Cell Tissue Res (1981) 214:107–118

Cell and Tissue Research

Inhibition of Glycoconjugate Secretion by Colchicine and Cytochalasin B*

An in vitro Study of Human Airway

Stephen J. Coles and Lynne Reid

Department of Pathology Research, Children's Hospital Medical Center and Harvard Medical School, Boston, Massachusetts, USA

Summary. The effects have been analyzed of cytochalasin B and colchicine on the secretion of glycoconjugates by human bronchial explants labeled in vitro with radioactive glucosamine. Both cytochalasin B and colchicine had no effect on baseline ^{14}C-labeled glycoconjugate release but caused a dose-dependent ($10^{-7} - 10^{-4}$ M) inhibition of ^{14}C-glycoconjugate release and discharge of labeled macromolecules from mucous and serous cells induced by $5 \cdot 10^{-5}$ M methacholine.

Quantitative autoradiographic analyses showed that neither cytochalasin B nor colchicine inhibited ^{3}H-threonine or ^{3}H-glucosamine incorporation into mucous and serous cells of the submucosal glands or goblet cells of the airway epithelium. Colchicine (10^{-5} M) but not cytochalasin B significantly reduced the rate at which labeled macromolecules were transported through mucous, serous and goblet cells but this effect was not observed until 4 h after the addition of colchicine. Neither cytochalasin B nor colchicine affected the basal rate of labeled-macromolecule discharge from mucous, serous or goblet cells. At a concentration of 10^{-5} M, both agents completely inhibited the increase in labeled-macromolecule discharge induced in mucous and serous cells by methacholine.

Our results suggest that in the submucosal gland of human airways microtubules and microfilaments may be important in secretagogue-induced but not in baseline cellular glycoconjugate discharge, implying that the mechanisms of the two processes differ significantly. Furthermore, a role for microtubules is suggested in the transport of secretory granules through mucous, serous and goblet cells.

Key words: Secretion – Glycoconjugates – Human airway – Colchicine – Cytochalasin B.

Send offprint requests to: Dr. Stephen Coles, Department of Pathology Research, Children's Hospital Medical Center, 300 Longwood Avenue, Boston, Massachusetts 02115, USA

* Supported by National Institutes of Health Research Grant 5R01HL22444. The authors gratefully acknowledge the technical assistance of Mr. Tudor Williams, Mr. Eduardo Quintanilla and Ms. Maureen Hayes

0302-766X/81/0214/0107/$02.40

The recent development of techniques for maintaining airway mucosa in vitro makes possible the analysis of mechanisms regulating the secretion of epithelial glycoconjugates, the major macromolecular components of airway mucus. By radioactive labeling with various sugars and amino acids, the release of secreted glycoconjugates can be measured by scintillation counting (Yeager et al. 1977; Chakrin et al. 1973; Gallagher et al. 1975; Last et al. 1977). Furthermore, by use of quantitative autoradiography, the subcellular stages of secretion are analysed, i.e., precursor uptake and synthesis, intracellular transport and discharge of macromolecules (Sturgess and Reid 1972; Meyrick and Reid 1975; Coles and Reid 1978).

A number of studies have suggested that microtubules and microfilaments are involved in the secretion of various macromolecules including insulin (Lacy et al. 1968), thyroid hormones (Williams and Wolff 1971), growth hormone (Schofield 1971), and amylase from the parotid gland (Butcher and Goldman 1972) and exocrine pancreas (Bauduin et al. 1975). Usually the evidence that these intracellular contractile organelles are essential for normal secretion derives from studies with agents that destroy their structural integrity. Colchicine, and the vinca alkaloids, prevent the assembly of microtubules by interaction with the protein tubulin (Wilson et al. 1974), while cytochalasin B, a metabolite of the mold *Helminthosporum dematoideum* has been shown to disrupt microfilaments (Schroeder 1970; Wessels et al. 1971). These agents modify, usually inhibit, secretion from a wide range of cells in many species (Allison and Davies 1974). A brief report has shown that cytochalasin B and colchicine inhibit porcine airway secretion (Adler and Craighead 1978). The actions of these agents on glycoconjugate secretion by the human airway has not been studied nor have their effects on the various types of glycoconjugate-secreting cells been separately analysed.

The present study analyses the effects of cytochalasin B and colchicine on the secretory activity of the cell types that synthesise glycoconjugates in the human airway mucosa, i.e., the mucous and serous cells in the submucosal glands and the goblet cells in the surface epithelium. We have analysed glycoconjugate secretion from explants of human bronchial mucosa by two techniques: (a) scintillation counting of labeled glycoconjugates released after incubation with ^{14}C-glucosamine, and (b) quantitative autoradiographic analysis of mucous, serous and goblet cells after pulse-labeling with ^{3}H-glucosamine.

Materials and Methods

Tissue

Four main and 10 lobar bronchi were obtained from 14 lung specimens that had been surgically resected from 6 female and 8 male patients (mean age 58.3 ± 7.8 years) with bronchial carcinoma. Each specimen was collected within 1 h of resection and transported to the laboratory in cold preoxygenated Earle's Salts solution. The proximal ring of bronchus from each specimen was excised, fixed in phosphate-buffered 10% formal saline (pH 7.2) and sections were prepared for histologic assessment and mesurement of submucosal glands. No specimen contained neoplastic tissue and all had either normal sized or mildly hypertrophied glands according to the criteria of Coles and Reid (1978). Since no

statistical difference in secretory activity was shown between these two groups, they are presented together.

^{14}C-labeled Glycoconjugate Release

Bronchial mucosa was dissected free from cartilage as described previously (Sturgess and Reid 1972) and cut into approximately 1 mm^3 explants with fine dissecting scissors. Groups of 8–10 explants were allocated to separate 25 ml Erlenmeyer flasks and equilibrated for 30 mins in 1.5 ml of incubation medium under constant gassing with oxygen/CO_2 (95 : 5) in a shaking water bath at 37°. The incubation medium consisted of Medium 199 with L-glutamine (GIBCO, Grand Island, New York) containing 100 units/ml penicillin and 100 µg/ml streptomycin. After equilibration, the medium was replaced with fresh Medium 199 containing 1 µCi/ml D-1-^{14}C-glucosamine hydrochloride (specific activity 60.8 mCi/mmol; Amersham, Arlington Heights, Illinois) and the flask incubated for 24 h. The "labeling" medium was discarded and the explants were washed extensively for 15 mins with Medium 199 containing no label. The explants were then incubated for two consecutive 60-min periods (Periods I and II) in Medium 199 containing no label. Media were harvested at the end of each period for determination of ^{14}C-labeled glycoconjugate release. "Control" flasks contained only Medium 199 during Periods I and II. During Period I, "test" flasks contained Medium 199 and during Period II they contained Medium 199 with either colchicine or cytochalasin B, singly or in combination with methacholine.

Glycoconjugates were extracted from incubation media by precipitation with 10% trichloracetic acid (TCA)/1% phosphotungstic acid (PTA) overnight at 4°. Our preliminary observations (Coles and Bhaskar, unpublished data) indicate that human bronchial explants, which have been incubated with ^{14}C-glucosamine, release both labeled glycoproteins (mucus and serum-type) and labeled proteoglycans (possibly contaminants released by connective tissue and/or basement membrane). We have shown that 10% TCA/1% PTA will precipitate the glycoprotein but not the proteoglycan components. In this study, the TCA/PTA precipitate is referred to as ^{14}C-acid precipitable glycoconjugates (^{14}C-APG).

The precipitates were separated by centrifugation at 2,000 g for 10 mins and acid washed 3–4 times to remove unbound ^{14}C-glucosamine. They were then dissolved in 0.1 N NaOH at 30° for 18 h. Radioactivity was determined by counting in Ultrafluor (National Diagnostics, Laneville, New Jersey) in a Packard Tri-Carb scintillation counter. Quench corrections were made by the Channels-Ratio method.

The ratio of Period II dpm to Period I dpm was calculated and termed the ^{14}C-APG release ratio. This ratio was used as an index of glycoconjugate release in this study. The ratio for "control" flasks (approximately 1.0) was compared with the ratio for "test" flasks by use of the Student's *t* test for unpaired observations.

Analysis of Cellular Secretion

Explants of bronchial mucosa were prepared as described by Sturgess and Reid (1972) and incubated (3–4 explants/flask) in 25 ml Erlenmeyer flasks at 37° under constant gassing with oxygen/CO_2 (95 : 5). "Pulse" medium consisted of glucose-free Medium 199 (prepared by GIBCO) containing antibiotics described above. "Chase" medium was Medium 199 containing 1 g/l glucose.

At the end of each study, explants were fixed in Karnovsky's fluid, post-fixed in 1% OSO_4 and embedded in Epon/Araldite mixture. Sections of 1 µm were stained with Regaud's hematoxylin mordanted with iron alum, dipped in Ilford K5 emulsion (diluted 1 : 1) and exposed for 4 weeks. Suitable controls were prepared with each batch of autoradiographs for assessment of background radiation and positive or negative chemography (Rogers 1973).

Before scoring, slides were coded by a colleague with no knowledge of the experimental details. With the exception of the grain density analysis (see below), all suitable cells in 2 sections of each explant were scored (usually 120–250 mucous cells, 80–150 serous cells and 50–100 goblet cells). Cells that showed poor histologic preservation were sectioned tangentially; those that contained no silver grains were not scored. For each experimental group (6–8 explants) mean values and standard errors were obtained. Means were compared statistically by the Student's *t* test.

Labeled Precursor Incorporation

The incorporation of labeled precursors into mucous, serous and goblet cells of bronchial mucosa was quantified by autoradiographic grain density analysis as described previously (Coles and Reid 1978).

Explants were incubated for 1 h in Medium 199 containing colchicine or cytochalasin B and then incubated for 30 min in "pulse" medium containing either 50 μCi/ml D-6-^{3}H glucosamine hydrochloride (specific activity 20.6 Ci/mmol) or 20 μCi/ml DL-^{3}H-threonine (410 mCi/mmol). As a positive control for this analysis, some explants were incubated pre-pulse with cycloheximide, which has been shown to reduce precursor incorporation into mucous, serous and goblet cells (Coles and Reid 1978). After pulse-labeling, the explants were washed briefly and then incubated in "chase" medium for 1 h before fixation and autoradiography.

Autoradiographs were viewed with a × 100 oil immersion objective. For each section 30–50 cells of each type were randomly selected and the number of silver grains overlying each cell counted visually. Scoring more than 50 cells/section failed to produce an improvement in the variance in grain counts between cells. Regional differences in grain density within a single section were not normally observed. Cells which were discharging label were not scored. For each explant an average grain density for each cell type was obtained. Background grain counts were made over 10 cells of each type in an autoradiograph of a section of unlabeled tissue, and the mean background count subtracted from each experimental value.

Intercellular Transport of Label

Explants were pulse-labeled for 30 min with 150 μCi/ml ^{3}H-glucosamine. They were then chased for 1, 4, 7 and 10 h periods either with chase medium alone or containing cytochalasin B or colchicine. At the end of each chase period, explants were fixed and autoradiographed.

The transport of label through each cell type was assessed by the method of Neutra et al. (1977) as follows: cells were visually divided into 4 levels and the level of label closest to the apical pole recorded. Thus cells in which label was located in the perinuclear region were scored "level 1" while those with label in the upper quarter of the cell close to the luminal membrane were scored "level 4." Cells which were discharging label or were considerably depleted of secretory granules were not scored. For each explant an average grain level was obtained for each cell type.

Cell Discharge

Explants were pulse-labeled for 30 min with 150 μCi/ml ^{3}H-glucosamine and then chased for 3.5 h before fixation and autoradiography. Colchicine and cytochalasin B, either singly or together with methacholine, were added for the final hour of the chase period.

Discharged cells could be identified since (a) they contained no secretory granules, and (b) they contained no aggregates of intracellular silver grains. In addition, in mucous cells a considerable reduction in height accompanied discharge, while in serous cells discharge was characterised by the presence of numerous canaliculi extending into the cytoplasm. The loss of secretory granules from goblet cells made it impossible to distinguish them from other nonciliated epithelial cell types; therefore this feature was no quantified. For each explant the number of discharged cells was expressed as a percentage of the total cells scored.

Drugs

Colchicine, cytochalasin B, methacholine chloride, and cycloheximide were obtained from the Sigma Chemical Company, St. Louis, Missouri. Cytochalasin B was prepared as a stock solution of 5 mg/ml in

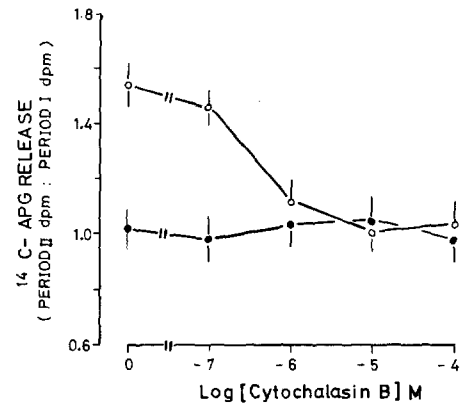

Fig. 1. Effect of cytochalasin B on baseline and methacholine-stimulated ^{14}C-labeled glycoconjugate (^{14}C-APG) release. Cytochalasin B was added during Period II, alone or together with $5 \cdot 10^{-5}$ M methacholine. Each point is the mean ± SE of 6–8 separate determinations. Baseline release (●—●); methacholine-stimulated release (○—○)

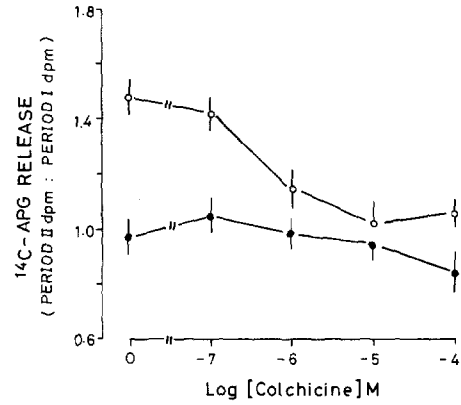

Fig. 2. Effect of colchicine on baseline and methacholine-stimulated ^{14}C labeled glycoconjugate (^{14}C-APG) release. Colchicine was added during Period II, alone or together with $5 \cdot 10^{-5}$ M methacholine. Each point is the mean ± SE of 6–8 separate determinations. Baseline release (●—●); methacholine-stimulated release (○—○)

dimethylsulfoxide and diluted with culture medium at the time of the experiment. Studies of appropriate dimethylsulfoxide concentrations indicated that it was without effect on baseline or methacholine-stimulated secretion. Colchicine and methycholine were dissolved directly in culture medium.

Results

Effect of Cytochalasin B and Colchicine on ^{14}C-labeled Glycoconjugate Release

Cytochalasin B. The cholinergic analogue, methacholine (5.10^{-5} M) caused a 52% increase ($p < 0.01$) in the release of ^{14}C-labeled acid precipitable glycoconjugates (^{14}C-APG) from bronchial explants. When cytochalasin B was present in the incubation medium, methacholine had a less marked effect on ^{14}C-APG release. (Fig. 1). This effect of cytochalasin B was dose-dependent. In the presence of cytochalasin B concentrations greater than 10^{-5} M, methacholine did not produce an increase in ^{14}C-APG release above the control baseline value. Over the concentration range used in this study (10^{-7}–10^{-4} M), cytochalasin B did not affect the baseline release of ^{14}C-APG from bronchial explants.

Table 1. Effect of methacholine, cytochalasin B and colchicine on the specific activity of ^{14}C-labeled glycoconjugates (^{14}C-APG) released by human bronchial explants

Drugs added	Total radioactivity (dpm $\times 10^{-3}$)[a]			Specific activity (dpm $\times 10^{-3}$/μg protein)[c]		
Period II	Period I	Period II	Ratio II : I	Period I	Period II	Ratio II : I
None	54.6[b]	53.2	0.97	8.2	8.8	1.07
Methacholine ($5 \cdot 10^{-5}$ M)	45.9	67.5	1.47	9.1	8.6	0.95
Cytochalasin B (10^{-5} M)	42.3	44.0	1.04	8.0	8.4	1.05
Colchicine (10^{-5} M)	51.6	46.7	0.91	7.9	8.7	1.10
Methacholine + Cytochalasin B	39.2	42.3	1.08	8.4	8.5	1.01
Methacholine + Colchicine	39.9	38.3	0.96	9.3	8.6	0.92

[a] Each count was made of ^{14}C-APG content of the media pooled from 5 flasks
[b] Each value represents a single determination
[c] Protein was determined by the method of Lowry et al. (1951)

Colchicine. When colchicine was present in the incubation medium, methacholine had a less marked effect on ^{14}C-APG release by bronchial explants (Fig. 2). This effect of colchicine was dose-dependent and in the presence of colchicine concentrations greater than 10^{-5} M, methacholine did not increase ^{14}C-APG release significantly above baseline release. At the highest concentration used in this study (10^{-4} M), colchicine caused a small but significant decrease in baseline ^{14}C-APG release by bronchial explants (Fig. 2; $p<0.05$). Lower concentrations of colchicine had no effect on baseline release.

Effect of Cytochalasin B and Colchicine on the Specific Activity of ^{14}C-labeled Glycoconjugates

The specific activities (in dpm/μg protein) of ^{14}C-APG released by bronchial explants during incubation Periods II and I (the ratio of which was used as index of glycoconjugate release in this study) were measured (Table 1). For "control" experiments in which no drug was present during Periods I and II, both the total radioactive count and the specific activities of the released ^{14}C-APG were similar in the two periods. Methacholine (5.10^{-5} M), when added to the incubation medium during Period II, caused an increase in the total radioactive count as compared with Period I but the specific activities ^{14}C-APG released in each period were similar. Neither cytochalasin B nor colchicine added during Period II, either singly or in combination with methacholine, altered the specific activity of ^{14}C-APG released as compared with Period I. The effects of methacholine, cytochalasin B and colchicine on ^{14}C-labeled glycoconjugate release (Figs. 1, 2) were thus apparently not the result of alterations in the rates of glycoconjugate synthesis or their intracellular of extracellular degradation. Such effects would have been expected to alter the specific activity of ^{14}C-labeled glycoconjugates released by the bronchial explants.

Table 2. Effect of colchicine and cytochalasin B on labeled precursor incorporation into mucous cells[a]

Additions to medium	Pre-pulse incubation time (h)	Mean grain density/cell[b]	
		^{3}H-glcN	^{3}H-thr
None	1	13.9± 2.1[c]	19.8± 3.6
Colchicine (10^{-5} M)	1	14.6± 2.8	23.7± 3.9
Cytochalasin B (10^{-5} M)	1	11.6± 2.2	22.9± 2.4
Cycloheximide (10^{-4} M)	1	5.9[d]± 1.6	3.7[e]± 2.3

[a] Drugs were incubated with explants before pulse-labeling with ^{3}H-glucosamine (^{3}H-glcN; 50 µCi/ml) or ^{3}H-threonine (^{3}H-thr; 20 µCi/ml) for 0.5 h. Explants were chased for 1 h with Medium 199
[b] Mean grain density/mucous cell was quantified from autoradiography as described in Methods
[c] Values represent means of 6 explants± SE
Values significantly different from explants incubated only with medium 199 before pulse-labeling:
[d] $p<0.05$. [e] $p<0.01$

Effect of Cytochalasin B and Colchicine on Labeled Macromolecule Secretion in Mucous, Serous, and Goblet Cells

Labeled Precursor Incorporation. Preincubation of bronchial explants with cytochalasin B or colchicine (both at concentrations of 10^{-5} M) had no effect on the density of silver gains overlying mucous cells of the submucosal glands labeled with either ^{3}H-glucosamine or ^{3}H-threonine (Table 2). This indicated that these agents did not affect the incorporation of the labeled precursors into intracellular macromolecules.

Neither cytochalasin B nor colchicine affected precursor incorporation into serous cells of the submucosal glands or goblet cells of the surface epithelium (data not shown).

By contrast, cycloheximide (10^{-4} M), which is known to inhibit glycoconjugate synthesis in mucous, serous and goblet cells of the human airway mucosa (Coles and Reid 1978), reduced the grain density of mucous cells labeled with ^{3}H-glucosamine by 58% ($p<0.05$) and with ^{3}H-threonine by 81% ($p<0.01$). Similar effects were observed with serous and goblet cells (data not shown).

Intracellular Transport of Labeled Macromolecules. One hour after pulse-labeling, the majority of mucous, serous and goblet cells contained radioactive label in their bottom quarter (level 1), i.e., in the peri- and supranuclear regions of the cell (Figs. 3a, b, c). With increasing time after pulse-labeling, label progressively moved towards the cell apex migrating, in mucous and goblet cells, as an aggregate (or several aggregates) of silver grains, and in serous cells as a more diffuse band of silver grains. In the submucosal gland, most mucous and serous cells contained label in their apical quarter (level 4) by 7 h after pulse-labeling (Figs. 3a and b).

In goblet cells, label transport was slower than in either mucous or serous cells and by 10 h after pulse-labeling. label had reached only halfway up the cell (levels 2–3; Fig. 3c). In addition, by 10 h after pulse-labeling the intracellular location of label

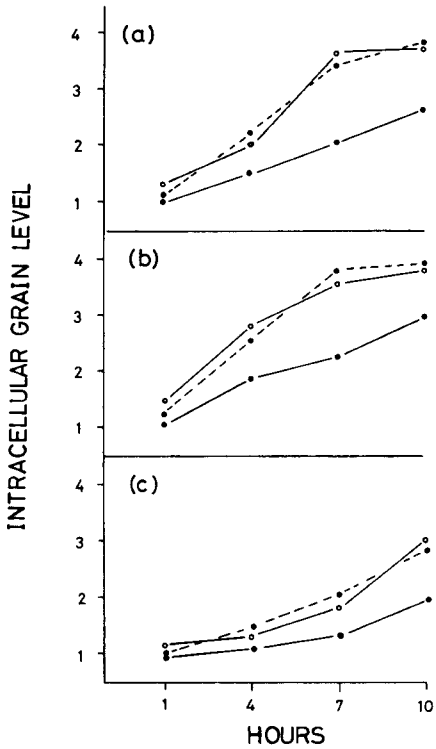

Fig. 3. Effect of cytochalasin B and colchicine on transport of ^{3}H-labeled macromolecules through (**a**) mucous, (**b**) serous, and (**c**) goblet cells. Intracellular grain level assessed as described in Methods. Abscissa indicates time after pulse-labeling with ^{3}H-glucosamine. Each point is the mean grain level calculated from 3–5 explants. Control (●---●); 10^{-5} M cytochalasin B (○—○); 10^{-5} *M* colchicine (●—●)

varied widely among individual goblet cells. By contrast, individual mucous and serous cells transported label at similar rates.

When 10^{-5} M cytochalasin B was present in the chase medium, mucous, serous and goblet cells transported label at rates which were indistinguishable from the controls (Fig. 3a, b, c). When 10^{-5} M colchicine was present in the chase medium, however, the rate of label transport was markedly reduced in all three cell types (Fig. 3a, b, c). By 4 h after pulse-labeling, a small difference in label transport was observed in colchicine-treated explants. This was considerably more marked at 7 and 10 h in each cell type ($p < 0.05$ in all cases), the label having migrated no further than the lower half of the cell (levels 1 and 2) in most cases.

No evidence of cytoxicity caused by cytochalasin B or colchicine was observed in these studies.

Discharge of Labeled Macromolecules. Four h after the addition of a pulse of ^{3}H-glucosamine (0.5 h pulse + 3.5 h unlabeled chase) to bronchial explants, 21–27% of mucous cells and 13–16% of serous cells had discharged radioactive label and were considerably depleted of secretory granules. This "baseline" discharge appears to be caused partially be release of endogeneous acetylcholine since it may be partially inhibited by atropine and stimulated by physostygmine (Sturgess and Reid 1972; Coles 1976). A large component of it, however, appears to be "spontaneous" cellular discharge.

Table 3. Effect of cytochalasin B and colchicine on discharge of labeled macromolecules

Additions to medium	% Discharged cells[b]			
	Cytochalasin B (10^{-5} M)		Colchicine (10^{-5} M)	
	mucous	serous	mucous	serous
None	26.9± 4.1[c]	16.0± 2.9	21.2± 4.7	13.6± 3.2
Drug alone	34.0± 4.9	15.9± 2.8	30.2± 4.9	15.1± 3.8
Methacholine ($5 \cdot 10^{-5}$ M)	73.6± 5.6	58.2± 3.3	76.6± 6.3	65.7± 5.2
Methacholine+ drug	36.3± 3.9	19.7± 2.9	30.8± 5.2	21.3± 5.0

[a] Explants were pulse-labeled with ^{3}H-glucosamine for 0.5 h and chased for 3.5 h with Medium 199 containing no label. Drugs were added singly or in combination for the last hour of the chase period
[b] Discharged mucous and serous cells quantified as described in Methods
[c] Values are means of 6–8 explants ± SE

When cytochalasin B (10^{-5} M) or colchicine (10^{-5} M) were incubated with bronchial explants during the last h of the chase period the percentages of mucous and serous cells which had discharged label were indistinguishable from control values (Table 3). Methacholine ($5 \cdot 10^{-5}$ M) added during the last h of the chase period caused a large increase in the percentage of discharged mucous and serous cells ($p < 0.001$ in each case). When colchicine or cytochalasin B (each 10^{-5} M) were incubated together with methacholine, the methacholine-induced increase in mucous and serous cell discharge was almost completely prevented ($p < 0.001$ in each case).

Qualitative assessment of autoradiographs indicated that cytochalasin B, colchicine, and methacholine did not substantially affect the discharge of label by goblet cells in the surface epithelium.

Discussion

To the best of our knowledge, this is the first study of the effects of cytochalasin B and colchicine on mucus secretion by human airway. In mucous and serous cells, colchicine, but not cytochalasin B, reduces the intracellular transport of labeled macromolecules though neither agent has any effect on baseline discharge. Both agents, at concentrations known to disrupt microtubules and microfilaments (Allison and Davies 1974), are inhibitory to increases in labeled macromolecule discharge induced by methacholine, though it is clear that they act at different intracellular sites. Cytochalasin B interferes primarily with secretagogue-induced discharge, while colchicine blocks both intracellular transport and discharge of macromolecules. Neither agent inhibits incorporation of labeled-glycoconjugate precursors into mucous or serous cells. In goblet cells of the surface epithelium, colchicine, but not cytochalasin B, reduces intracellular transport of macromolecules while neither agent affects precursor incorporation or discharge. The effects of cytochalasin B and colchicine on macromolecular secretion at the cellular level

correlate well with their effects on glycoconjugate release by the bronchial explant, i.e., neither agent reduced baseline glycoconjugate release but both antagonised the effect of methacholine.

Cytochalasin B has not been shown to inhibit baseline secretion at any site studied (Schofield 1971; Williams and Wolff 1971; Williams 1977; Stock et al. 1978). In other exocrine, including bronchial glands, it has been shown to inhibit stimulated secretion, e.g., epinephrine-induced amylase release by rat parotid glands (Butcher and Goldman 1972) and bethanechol-stimulated enzyme secretion by mouse pancreas (Williams 1977). The fact that in the present study of human bronchi cytochalasin B inhibited the methacholine-induced increase in ^{14}C-labeled glycoconjugate release seems to be the result of the inhibitory effect of cytochalasin B on macromolecular discharge induced by methacholine in mucous and serous cells of the submucosal glands.

In a recent study of mast cell histamine secretion, Nemeth and Douglas (1978) were able to show that the anti-secretory effects of cytochalasin B resulted only because of inhibition of hexose uptake. Certainly, cytochalasin B has been shown to inhibit monosaccharide transport across the plasma membranes of several culture cell lines (Kletzien et al. 1972; Mizel and Wilson 1972). Bauduin and coworkers (1975), however, showed that reduced hexose uptake did not account for the cytochalasin B inhibition of pancreatic enzyme release which probably resulted from microfilament disruption. The present study indicates that this may also be true in the airway since cytochalasin B did not inhibit the incorporation of either glucosamine or threonine into the cells responsible for glycoconjugate synthesis. It is also improbable that inhibition of hexose uptake would account for the reduction in discharge caused by cytochalasin B since, over short periods, the rates of hexose uptake and glycoconjugate discharge are independent of each other (Coles and Reid 1978).

In the present study, colchicine reduced the rate of intracellular transport of macromolecules but failed to reduce the baseline release of labeled-glycoconjugates by bronchial explants (except at the highest concentration studied). This is surprising since the release of labeled macromolecules from the cell apex would be expected to be reduced if the migration of label through the cell is inhibited. Our studies indicate, however, that colchicine appeared to inhibit transport of macromolecules only after several hours incubation (reduction in transport rate was observed after 4–7 h incubation with colchicine). This may have been due to the failure of our method of analysis to detect small changes in transport rate, but is more probably the result of needing to incubate the tissue for several hours with colchicine before it becomes effective. Colchicine treatment for several hours is necessary for secretory inhibition in other organs, such as the exocrine pancreas (Patzelt et al. 1977), parotid gland (Butcher and Goldman 1972), and in mast cells (Gillespie et al. 1968); the degree of secretory inhibition is proportional to the duration of colchicine pretreatment. Williams and Lee (1976) have shown that while colchicine is rapidly taken up by pancreatic cells, it has no effect on secretion for between 2 and 3 h. They ascribed this delay to the resistance of pancreatic microtubules to disruption with colchicine. This may also be the case in the airway secretory cells.

Colchicine did inhibit glycoconjugate release induced by methacholine and this can be related to colchicine inhibition of methacholine-induced discharge from mucous and serous cells in the submucosal glands. While colchicine may inhibit secretagogue-induced discharge and intracellular transport of secretory granules by entirely different mechanisms, it is not inconceivable that the effects are mediated in some way by a similar mode of action, both involving microtubules.

Previous studies have shown that mucous and serous cells of the submucosal glands are similar in their response to secretagogues and metabolic inhibitors (Coles and Reid 1978). The goblet cell of the surface epithelium differs significantly in that it is insensitive to all physiological secretagogues examined so far. The present study adds to our knowledge of goblet cell secretion in that it demonstrates that colchicine inhibits intracellular transport of macromolecules in this cell type while neither colchicine nor cytochalasin B inhibit its baseline discharge. In its response to cytochalasin B and colchicine, the goblet cell thus resembles mucous and serous cells, at least as regards its baseline secretion.

That the effects of colchicine and cytochalasin B in the present study are mediated by effects primarily on microtubules and microfilaments is not certain though the results suggest a role for these organelles in the cellular process of glycoconjugate secretion in the human airway. Only microtubule integrity seems important for baseline glycoconjugate secretion since colchicine inhibits intracellular transport of macromolecules in all three cell types. A role for microtubules in intracellular transport of secretory granules has been suggested by other studies (Allison and Davies 1974). In addition, a role for microtubules in secretagogue-induced discharge of macromolecules from mucous and serous cells is suggested by this study.

Microfilament integrity is apparently not necessary for baseline glycoconjugate secretion by any cell type in the human bronchial mucosa. These structures are implicated, however, in the process of macromolecule discharge from mucous and serous cells stimulated with methacholine suggesting a difference in mechanism between baseline and stimulated discharge in these cell types.

References

Adler KB, Craighead JE (1978) Function of microtubules and microfilaments in the secretion of mucin by porcine tracheobronchial tissue in organ culture. Fed Proc 37:266

Allison AC, Davies P (1974) In: Ceccarelli B, Clementi F, Meldolesi J (eds) Interaction of membranes, microfilaments and microtubules in endocytosis and exocytosis. Advances in Cytopharmacology, Vol II, New York, pp 237–248

Bauduin HC, Stock C, Vincent D, Grenier JF (1975) Microfilamentous system and secretion of enzyme in the exocrine pancreas. Effect of cytochalasin B. J Cell Biol 66:165–181

Butcher FR, Goldman RH (1972) Effect of cytochalasin B and colchicine on the stimulation of α-amylase release from rat parotid tissue slices. Biochem Biophys Res Commun 48:23–29

Chakrin LW, Baker AP, Christian P, Wardell JR Jnr (1973) Effect of cholinergic stimulation on the release of macromolecules by canine trachea in vitro. Am Rev Respir Dis 108:69–76

Coles SJ (1976) Regulation of the secretion cycles of mucous and serous cells in the human bronchial gland. In: Elstein M, Parke DV (eds) Mucus in Health and Disease. Plenum Press, New York London, p 155

Coles SJ, Reid L (1978) Glycoprotein secretion in vitro by human airway: Normal and chronic bronchitis. Exp Mol Pathol 29:326–341

Gallagher JT, Kent PW, Passatore M, Phipps RJ and Richardson PS (1975) The composition of tracheal mucus and the nervous control of its secretion in the cat. Proc R Soc Lond B 192:49–76

Gillespie E, Levine RJ, Malawista SE (1968) Histamine release from rat peritoneal mast cells: inhibition by colchicine and potentiation by deuterium oxide. J Pharmacol Exp ther 164:158–165

Kletzien RF, Perdue JF, Springer A (1972) Cytochalasin A and B. Inhibition of sugar uptake in cultured cells. J Biol Chem 247:2964–2966

Lacy PE, Howell SL, Young DA, Fink CJ (1968) New hypothesis of insulin secretion. Nature 219:1177–1179

Last JA, Jennings MD, Schwartz LW, Cross CE (1977) Glycoprotein secretion by tracheal explants cultured from rats exposed to ozone. Am Rev Respir Dis 116:695–703

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin-phenol reagent. J Biol Chem 193:265–275

Meyrick B, Reid L (1970) Ultrastructure of cells in the human bronchial submucosal glands. J Anat 107:281–299

Meyrick B, Reid L (1975) In vitro incorporation of [^{3}H] threonine and [^{3}H] glucose by the mucous and serous cells of the human bronchial submucosal gland. J Cell Biol 67:320–344

Mizel SB, Wilson L (1972) Inhibition of the transport of several hexoses in mammalian cells by cytochalasin B. J Biol Chem 247:4102–4105

Nemeth EF, Douglas WW (1978) Effects of microfilament-active drugs, phalloidin and the cytochalasins A and B, on exocytosis in mast cells evoked by 48/80 or A23187. Naunyn-Schmiedeberg's Arch Pharmacol 302:153–163

Neutra MR, Grand JF, Trier JS (1977) Glycoprotein synthesis, transport and secretion by epithelial cells of human rectal mucosa. Normal and cystic fibrosis. Lab Invest 36:535–546

Patzelt C, Brown D, Jeanrenaud B (1977) Inhibitory effect of colchicine on amylase secretion by rat parotid glands. Possible localization in the Golgi area. J Cell Biol 73:578–593

Rogers AR (1973) Techniques of Autoradiography. Elsevier, Amsterdam London New York, p 89

Schofield JG (1971) Cytochalasin B and release of growth hormone. Nature 234:215–216

Schroeder TE (1970) The contractile ring I. Fine structure of dividing mammalian (HeLa) cells and the effects of cytochalasin B. Z Zellforsch 109:431–449

Stock C, Launay JF, Grenier JF, Bauduin H (1978) Pancreatic acinar cell changes induced by caerulein, vinblastine, deuterium oxide and cytochalasin B in vitro. Lab Invest 38:157–164

Sturgess J, Reid L (1972) Secretory activity of the human bronchial mucous glands in vitro. Exp Mol Pathol 16:362–381

Wessels NK, Spooner BS, Ash JF, Bradley MO, Luduena MA, Taylor EL, Wrenn JT, Yamada KM (1971) Microfilaments in cellular and developmental processes. Science 171:135–143

Williams JA (1977) Effects of cytochalasin B and pancreatic acinar cell structure and secretion. Cell Tissue Res 179:453–466

Williams JA, Lee M (1976) Microtubules and pancreatic amylase release by mouse pancreas in vitro. J Cell Biol 71:795–806

Williams JA, Wolff J (1971) Cytochalasin B inhibits thyroid secretion. Biochem Biophys Res Commun 44:422–425

Wilson L, Bamburg JR, Mizel SB, Grisham LM, Cresswell KM (1974) Interaction of drugs with microtubule proteins. Fed Proc 33:158–166

Yeager H, Massaro G, Massaro D (1971) Glycoprotein synthesis by the trachea. Am Rev Respir Dis 103:188–197

Accepted September 8, 1980