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Modulation of albumin-like protein and lysozyme production by bovine tracheal gland serous cells

Dependence on culture conditions

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Bovine tracheal submucosal gland serous cells were cultured in medium supplemented with either 10% fetal calf serum or 2% Ultroser G, a commercial serum substitute for cell culture. The proteins synthesized and secreted into the culture medium during [^{35}S]methionine pulse, chase and isoproterenol-stimulated periods were analyzed. Marked differences in the patterns of secretory radiolabeled proteins with M_r values ranging from 15000 to 95000 were observed between pulse and chase media of cells cultured in fetal calf serum and Ultroser G. In the presence of Ultroser G, albumin-like protein production was inhibited 95% as compared to cultures incubated with fetal calf serum. A bovine lysozyme-type enzymatic activity was detected only in medium from stimulated cells cultured in Ultroser G. The results suggest that bovine tracheal serous cells synthesize different proteins according to the composition of culture medium and release certain proteins when adrenergically stimulated.

Albumin; Lysozyme; Trachea; Gland; Cell culture

1. INTRODUCTION

The presence of albumin in airway secretions has been attributed to either serum-transudation from local vessels [1] or specialized vesicular transport mechanisms across airways epithelia [2,3]. Recently, we have demonstrated that an albumin-like protein is synthesized de novo by cultured bovine tracheal gland serous cells maintained in serum-supplemented medium [4]. The major site of albumin synthesis is the liver [5]. Several reports have shown that the albumin synthesis and secretion from cultured fetal and adult human hepatocytes and hepatoma cells is greatly dependent on hormonal composition of culture medium [6–8]. Interestingly, the albumin production by cultured fetal rat hepatocytes is about 20-fold higher in Ultroser G-supplemented medium (a commercial serum substitute) compared with fetal calf serum-supplemented medium [9]. In a previous study [10], it was observed that, whatever the culture medium (fetal calf serum vs Ultroser G), bovine tracheal serous cells synthesize and secrete a similar pattern of hyaluronic acid, chondroitin sulfate proteoglycans and asparagine-linked glycoproteins. This prompted us to investigate whether albumin production by bovine tracheal serous cells is affected by culture conditions. In addition, we have examined the production of lysozyme, an antibacterial enzyme considered as a specific marker for serous cells of airway submucosal glands [11,12].

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2. MATERIALS AND METHODS

2.1. *Cell culture*

Bovine tracheal gland serous cells were isolated by enzymatic digestion as previously described [13]. Cells between passages 15 and 25 were grown in RPMI 1640 medium supplemented with either 10% fetal calf serum and referred to as RPMI/10% FCS or 2% Ultroser G (a serum substitute for cell culture from IBF Laboratories, France) and referred to as RPMI/2% UG. All culture media contained 2 mM L-glutamine, streptomycin (100 μg/ml) and penicillin (100 units/ml). Bovine tracheal serous cells were seeded onto human placenta collagen-coated (15 μg/cm^2) T-75 flasks, maintained at 37°C under 5% CO_2 in air. Medium was changed every 3 days.

2.2. *Radiolabeling of proteins*

Confluent bovine tracheal serous cells (Day 10) were rinsed 3 times with PBS, incubated for 1 h in methionine-free RPMI 1640 medium with no supplement, and pulsed with 10 ml medium containing 20 μCi/ml [35S]methionine (1000 Ci/mmol, Amersham International) for 3.5 h. Then, cells were washed once quickly with fresh RPMI 1640 medium containing an excess of unlabeled methionine, chased for 1 hour in fresh RPMI 1640 medium (unstimulated control period) and for an additional 1 h period in fresh RMPI 1640 medium containing 10^{-5} M isoproterenol, and β-adrenergic agonist known to increase release of glycoconjugates between 200% and 300% from bovine tracheal serous cells in culture [13]. All harvested media were dialyzed exhaustively (Spectropor tubing; MW cutoff 6000–8000; Spectrum Medical Industries, L.A.) against deionized water for 8 days (2 changes a day), lyophilized and then stored at −20°C until used for biochemical analysis. Total protein content was measured by the Bio-Rad Protein Microassay Procedure [14] using bovine serum albumin (BSA) as a standard.

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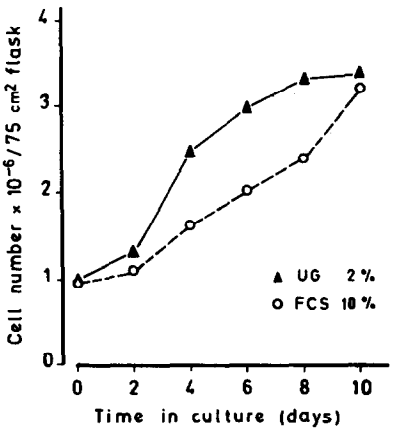

Fig. 1. Comparative effect on cell growth of 10% fetal calf serum and 2% Ultroser G-supplemented culture medium. Triplicate cell counts were made using a hemacytometer.

2.3. *Lysozyme assay*

Lysozyme lytic activity was evaluated spectrophotometrically by measuring the initial rate of lysis of a *Micrococcus luteus* cell suspension (Worthington, Biochemicals Corp., Freehold, NJ) according to Shugar's method [15] modified as previously described [16]. The bovine lysozyme-type enzymatic activity was defined as units of enzyme activity per microgram of protein and expressed as equivalent of hen egg-white lysozyme (Worthington).

2.4. *SDS polyacrylamide gel electrophoresis and chromatographic procedures*

Newly synthesized proteins in media were analyzed by electrophoresis and chromatography. SDS polyacrylamide gel electrophoresis (SDS-PAGE) was performed in 10–15% gradient Phast gels according to the manufacturer's instructions (Pharmacia, France). Samples were dissolved in 0.01M Tris/HCl, pH 8.0, containing 1 mM EDTA, 2.5% SDS, 5% β-mercaptoethanol and treated at 100°C for 3 min. After electrophoresis, the dried gels were exposed to X-Omat AR films (Kodak Co., France). Relative protein quantitation of fluorograms was performed using a TV-camera microcomputer system. Software for 1-D gel analysis was developed in our laboratory and driven by a PC computer (VICTOR V286C). Cation-exchange chromatography was performed using a Mono S HR 5/5 FPLC column (Pharmacia) equilibrated in 0.05 M phosphate buffer pH 7.2. Gel filtration chromatography was performed using a Superose 12 HR 10/30 FPLC column (Pharmacia) equilibrated in 0.05 M phosphate 0.15 M NaCl buffer, pH 7.2.

3. RESULTS AND DISCUSSION

As shown in Fig. 1, more rapid growth occurred when bovine tracheal serous cells were cultured in RPMI/2% UG medium than in RPMI/10% FCS medium. However, in both cases, the density of cells was similar after 10 days in culture (at confluency). Whatever the culture medium, cells assumed an epithelial morphology, forming dense monolayer colonies of tightly packed polygonal cells, as previously shown [17]. These epithelial characteristics were also observed for human tracheal gland cells cultured in Ultroser G medium [18]. The proteins released by bovine tracheal serous cells during pulse and chase periods were analyzed from cells grown in RPMI/2% UG medium and in RPMI/10% FCS medium. Marked

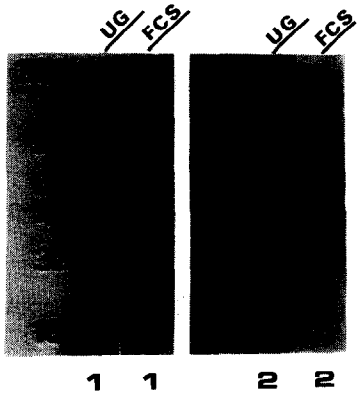

Fig. 2. Fluorogram of [^{35}S] methionine pulse-chase labeled secretory proteins from bovine tracheal serous cells in culture. Lyophilized culture media from the 3.5 h pulse (lane 1) and 1 h chase (lane 2) periods were solubilized in reducing buffer. Following SDS-PAGE (10–15%), the dried gel was exposed for 4 days. UG cells were grown in RPMI/2% Ultroser G medium; FCS cells were grown in RPMI/10% fetal calf serum.

differences in the patterns of secretory radiolabeled proteins with M_r values ranging from 15 000 to 95 000 were observed between media of bovine tracheal serous cells cultured in RPMI/2% UG and in RPMI/10% FCS (Fig. 2). Three major polypeptides of apparent M_r values of 67 000, 47 000 and 32 000 respectively (lane 1, FCS), were found at greatly increased levels in the medium from cells cultured in RPMI/10% FCS compared to those of medium from cells cultured in RPMI/2% UG (lane 1, UG). Based on the molecular weight, immunoreactive cross-reactivity and surface molecular charge characteristics, the labeled M_r 67 000 protein band has previously been identified as an

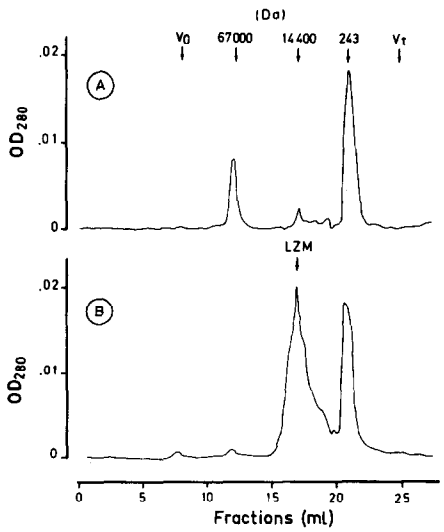

Fig. 3. Gel-filtration FPLC on a Superose 12 column of media from stimulated tracheal serous cells cultured in RPMI/10% FCS (A) and in RPMI/2% UG (B). The column was eluted with 0.05 M phosphate 015 M NaCl buffer, pH 7.2, at a flow rate of 0.4 ml/min and calibrated with Blue Dextran (Vo) and M_r-standard bovine albumin (M_r 67 000), HEWL (M_r 14 400) and cytidine (M_r 243).

albumin-like protein [4]. The amount of albumin-like protein released during the 3.5 h pulse (lane 1, FCS) and 1 h chase (lane 2, FCS), was approximately 25 ng and 10 ng/10^6 cells, respectively. As yet, the identity of two other major polypeptides (47 and 32 kDa) is unknown. When bovine tracheal serous cells were grown in RPMI/2% UG medium, a major 15–18 kDa protein band (lane 1, UG) was clearly observed, compared to the faint band observed at the same level in medium from cells cultured in RPMI/10% FCS (lane 1, FCS). In other to obtain medium samples enriched in secretion products, cultured cells were stimulated with 10^{-5} M isoproterenol for 1 h. Fig. 3 shows the comparison of gel-filtration FPLC patterns between medium from stimulated cells cultured in RPMI/10% FCS (A) and RPMI/2% UG (B). An enhanced amount of low molecular weight components (ranging from 15 to 20 kDa) was evident in the medium from cells cultured in RPMI/2% UG (B). The M_r 15–20 kDa fraction peak exhibited a strong lysozyme-type enzymatic activity evaluated by a turbidimetric method using *Micrococcus luteus* as substrate. When analyzed by Mono S-cation exchange chromatography, the medium from stimulated cells cultured in RPMI/2% UG was found to contain a major component eluting at 0.35 M NaCl (Fig. 4), an elution position required for the elution of human airway lysozyme [19]. The lysozyme content in the 0.35 M NaCl fraction (3.0 μg/10^6 cells expressed as equivalent of hen egg white lysozyme) was 37% of the total protein content released by isoproterenol-stimulated serous cells. This finding suggests that lysozyme activity, previously identified in bovine lung tissue extracts and defined as a g-type lysozyme with a M_r value of around 20 000 [20,21] originates, at least in part, from tracheal gland serous cells.

Our data clearly demonstrate the divergent effects of culture medium supplements on the biosynthesis and production of secretory proteins in bovine tracheal serous cells. Referring to neosynthesized protein pattern from cells cultured in fetal calf serum-supplemented medium, albumin-like protein is inhibited 95% whereas lysozyme synthesis is stimulated in Ultroser G-supplemented medium. This suggests that albumin-like protein and lysozyme synthesis depends on different specific factors present in culture medium and/or the secretion of each protein is controlled by two different secretory pathways. These data are consistent with previous studies showing that the relative rate of synthesis of four proteins (i.e. ovalbumin, conalbumin, ovomucoid and lysozyme) is not coordinated in chick oviduct tubular gland cells [22], and the expression of albumin gene in a rat hepatoma cell line [23] is dependent on specific serum factors. It has been reported that Ultroser G medium induces an active synthesis and secretion of lysozyme and bronchial inhibitor in cultured human tracheal gland cells [18]. It also maintains albumin release from cultured fetal hepatocytes [9]. The latter contrasts with our observations showing that Ultroser G medium greatly decreased albumin-like protein secretion from cultured bovine tracheal serous cells. The mechanisms by which Ultroser G modifies the protein synthesis remain to be elicited. Recently [24], it has been shown that Ultroser G medium induces extensive modifications of the cytoskeletal protein organization of cultured epithelial cells. Evidence now indicates a strong connection between cytoskeletal structures and protein synthesis and delivery in epithelial cells [25,26]. Recently, we showed that lysozyme production by bovine tracheal gland cells cultured in serum-free (or Ultroser G-free) defined medium was 7–10-fold higher compared to that obtained from cells cultured in fetal calf serum or Ultroser G medium [27]. Moreover, changes in the morphology and secretion of bovine tracheal gland cells cultured in serum-free defined medium were induced by the presence of specific extracellular matrix proteins [28].

These data emphasize the importance of tracheal gland serous cell cultures for studies of the regulation of the biosynthesis of proteins in airways. Studies of the rates of specific gene transcription and mRNA translation are now underway to identify more precisely the factors involved in the secretory function of gland serous-type cell.

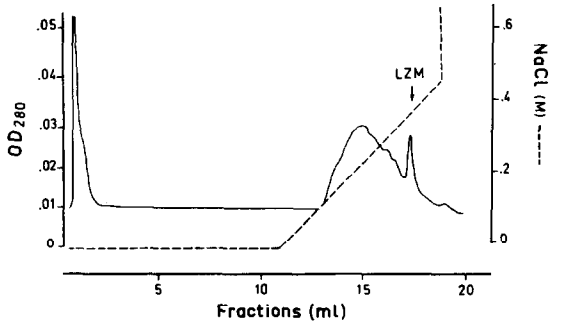

Fig. 4. Cation-exchange FPLC on a Mono S column of medium from stimulated tracheal serous cells cultured in RPMI/2% UG. The column was eluted with a gradient of NaCl (0–1.0 M) in 0.05 M phosphate buffer, pH 7.2; LZM lysozyme activity.

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