed with Karnovsky's fixative, rinsed with phosphate buffered saline, washed with 5% trichloroacetic acid, and dehydrated with methanol prior to histoautoradiographic labelling. Explant mitogenicity was estimated by determining the percentage of labelled MAC-T cells (i.e., labelling index) in three separate randomly selected microscope fields within a chamber and averaging the percentage of labelled cells in duplicate chambers.

Statistical analyses were performed using SAS® (Cary, N.C., U.S.A.). Main effects and interactions of oestrogen

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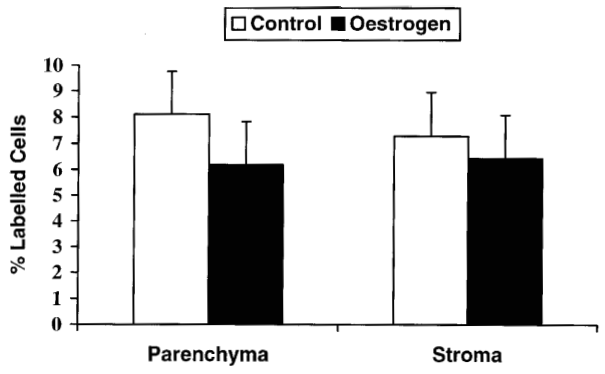
(treated or control) and tissue type (parenchymal or stromal) on the labelling index of MAC-T cells were analysed. Effects were deemed significant at $P < 0.10$. Data presented are least squares means (LSM) ± standard error of the mean (SEM).

RESULTS

Oestrogen treatment increased the percentage of ewe mammary epithelial cells labelled with [^{3}H]-thymidine more than 3-fold (oestrogen treated = 8.4 ± 1.4%, control = 2.5 ± 1.4% $P < 0.02$), demonstrating a typical mammary growth response to oestrogen stimulation, *in vivo*. Studies *in vitro* revealed a labelling index of 1.1 ± 0.2% for MAC-T cells cultured in basal medium alone. Co-culture of ewe mammary explants with MAC-T cells increased the labelling index by approximately 7-fold ($P < 0.01$) but there was no difference between stromal and parenchymal explants ($P > 0.10$; Figure 1). This stimulation of MAC-T proliferation by co-cultured explants was about one-half of that observed upon addition of an optimal dose (5%) of foetal calf serum (labelling index = 17.1 ± 3.5%). In contrast to its effect on ewe mammary tissue *in vivo*, oestrogen treatment had no effect on the ability of these explants to stimulate growth of MAC-T cells in co-culture ($P > 0.10$; Figure 1). Nor was there an interaction between oestrogen treatment and tissue type ($P > 0.10$). Furthermore, the correlation between explant weight and labelling index in a given culture well was not significant ($P > 0.40$). However, there was a negative correlation between the labelling index of ewe mammary explants and the proliferation of the MAC-T cells in co-culture ($r = -0.41$; $P < 0.07$).

FIGURE 1: Labelling index of MAC-T cells co-cultured with explants of mammary parenchyma or stroma from oestrogen treated or control lambs. No significant differences due to oestrogen treatment, tissue type, or their interactions were detected ($P > 0.10$).



DISCUSSION

Previous research has shown that explants of mammary fat pad (stroma) markedly stimulate proliferation of mammary epithelial cells in co-culture indicating that mammary stroma may play a role in regulating epithelial development. These studies utilised mouse mammary explants with a murine mammary epithelial cell line, Comma D, (Hovey *et al.*, 1994) or bovine mammary explants with the bovine line, MAC-T, (McFadden and Cockrell, 1994). Thus, the results of the present study are consistent with these earlier findings. However, the lack of difference in the mitogenicity of stromal and parenchymal explants was surprising, given that stromal fibroblasts have been suggested to mediate the mitogenic signal from oestrogen to mammary epithelia (Haslam and Levely, 1985). Indeed, in a murine system differences between stromal and parenchymal explants were consistently observed and were dependent on hormonal status of the donor and hormones added to cultures (Hovey *et al.*, 1994). It is presently unclear why the same was not true for explants of ovine mammary tissue, but we have previously observed differences in the mitogenic activity of ovine and murine mammary stromal explants (Hovey *et al.*, 1995). Furthermore, we did not observe the expected enhancement of mitogenicity of explant tissues after *in vivo* treatment with exogenous oestrogen. Oestrogen treatment did increase the epithelial labelling index of ewe mammary tissues, confirming that a typical mammary response occurred. However, the oestrogen mediated stimulation *in vivo* did not carry over to co-cultures, *in vitro*. This result also differs from the murine system, in which stimulatory effects of oestrogen treatment *in vivo* were also observed in co-culture (Hovey *et al.*, 1994).

There are several possible explanations for the observed results. First, the amount of explant (in mg tissue / chamber) could account for some variation in the labelling. However, explant weight did not differ between tissue types or *in vivo* treatment. Also, correlations between explant weight in a given chamber and the labelling index for that chamber were not significant. It may also be that the method used to measure co-culture responses had limitations. Because the method only determined responses during the final 4 hrs of the co-culture period prior effects could have been missed. Such differences could have been detected by an earlier labelling period or by quantifying total DNA or cell number / chamber. The latter approach accounts for cumulative cell growth during the co-culture period and was used successfully in previous studies (Hovey *et al.*, 1994, 1995). Against this possibility however, is the observation that MAC-T cells cultured with 5% FCS exhibited a labelling index nearly twice that of co-cultured cells indicating that the ability to respond to mitogens was not limiting. A final possibility is that the dynamics of co-culture mitogenicity may differ with tissues derived from different species. We have previously observed that ovine and murine mammary fat pads differ in mitogenicity and interactive effects with growth factors (Hovey *et al.*, 1995). Such differences may relate to the marked structural and histologic differences between ruminant and rodent mammary glands (Akers, 1990). Thus far, there has been little characterisation of growth factors present and active in ruminant mammary tissues. Species differences in oestrogen responsiveness could be addressed with further co-culture studies.

REFERENCES

Akers, R.M. 1990. Lactation Physiology: A Ruminant Animal Perspective. *Protoplasma* **159:**96-111.

Bates, S.E.; Davidson, N.E.; Valverius, E.V.; Freter, C.E.; Dickson, R.B.; Tam, J.P.; Kudlow, J.E.; Lippman, M.E.; Solomon, D.S. 1988. Expression of transforming growth factor α and its messenger ribonucleic acid in human breast cancer: Its regulation by oestrogen and its possible functional significance. *Molecular Endocrinology* **6:** 543-555.

Haslam, S. Z. 1988. Local versus systemically mediated effects of estrogen on normal mammary epithelial cell deoxyribonucleic acid synthesis. *Endocrinology* **122:** 860-867.

Haslam, S.Z.; and Levely, M.L. 1985. Estrogen responsiveness of normal mouse mammary cells in primary cell culture: Association of mammary fibroblasts with estrogenic regulation of progesterone receptors. *Endocrinology* **116:**1835-1844.

Hovey, R.C.; McFadden, T.B.; and Mackenzie, D.D.S. 1994. Response of mammary epithelial cells to ovarian steroids is modulated by the mammary fat pad during the estrous cycle. *Journal of Dairy Science* **77(Suppl. 1):** 8.

Hovey, R.C.; McFadden, T.B.; and Mackenzie, D.D.S. 1995. Mitogenic activity of lamb mammary fat pad is distinct from that of mouse mammary fat pad. *Journal of Dairy Science* **78(Suppl. 1):** 153.

Huynh, H.T.; Robitaille, G.; Turner, J.T. 1991. Establishment of bovine mammary epithelial cells (MAC-T): an *in vitro* model for bovine lactation. *Experimental Cell Research* **197:**191-199.

Lyons, W.R. ; Li, C.H.; and Johnsson, R.E. 1958. The hormonal control of mammary development and lactation. *Recent Progress in Hormone Research* **14:**219-254.

McFadden, T.B.; and Cockrell, D.C. 1994. Mammary fat pad from heifers fed polyunsaturated fat stimulates growth of Mac-T bovine mammary epithelial cells in co-culture. *Journal of Dairy Science* **77(Suppl. 1):** 116.

Silberstein, G.B.; van Horn, K.; Shyamala, G.; Daniel, C.W. 1994. Essential role of endogenous estrogen in directly stimulating mammary growth demonstrated by implants containing pure antiestrogens. *Endocrinology* **134:** 84-90.

Sirbasku, D. A. and Benson, R.H. 1980. Proposal Of An Indirect (Estromedin) Mechanism Of Estrogen-Induced Mammary Tumor Cell Growth. Cell Biology Of Breast Cancer. C. McGrath and M. Rich, Eds. Academic Press, N.Y. , pp. 289-314.

Smith, J.J.; Capuco, A.V.; Beal, W.E.; and Akers, R.M. 1989. Association of prolactin and insulin receptors with mammogenesis and lobulo-alveolar formation in pregnant ewes. *International Journal of Biochemistry* **21:**73-81.

Woodward, T.L.; Beal, W.E.; and Akers, R.M. 1993. Cell interactions in initiation of mammary epithelial proliferation by oestradiol and progesterone in prepubertal heifers. *Journal of Endocrinology* **136:**149-157.

Woodward, T.L.; Akers, R.M.; and Turner, J.D. 1994. Lack of mitogenic response to EGF, pituitary and ovarian hormones in bovine mammary epithelial cells. *Endocrine* **2:** 529-535.