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Effects of Ambroxol, S-carboxymethylcysteine, Dextromethorphan and Noscapine on Mucin Release from Airway Goblet Cells

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In the present study, we investigated whether ambroxol, S-carboxymethyl-L-cysteine, dextromethorphan and noscapine affect mucin release from airway goblet cells. Confluent primary hamster tracheal surface epithelial cells were metabolically radiolabeled and chased for 30 min in the presence of varying concentrations of the above agents to assess the effects on ^{3}H-mucin release. Noscapine stimulated mucin release during 30 min of treatment period in a dose-dependent manner. However, ambroxol, S-carboxymethyl-L-cysteine and dextromethorphan showed no significant effect on mucin release during 30 min of treatment period. We conclude that noscapine can affect mucin release by acting on airway mucin-secreting cells.

Key Words: Airway, Mucin, Ambroxol, S-carboxymethyl-L-cysteine, Dextromethorphan, Noscapine

INTRODUCTION

Mucus in the airway plays an important role in host defense against airborne chemicals, particles and invading microorganisms through a mechanism called the mucociliary clearance. Its protective function is mainly due to the viscoelastic property of mucous glycoproteins or mucins (Ellis, 1985). Mucins are high molecular weight glycoproteins produced by goblet cells in the surface epithelium as well as mucous cells in the submucosal gland. Therefore, any abnormality in the quality or quantity of mucins not only causes altered airway physiology, but also impairs host defenses, often leading to serious airway pathology as exemplified in chronic bronchitis, cystic fibrosis, asthma, and bronchiectasis (Kim et al, 1997). To remove an excess mucus from the airway, there could be two ways; i) getting rid of the mucus by physical means, that is to say, aspiration after dilution of mucus, and ii) suppression of secretion and/or production of mucus by pharmacological means. However, clinically, the physical method induces irritation of airway luminal wall and leads to hypersecretion of mucus through a reflex mechanism. Thus, the pharmacological means to inhibit mucin secretion and/or production has become an important approach to regulate the hypersecretion of airway mucus (Mutschler et al, 1995). Secretion of airway mucin is generally stimulated by various agents whereas glucocorticoids inhibits the hypersecretion of airway mucins (Mutschler et al, 1995; Lee et al, 2002). However, since glucocorticoids have various limitations in the application to pharmacotherapy of human diseases with airway mucus hypersecretion, it is highly desirable to find a way to inhibit the excessive mucin release by agents that have clinically been used for the management of diverse diseases. Ambroxol and S-carboxymethyl-L-cysteine (SCMC) are clinically-used mucolytics, and dextromethophan and noscapine are clinically-used non-narcotic antitussives (Mutschler et al, 1995). However, to the best of our knowledge, there are no reports about direct effects of ambroxol, SCMC, dextromethophan and noscapine on basal-physiological or constitutive-mucin release from airway goblet cells, and their mechanisms of action on airway mucin-secreting cells are not clearly understood, yet. Therefore, we attempted to investigate whether these agents affect basal mucin release from airway goblet cells, using a primary hamster tracheal surface epithelial (HTSE) cell culture-an established *in vitro* model for secretory cell metaplasia (Wasano et al, 1988). Indeed, we found that noscapine can stimulate mucin release, in a dose-dependent manner.

METHODS

Materials

All the chemicals and reagents used in this experiment were purchased from Sigma (St. Louis, MO. USA), unless otherwise specified.

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ABBREVIATIONS: PLL, poly-L-lysine; ATP, adenosine triphosphate; HTSE, hamster tracheal surface epithelial; PBS, phosphate-buffered saline; SCMC, S-carboxymethyl-L-cysteine.

Primary hamster tracheal surface epithelial (HTSE) cell culture

The animals were cared in accordance with the Guide for the Care and Use of Laboratory Animals as promulgated and regulated by Chungnam National University. Tracheas were obtained from Golden Syrian male hamsters, 8 weeks of age (Harlan Sprague Dawley, Indiana, USA). Hamster tracheal surface epithelial cells were harvested and cultured on a thick collagen gel substratum as previously reported (Wasano et al, 1988). Briefly, animals were euthanized in a CO_2 chamber and the tracheas were exposed under aseptic conditions. The tracheas were canulated using a polyethylene tube through which the tracheal lumen was filled with 0.1% pronase (Type XIV) prepared in Ca^{++}, Mg^{++}-free Minimum Essential Medium (MEM, GIBCO) and incubated at 4°C for 16 hr. The luminal contents were flushed, and cells were washed twice with Ca^{++}, Mg^{++}-free MEM containing 10% fetal bovine serum by centrifuging at 200 ×g. The washed cell pellets were dissociated in a growth medium containing Medium 199 and Dulbecco's Modified Eagle's medium (DME) (1 : 1) supplemented with insulin (5 μg/ml), epidermal growth factor (12.5 ng/ml), hydrocortisone (0.1 μM), fetal bovine serum (5% v/v, Hyclone, Logan, UT, USA), sodium selenite (0.01 μM), retinoic acid (0.1 μM), penicillin G (100 U/ml, GIBCO), streptomycin (100 μg/ml, GIBCO), and gentamicin (50 μg/ml) ("complete" medium). At this stage, most of the cells were in small aggregates and plated at a density of 10^4 cells/cm^2 into tissue culture dishes containing thick collagen gel (0.15 ml/ cm^2) made of collagen type I (Regenmed, Seoul, Korea). Cultures were incubated at 37°C in a humidified atmosphere containing 95% air and 5% CO_2 and culture medium was changed on day 1, 3, 5 and 7.

Metabolic labeling of mucins and treatment of cultures

Mucins were metabolically radiolabeled for 24 hr by incubating confluent cultures (24 well plate, 5×10^5 cells/well) with 0.2 ml/well of a "complete" medium containing 10 Ci/ml of [6-^{3}H] glucosamine (39.2 Ci/mmol, New England Nuclear) for 24 hr, as previously reported (Kim et al, 1987). At the end of the 24 hr of incubation, the spent media (the pretreatment sample) were collected, and the labeled cultures were washed twice with Dulbecco's phosphate-buffered saline without Ca^{++} and Mg^{++} before chasing for 30 min or 24 hr in Dulbecco's phosphate-buffered saline containing varying concentrations of each agent (the treatment sample). Ambroxol, SCMC, dextromethorphan, noscapine, ATP and poly-L-lysine (PLL) (average molecular weight 7,500 Da) were dissolved and administered in Dulbecco's phosphate-buffered saline. The final pH values of these solutions were between 7.0 and 7.4. Dulbecco's phosphate-buffered saline in this range did not affect mucin release from cultured hamster tracheal surface epithelial cells (Kim et al, 1997). Floating cells and cell debris were removed by centrifugation at 12,000 × g for 5 min. The samples were stored at −80°C until assayed for their ^{3}H-mucin contents.

Quantitation of ^{3}H-mucins

High molecular weight glycoconjugates excluded through Sepharose CL-4B gel-filtration column chromatography and resistant to hyaluronidase were defined as mucins and measured by the column chromatography as previously reported (Kim et al, 1985). Media samples were adjusted to pH 5.0 with 0.1 M citric acid and treated with 100 U/ml of testicular hyaluronidase (Type VI-S) at 37°C for 16 hr. At the end of the incubation, the digestion mixtures were neutralized to pH 7.4 using 0.2 M NaOH, boiled for 2 min and centrifuged. The supernatants were applied to Sepharose CL-4B columns (1×50 cm) equilibrated with Dulbecco's phosphate-buffered saline containing 0.1% (w/v) sodium dodecyl sulfate. Columns were eluted with the same buffer at a constant flow rate of 0.336 ml/min and 0.42 ml fractions were collected. Void volume fractions (4 peak fractions) were mixed with 4 ml of scintillation cocktail (Hydrofluor; National Diagnostic), and radioactivity was counted using a liquid scintillation counter. The sum of radioactivity in four peak fractions was defined as the amount of mucin in the sample. The effect of agents on mucin release was measured as follows: the amount of mucin released during the treatment period was divided by the amount of mucin released during the pretreatment period, and the ratio was expressed as a secretory index. Means of secretory indices of each group were compared, and the differences were assessed using statistics.

Statistics

Means of individual group were converted to percent control and expressed as mean±S.E.M. The difference between groups was assessed using Student's t-Test for unpaired samples. $p<0.05$ was considered as significantly different.

RESULTS

Effect of ambroxol on mucin release

Ambroxol did not affect mucin release during 30 min of treatment period. The amounts of mucin in the spent media of ambroxol-treated cultures were 100±7%, 109±7%, 107 ±11% and 93±9% for control, 10^{-6} M, 10^{-5} M and 10^{-4} M, respectively. For comparison, both 2.0×10^{-4} M ATP and 10^{-5} M PLL (MW 7,500) were used as positive controls (Fig. 1).

Effect of SCMC on mucin release

S-carboxymethylcysteine (SCMC) did not affect mucin release during 30 min of treatment period. The amounts of mucin in the spent media of SCMC-treated cultures were 100±7%, 97±5%, 94±3% and 89±4% for control, 10^{-6} M, 10^{-5} M and 10^{-4} M, respectively. For comparison, both 2.0×10^{-4} M ATP and 10^{-5} M PLL (MW 7,500) were used as positive controls (Fig. 2).

Effect of dextromethorphan on mucin release

Dextromethorphan did not affect mucin release during 30 min of treatment period. The amounts of mucin in the spent media of dextromethorphan-treated cultures were 100± 13%, 99±11%, 92±13% and 141±30% for control, 10^{-6} M, 10^{-5} M and 10^{-4} M, respectively. For comparison, both 2.0×10^{-4} M ATP and 10^{-5} M PLL (MW 7,500) were used as positive controls (Fig. 3).

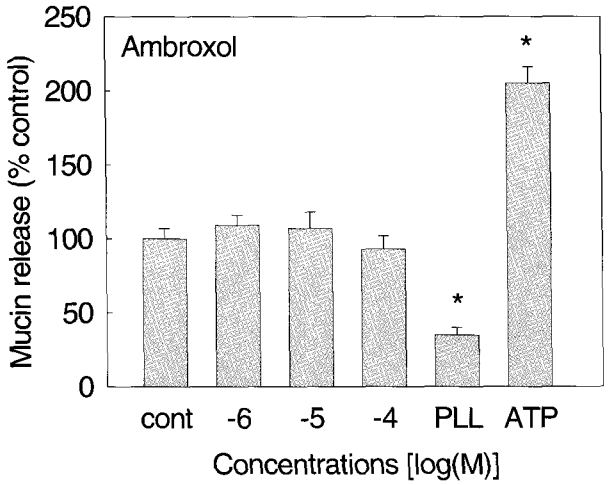

Fig. 1. Effect of ambroxol on mucin release. Confluent HTSE cells were metabolically radiolabeled with ^{3}H-glucosamine for 24 hr and chased for 30 min in the presence of varying concentrations of ambroxol. The amounts of ^{3}H-mucins in the spent media were measured as described in Methods. Each bar represents mean±S.E.M. of 3~4 culture wells in comparison with that of control set at 100%. For comparison, both 2.0×10^{-4} M ATP and 10^{-5} M PLL (MW 7,500) were used as positive controls. *significantly different from control ($p<0.05$).

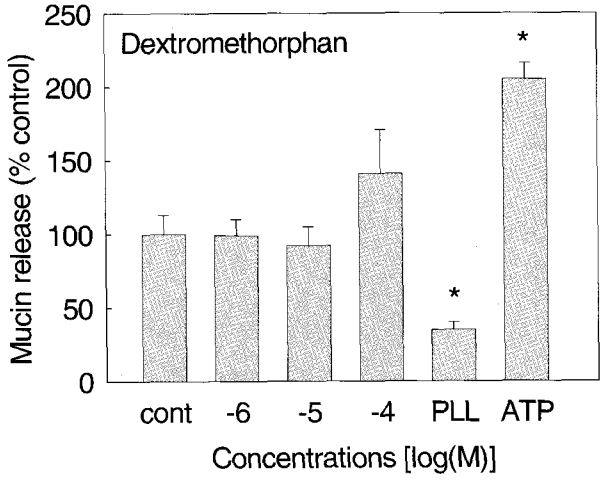

Fig. 3. Effect of dextromethorphan on mucin release. Confluent HTSE cells were metabolically radiolabeled with ^{3}H-glucosamine for 24 hr and chased for 30 min in the presence of varying concentrations of dextromethorphan. The amounts of ^{3}H-mucins in the spent media were measured as described in Materials and Methods. Each bar represents mean±S.E.M. of 3~4 culture wells in comparison with that of control set at 100%. For comparison, both 2.0×10^{-4} M ATP and 10^{-5} M PLL (MW 7,500) were used as positive controls. *significantly different from control ($p<0.05$).

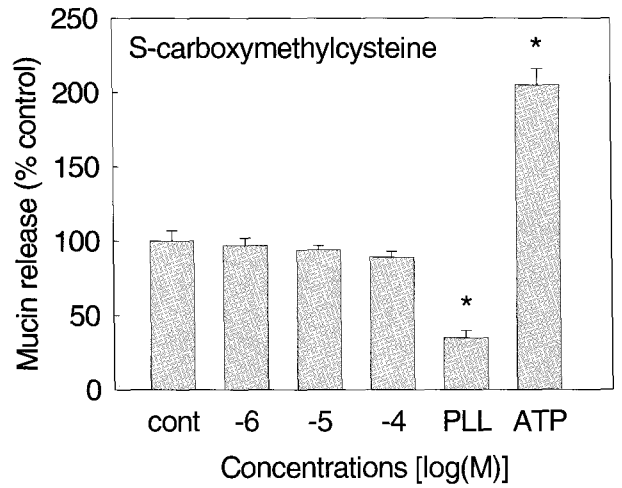

Fig. 2. Effect of SCMC on mucin release. Confluent HTSE cells were metabolically radiolabeled with ^{3}H-glucosamine for 24 hr and chased for 30 min in the presence of varying concentrations of SCMC. The amounts of ^{3}H-mucins in the spent media were measured as described in Methods. Each bar represents mean± S.E.M. of 3~4 culture wells in comparison with that of control set at 100%. For comparison, both 2.0×10^{-4} M ATP and 10^{-5} M PLL (MW 7,500) were used as positive controls. *significantly different from control ($p<0.05$).

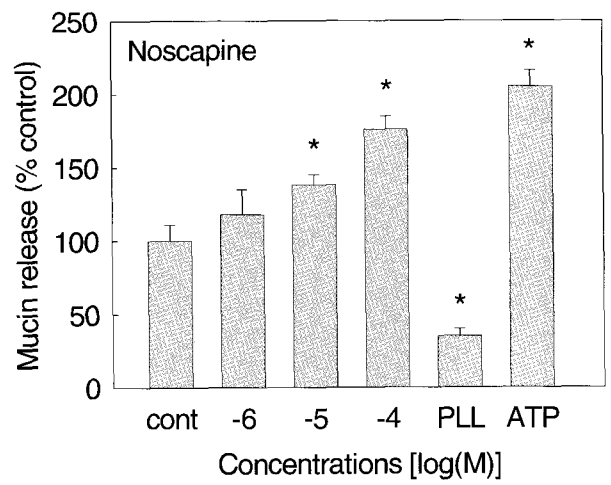

Fig. 4. Effect of noscapine on mucin release. Confluent HTSE cells were metabolically radiolabeled with ^{3}H-glucosamine for 24 hr and chased for 30 min in the presence of varying concentrations of noscapine. The amounts of ^{3}H-mucins in the spent media were measured as described in Materials and Methods. Each bar represents mean±S.E.M. of 3~4 culture wells in comparison with that of control set at 100%. For comparison, both 2.0 ± 10^{-4} M ATP and 10^{-5} M PLL (MW 7,500) were used as positive controls. *significantly different from control ($p<0.05$).

Effect of noscapine on mucin release

Noscapine significantly stimulated mucin release during 30 min of treatment period. The amounts of mucin in the spent media of noscapine-treated cultures were 100±11%, 118±17%, 138±7% and 176±9% for control, 10^{-6} M, 10^{-5} M and 10^{-4} M, respectively. For comparison, both 2.0×10^{-4} M ATP and 10^{-5} M PLL (MW 7,500) were used as positive controls (Fig. 4).

DISCUSSION

In this study, we used a primary Hamster Tracheal Surface Epithelial cell culture system to elucidate the effects of ambroxol, SCMC, dextromethorphan and noscapine on mucin release. Hamster tracheal surface epithelial cells grown on a thick collagen gel synthesize and secrete mucins at confluence, which are indistinguishable from in vivo mucins in terms of both size and charge. Using this cell culture system, an optimum condition was established to study the pharmacology of mucin release of airway goblet cells (Kim et al, 1985). Ambroxol was reported to increase the secretion of pulmonary surfactant and this increase is likely to be involved in the expectorant action of ambroxol (Miyata et al, 1986). Ambroxol stimulated serous and mucous secretion and restored normal production of mucin in patients suffering from chronic bronchopneumopathy (Aliperta et al, 1986). Based on these reports, we expected that ambroxol might increase, or, at least in part, change the quantity of secretion of airway mucin, by directly acting on mucin-secreting cells. However, as shown in Fig. 1, ambroxol did not show any activity on mucin release from airway goblet cells, during 30 min of treatment period. There are several reports about the activity of sulfur-containing mucolytics on airway diseases (Cortijo et al, 1999; Houtmeyers et al, 1999, Rubio et al, 2000; Sun et al, 2002). Sulfur-containing mucolytics have been suggested as a cause of clinical improvement in patients suffering from airway diseases with mucus hypersecretion (Houtmeyers et al, 1999). Sun et al. reported sulfur-containing mucolytics increased transportability of sputum of cystic fibrosis patients (Sun et al, 2002). Sueyoshi et al. reported that SCMC-one of sulfur-containing mucolytics-decreased both the expression of MUC5AC (mucin) protein and airway inflammation, in the airway of rats exposed to sulfur dioxide (Sueyoshi et al, 2004). However, in our results, as shown in Fig. 2, SCMC did not inhibit mucin release, during 30 min of treatment period. Thus, it is highly likely that both ambroxol and SCMC regulate mucin release clinically, not by acting directly on mucin-secreting cells but by different mechanisms. Dextromethorphan inhibited both neurogenic and acetylcholine-mediated contractions of isolated bronchial muscle of guinea pigs (Kamikawa and Shimo, 1991). Noscapine suppressed angiotensin converting enzyme inhibitor-induced cough by interfering with the mediation of bradykinin (Ebrahimi et al, 2003). Therefore, if dextromethorphan and noscapine have anti-bronchoconstricting/antitussive effects with anti-hypersecretory effects, they might be used as ideal drugs for the management of bronchial asthma. On the basis of these reports and hypothesis, we tested the possible effects of dextromethorphan and noscapine on airway mucin release. In our results, as shown in Fig. 3, dextromethorphan did not significantly affect basal mucin release from airway goblet cells during 30 min of treatment period. However, as shown in Fig. 4, noscapine significantly stimulated basal mucin release, during 30 min of treatment period. Since noscapine showed the stimulatory effect on mucin release with its mild antitussive action, it can be used as an expectorant in the patients with upper respiratory tract infections, though further studies are needed. Taken together, we failed to find a drug that showed a robust inhibitory action on mucin release. Nonetheless, from the viewpoint of management of airway mucus hypersecretion, it is of a great interest to find the inhibitory effects of clinically-used drugs on mucin release as another pharmacological activity.

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