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ALBUMIN A MAMMARY GLAND SECRETING CELL KEEPER

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Introduction

Albumin, a well-characterized product of the liver is the most abundant extracellular protein. It is a single polypeptide with 585 amino acids and a molecular weight of 66,200D. Albumin is manufactured in the liver at a rate 9-12g/day and there is no storage and no reserve. Albumin is catabolized at a rate of 9 - 12 g/day (the same rate as it was produced) by pinoctosis in cells adjacent to the vascular endothelium. Albumin is a major plasma protein, responsible for binding and transport of many biologically active molecules (Peters, 1985). Albumin is synthesized largely in the liver, though non-hepatic expression has been documented in several other tissues, such as the mouse retina (Dodson et al., 2001), the mouse skeletal muscle (Wagatsuma and Stromberg, 2001; Wagatsuma et al., 2002) human ovarian epithelial cells (Varricchio et al., 1994) and bovine tracheal gland serous cells (Jacquot et al., 1988). Milk whey albumin has the same amino acid sequence as the blood serum molecule. Therefore, increase in milk albumin was taken as evidence for tight junction disruption (Nguyen and Nevill, 1998). Albumin concentration in milk increases during functional transitions from lactation to involution and from involution to lactogenesis (Sordillo et al., 1987) and during inflammation (Riollet et al., 2000; Watanabe et al., 2000). During these periods albumin may augment the immune defenses of the gland (Bounous, 2000).

Materials and Methods

Preparation of mammary gland culture

Explants: Mammary gland tissue from cows was obtained from the slaughterhouse. The tissue was transferred into medium M-199 containing 100 U penicillin, 100 ug streptomycin, 0.25 μg fungizone and insulin at 1 μg/mL and was brought to the laboratory. Explants were prepared as previously described (Shamay et al., 1987). The mammary gland tissue was cut into small pieces (3 to 6 mg) and placed on an impregnated lens paper in a 50-mm plastic dish. Five mL medium M-199 with 1 μg insulin, 10,000 U penicillin, 10 mg streptomycin, 0.025 mg fungizone and 0.5 μg cortisol/ml and the experimental additives.

Cells: Bovine mammary gland tissue was transferred to the laboratory, cut into small pieces and placed in a 500-mL Erlenmeyer flask containing medium M-199 supplemented with collagenase (1 mg/mL), hyaluronidase (1mg/mL), and bovine insulin (1μg/mL) in a ratio of 10 mL medium to 1 g tissue. Cells were washed 3 to 5 times and grown in a 10-mm plastic dish in DMEM F-12 (HAM) 1:1. The cells were used for RNA isolation. Albumin secretion to the medium was determined by ELISA assay.

Real Time RT-PCR Assay

Real time Reverse Transcription-PCR for albumin mRNA was carried out with forward (5' AGG GAG GTC TGG GCT ATC ATC 3') and reverse (5' TTC GTG AAA CCT ATG GTG ACA TG 3') primers employing a SYBR green measurement of the accumulation of double-stranded DNA.

ELISA Assay

Analysis of BSA was done as described by Stelwagen et, al 1994. Enzyme linked immunosorbent assay (ELISA) was performed on medium collected from tissue cultures of healthy, mastitic and dry mammary gland tissue explants and on medium collected from primary culture cells, with or without LPS (lipopolysaccharide; Sigma, St. Louis MO USA). The ELISA was carried out with two 96-well polystyrene microtiter plates coated with BSA incubated with mouse monoclonal anti-bovine serum (Sigma, St. Louis MO USA B201) at a final dilution of 1:8000. After washing the plate was incubated goat anti-mouse IgG peroxidase conjugate (Sigma, St. Louis MO USA) at a final dilution 1:2500 in ELISA diluent for 1.5 h at 37°C. A substrate solution (0.1% 2' 2'-azino-diethylbenzo-thiazoline in citrate-phosphate buffer, pH 4.0, containing 0.003% (wt/vol) H_2O_2) was added to each well. After a final incubation at 20°C for 30 min, absorbance was measured at 405 nm.

Results

Albumin synthesis: Incubation of mammary gland explants with the labeled amino acid L- [^{35}S] methionine resulted in formation of labeled immunoprecipitable albumin, newly synthesized in the explant. Immunoprecipitable albumin in the medium verified that newly synthesized albumin was also secreted into the medium. This shows that the gland itself is a source of milk albumin.

Albumin expression and secretion: Mammary gland tissue from healthy and mastitic dairy cows were examined for albumin mRNA expression by means of Real-Time RT-PCR. Albumin mRNA expression was approximately 4 times higher in mastitis mammary gland tissue compared to expression in healthy tissue. Mammary gland explants from healthy, mastitis, and dry dairy cows were incubated for 3 d in medium. The effect of mastitis on the secretion of albumin from the mammary gland tissue was determined by ELISA. The secretion of albumin was increased 3.5-fold ($P < 0.05$) in the mastitic mammary gland tissue explants, relative to the healthy mammary gland tissue explants.

Hormonal effect on BSA: we demonstrate here for the first time that leptin in the presence of prolactin (simulation of lactation) enhance the expression and accumulation of albumin in the bovine mammary gland (fig 1).

Conclusion

The results shown here suggest that the synthesis and secretion of albumin by the mammary gland is part of the innate nonspecific defense system. Albumin synthesis and secretion in the mammary gland is influence by the tissue health status and by hormones.