Agents and Actions, vol. 22, 1/2 (1987)

0065-4299/87/020082-04 $ 1.50 + 0.20/0

The actions of methacholine, phenylephrine, salbutamol and histamine on mucus secretion from the ferret *in-vitro* trachea

S. E. Webber and J. G. Widdicombe

Department of Physiology, St. George's Hospital Medical School, Cranmer Terrace, London SW17 ORE, U.K.

Abstract

Methacholine, phenylephrine and histamine produced highly significant and salbutamol significant increases in the rate of mucus secretion from the ferret trachea.
Methacholine, phenylephrine and histamine all produced highly significant increases in the rate of output of lysozyme, but the concentration of lysozyme in the mucus was significantly increased only by phenylephrine.
Salbutamol produced no significant change in the output of lysozyme, and the concentration of lysozyme in the mucus was significantly decreased.
It is concluded that methacholine, phenylephrine and histamine are potent stimulators of serous cell secretion whereas salbutamol has only a weak secretory action on these cells. Methacholine, histamine and salbutamol probably stimulate secretion from mucous cells as well as from serous cells.
The increase in the concentration of lysozyme produced by phenylephrine may be due to stimulation of a fluid reabsorption mechanism.

Introduction

Histological studies have shown that the ferret trachea contains very few epithelial goblet cells [1] and so the secretion of mucus is almost entirely from mucous and serous cells in the submucosal glands. Therefore the ferret trachea is a good model to use for examining the effects of drugs on mucus secretion from these glands.
Immunocytochemical and morphometric studies have suggested that mucous cell secretion from the ferret trachea is stimulated more potently by β-adrenoceptor and cholinoceptor agonists than by α-adrenoceptor agonists, whereas serous cell secretion is stimulated more potently by α-adrenoceptor and cholinoceptor agonists than by β-adrenoceptor agonists [2, 3]. However, there is little evidence from functional or biochemical studies to indicate whether secretions produced by cholinergic and adrenergic agonists are from serous or mucous cells. Furthermore, there has been no attempt to investigate the cellular origin of the secretion produced by inflammatory mediators such as histamine.
Lysozyme is a bactericidal enzyme found in respiratory tract fluid. Immunocytochemistry has shown that lysozyme is located in the secretory granules of serous but not mucous cells in the submucosal glands of ferrets [4] and is therefore a useful marker for serous cell secretion. We have used a turbidimetric assay for lysozyme to examine further the actions of methacholine (musca-

Correspondence and requests for reprints to J. G. Widdicombe.

rinic agonist), salbutamol (β-adrenoceptor agonist), phenylephrine (α-adrenoceptor agonist) and histamine on mucous and serous cell secretion from the ferret *in vitro* whole treachea.

Methods

The preparation of the ferret *in vitro* whole trachea has previously been described in detail [5]. Briefly, the trachea of an anaesthetised ferret was exposed and cannulated 5 mm below the larynx with a special perspex cannula. The ferret was killed and the chest opened along the midline. The trachea was cleared of adjacent tissue, cannulated at the carinal end and was mounted, air-filled and laryngeal end-down, in an organ bath filled with Krebs-Henseleit buffer containing 0.1% (w/v) glucose. The buffer was maintained at 37 °C and gassed with 95% O_2/5% CO_2. Secretions were carried by gravity and mucociliary transport to the lower cannula where they collected and were periodically withdrawn into a polyethylene catheter. The catheters were sealed at both ends and stored frozen until required. After unfreezing the secretions were washed out of the catheters with 0.5 ml distilled H_2O into 1.5 ml vials. The vials were frozen and stored for use in the lysozyme assay. Secretion volumes were estimated by the differences in the weight of the catheters with secretions and dried without secretions, and the secretion rates were expressed as $\mu l\ min^{-1}$ (assuming 1 g is equivalent to 1 ml).

After two 30-min control periods one of the drugs under investigation was added to the buffer bathing the trachea. Any secretion was withdrawn 30 min after the addition of the drug. The trachea was then washed twice and fresh buffer containing no drugs was placed in the organ bath. Between two and four 30-min control periods were allowed before addition of the next drug, depending on how long it took for the secretion rate to return to the basal level; results for only the 30 min control periods immediately before and after the drug periods are assessed in Results. At least three of the four drugs to be examined were administered to each trachea. The concentrations of drugs used in these experiments have previously been shown to produce a highly significant ($p < 0.01$) increase in mucus secretion rate [6].

Assay for lysozyme

The lysozyme concentration of the mucus samples was determined using a turbidimetric assay which relies on the ability of lysozyme to break down cell walls from the bacterium *Micrococcus lysodeikticus* [7]. Addition of lysozyme to a solution of the bacteria reduces the turbidity of the solution, leading to a fall in the optical density (OD) at 450 nm.

A stock suspension of *M. lysodeikticus* was prepard which, when diluted 10-fold (the dilution in the assay), gave an OD of approximately 0.6 at 450 nm. Various known concentrations of hen egg white lysozyme (0.5 to 100 $ng\ ml^{-1}$) or 20 µl of a mucus sample were incubated in 1.5 ml potassium phosphate buffer (50 m*M*, pH 7.4) containing *M. lysodeikticus* (0.3 $mg\ ml^{-1}$), sodium azide (1 $mg\ ml^{-1}$) and bovine serum albumin (BSA, 1 $mg\ ml^{-1}$). The reaction mixtures were incubated for 18 h at 37 °C. After incubation the OD of each solution was measured at 450 nm with potassium phosphate buffer containing BSA (1 $mg\ ml^{-1}$) as a blank. A standard curve relating fall in OD to concentration of hen egg white lysozyme was constructed, and the concentration of lysozyme in the mucus samples was determined by reference to this standard curve. The rate of output of lysozyme was also determined by dividing the total amount of lysozyme in a mucus sample by the time period over which the sample was collected.

All comparisons between drug-induced and control periods were made using the Student's *t*-test for paired observations.

Results

The results are summarised in Figure 1. Methacholine (0.02 m*M*), phenylephrine (0.1 m*M*) and histamine (0.3 m*M*) produced highly significant ($p < 0.01$), and salbutamol (0.1 m*M*) significant ($p < 0.05$) increases in the rate of mucus secretion from the ferret trachea compared to the previous control values. With these concentrations the secretion rate produced by methacholine was significantly higher ($p < 0.05$) than that due to any of the other drugs, and there was no significant difference between the secretion rates due to the other drugs. The changes in secretion rate produced by methacholine and histamine were accompanied by highly

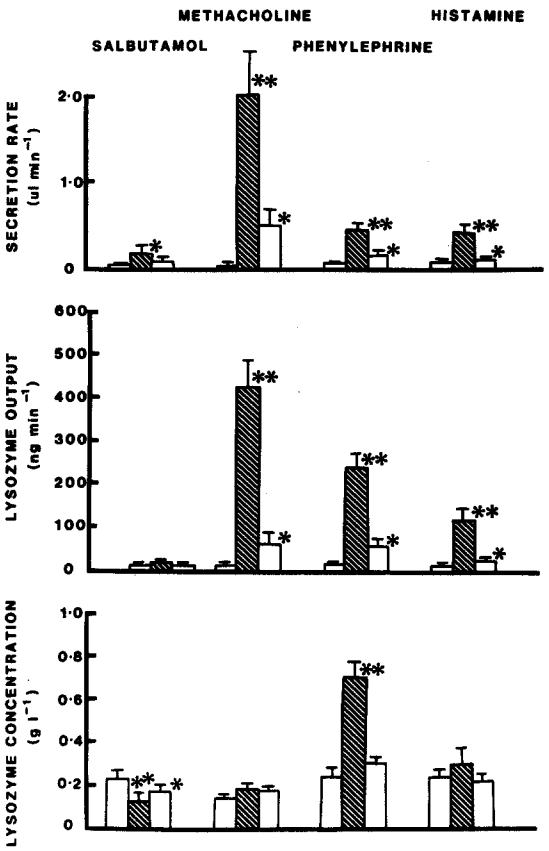

Figure 1
The effects of salbutamol (0.1 m*M*), methacholine (0.02 m*M*), phenylephrine (0.1 m*M*) and histamine (0.3 m*M*) on mucus secretion rate, lysozyme output and the lysozyme concentration of the mucus samples obtained from the ferret trachea. The open columns are results from control periods immediately before and immediately after addition of drug, and the shaded columns are from drug-induced periods, each column representing a 30 min period. Each column is the mean of six determinations, and the vertical bars represent s.e.m.'s. *Significantly different ($p < 0.05$) and **highly significantly different ($p < 0.01$) from control period before drug addition.

significant ($p < 0.01$) increases in the output of lysozyme but no significant changes in lysozyme concentrations of the mucus samples. Phenylephrine produced highly significant ($p < 0.01$) increases in both lysozyme output and concentration. Salbutamol produced a small non-significant ($p > 0.05$) increase in the output of lysozyme but a highly significant ($p < 0.01$) decrease in the lysozyme concentration.

The increases in lysozyme output produced by methacholine, phenylephrine and histamine were significantly reduced ($p < 0.05$) after removal of the drug from the bathing medium. However, the output of lysozyme was still significantly higher in the control period after removal of the drug than in the control period before addition of the drug.

Discussion

We have not obtained dose-response curves, but instead have chosen single concentrations of each agonist which are known from previous studies with the same preparation to give near-maximal increases in mucus secretion rate for each drug [6]. In this respect methacholine can produce a large flow of mucus, whereas salbutamol, although it can increase the output of labelled glycoprotein, has never been found to cause more than a very small increase in mucus volume flow rate. The concentrations used were unlikely to be within the therapeutic range, except possibly for salbutamol given as aerosol; however, we were concerned with secretory mechanisms rather than clinical significance.

The increases in the output of lysozyme produced by methacholine, phenylephrine and histamine suggest that these drugs produce a potent stimulation of serous cell secretion from the submucosal glands of the ferret trachea. Methacholine and histamine produced roughly proportionate increases in secretion flow rate and lysozyme output, the former drug being several times more active. Moreover, with methacholine and histamine there were no significant changes in the concentration of lysozyme associated with the increases in the mucus secretion rate; therefore these drugs probably produce secretion from mucous cells as well as from serous cells, and the magnitude of the secretory action is similar on both cell types. Similar conclusions have been reached from histological observations [2, 3].

Phenylephrine significantly increased the concentration of lysozyme as well as the output. It is not clear how this increase in concentration is produced. The release of lysozyme from serous cells may be proportionately higher than the increase in the output of water, thus leading to an increase in lysozyme concentration. However, if this were true, methacholine and histamine should also increase the lysozyme concentration by their ac-

tions on serous cells. It is possible that phenylephrine stimulates a fluid reabsorptive mechanism in the epithelium or the gland ducts of the ferret trachea, leading to a reduced output of water and an increase in the concentration of lysozyme.

The small, non-significant increase in lysozyme output produced by salbutamol suggests a weak secretory action on serous cells, which agrees with previous histological observations [3, 4]. However, the small significant increase in mucus secretion rate and the clear significant reduction in lysozyme concentration indicates that salbutamol produces secretion from sources other than serous cells. The source of this secretion is not known. However, it may be coming from mucous cells and there is histological evidence that β-adrenergic receptor stimulation results in mucous cell degranulation [2]. It is also possible that salbutamol has a stimulatory action on the ion-mediated movement of water across the tracheal epithelium into the lumen. Indeed, stimulation of β-adrenergic receptors has been shown to stimulate chloride transport across the dog tracheal epithelium [8], which would lead to increased output of water into the lumen [9].

Acknowledgements

S. E. W. was supported by a grant from Zyma SA. We are grateful to Ms. Jacqueline Disley and Ms. S. Mustafa for efficient technical assistance.

Received 5 November 1986; accepted 4 February 1987

References

[1] N. P. Robinson, L. Venning, H. Kyle and J. G. Widdicombe, *Quatitation of the secretory cells of the ferret tracheobronchial tree.* J. Anat. *145,* 173–188 (1986).

[2] A. A. Gashi, J. A. Nadel and C. B. Basbaum, *Morphometric studies of tracheal gland mucous cell stimulated with autonomic drugs.* Clin. Res. *32,* 429 A (1984).

[3] C. B. Basbaum, I. Ueki, L. Brezina and J. A. Nadel, *Tracheal submucosal gland serous cells stimulated in vitro with adrenergic and cholinergic agonists.* Cell Tiss. Res. *220,* 481–498 (1981).

[4] M. Tom-Moy, C. B. Basbaum and J. A. Nadel, *Localisation and release of lysozyme from ferret trachea: effects of adrenergic and cholinergic drugs.* Cell Tiss. Res. *228,* 549–562 (1983).

[5] H. Kyle, N. P. Robinson and J. G. Widdicombe, *Mucus secretion by tracheas of ferret and dog.* Eur. J. Respir. Dis. *70,* 14–22 (1987).

[6] H. Kyle, N. P. Robinson, N. R. Robinson and J. G. Widdicombe, *Simultaneous measurement of airway mucus secretion and smooth muscle tone in ferret trachea in vitro.* J. Physiol. *372,* 38 P (1986).

[7] M. E. Selsted and R. J. Martinez, *A simple and ultrasensitive enzymatic assay for the quantitative determination of lysozyme in the picogram range.* Anat. Biochem. *109,* 67–70 (1980).

[8] F. S. Al-Bazzaz and E. Cheng, *Effect of catecholamines on ion transport across dog tracheal epithelium.* J. Appl. Physiol. *47,* 397–403 (1979).

[9] M. J. Welsh and J. H. Widdicombe, *Pathways of ion movement in the canine tracheal epithelium.* Am. J. Physiol. *239,* F215–F221 (1980).