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The effect of vasoactive intestinal peptide on smooth muscle tone and mucus secretion from the ferret trachea

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1 The effect of vasoactive intestinal peptide (VIP) was examined on the smooth muscle contraction and mucus secretion produced by methacholine and phenylephrine in the ferret whole trachea *in vitro*.

2 VIP (0.5 to 800 nM) produced a concentration-dependent relaxation of the ferret trachea contracted by methacholine (1 μM) and phenylephrine (10 μM). The concentration-response curves for methacholine- and phenylephrine-induced contractions were both shifted to the right by VIP (0.1 μM).

3 Methacholine-induced secretion was inhibited in a concentration-dependent manner by VIP, whereas that due to phenylephrine was enhanced. The concentration-response curve for methacholine-induced secretion was shifted to the right by VIP, whereas the curve for phenylephrine was shifted to the left.

4 Methacholine produced a concentration-dependent increase in the rate of output of lysozyme from the ferret trachea with no corresponding increase in the concentration of lysozyme in the mucus. Phenylephrine produced a concentration-dependent increase in the rate of output and in the concentration of lysozyme.

5 VIP (0.1 μM) significantly increased the concentration of lysozyme in the mucus produced by methacholine with no increase in the rate of lysozyme output. However, the rate of lysozyme output due to phenylephrine was significantly increased by VIP (0.1 μM) with no increase in concentration.

6 We suggest that VIP inhibits secretion from mucous cells stimulated by methacholine, and enhances the secretion produced by phenylephrine from serous cells.

Introduction

The smooth muscle of the tracheobronchial tree is innervated by excitatory cholinergic and inhibitory adrenergic nerves, and a third nervous system to the airways has recently been demonstrated in some species. This non-adrenergic, non-cholinergic (NANC) system relaxes airway smooth muscle (both *in vitro* and *in vivo*) independently of adrenergic pathways (Coburn & Tomita, 1973; Coleman & Levy, 1974; Richardson & Beland, 1976; Diamond & O'Donnell, 1980).

Vasoactive intestinal peptide (VIP) is an endogenous peptide found in a large number of mammalian tissues including the lung (Said, 1980). It has a potent relaxant effect on *in vitro* airway smooth muscle from the cat (Altiere & Diamond, 1984a,b) and the guinea-pig (Wasserman *et al.*, 1982) and produces bronchodilatation *in vivo* in the cat (Diamond *et al.*, 1983). VIP also protects against histamine-induced bronchoconstriction in man *in vivo* (Barnes & Dixon, 1984; Altiere *et al.*, 1984). Furthermore, VIP mimics the effect of NANC nerve stimulation by producing a relaxation of the guinea-pig (Matsuzaki *et al.*, 1980), cat (Ito & Takeda, 1982) and bovine tracheal smooth muscle; it is released on intramural nerve stimulation in the last species (Cameron *et al.*, 1983). VIP-immunoreactive nerves have been identified in the respiratory tract of the guinea-pig, rabbit, cat, dog and man (Uddman *et al.*, 1978; Dey *et al.*, 1981). Therefore it is likely that VIP is a transmitter for the NANC nervous system in the airways.

Stimulation of muscarinic cholinoceptors and α- and β-adrenoceptors can produce mucus secretion in the airways of several species including the ferret (Phipps *et al.*, 1980; Basbaum *et al.*, 1981; Peatfield & Richardson, 1982; Tom-Moy *et al.*, 1983; Gashi *et al.*, 1984). Although VIP-immunoreactive nerves have been identified apposed to cells of submucosal glands in the airways of dog, cat and man (Uddman *et al.*, 1978; Dey *et al.*, 1981), there is little information concerning the effects of VIP on mucus secretion from

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the airways. Published results are conflicting; Coles *et al.* (1981) found that VIP inhibited baseline and methacholine-stimulated release of glycoprotein from explants of human bronchial mucosa, whereas Peatfield *et al.* (1983) showed that VIP increased [^{35}S]-sulphate macromolecular output from explants of ferret trachea.

The ferret *in vitro* whole trachea preparation (Kyle *et al.*, 1986) allows the simultaneous measurement of tracheal smooth muscle contraction and mucus secretion. VIP has no significant effect on the basal secretion rate or on the basal smooth muscle tone of the ferret trachea (Kyle *et al.*, 1986). However, any inhibitory action of VIP on secretion, or on smooth muscle tone, would not be apparent in these studies since there is little muscle tone present in the trachea in its resting state and usually no resting secretion. Therefore, in the present series of experiments, we have examined the effect of VIP on the increased secretion and smooth muscle tone produced by methacholine (a muscarinic agonist) and phenylephrine (an agonist which is relatively selective for α-adrenoceptors).

Methods

The ferret in vitro *trachea*

Ferrets of either sex, weighing 0.5–1.5 kg, were anaesthetized by an intraperitoneal injection of sodium pentobarbitone (Sagatal, May & Baker, 50 mg kg^{-1}). The trachea was exposed and cannulated about 5 mm below the larynx with a perspex cannula containing a conical collecting well (Kyle *et al.*, 1986). The ferret was then killed with an overdose of anaesthetic injected into the heart. The chest was opened along the midline and the trachea exposed to the carina, cleared of adjacent tissue, removed and cannulated just above the carina. The trachea was mounted, laryngeal end down, in a jacketed organ bath with Krebs-Henseleit buffer restricted to the submucosal side. The composition of the Krebs-Henseleit solution was (mM): NaCl 120.8, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ $7H_2O$ 1.2, $NaHCO_3$ 24.9, $CaCl_2$ 2.4, glucose 5.6. The buffer was maintained at 37°C and gassed with 95% O_2/5% CO_2. The lumen of the trachea remained air-filled. Secretions were carried by gravity and mucociliary transport to the lower cannula, where they pooled and could be withdrawn periodically into a polyethylene catheter which was inserted into the lower cannula to form an airtight seal. The catheters containing the secretions were sealed at both ends with bone wax, numbered and stored frozen until required.

After defrosting, the secretions were washed out of the catheters into labelled plastic vials using 0.5 ml distilled H_2O. The vials were frozen and stored for use in the lysozyme assay. Preliminary experiments had shown that frozen storage for up to 6 months does not affect the enzymatic activity of lysozyme. Secretion volumes were estimated by the differences in weights of the catheters with secretions and dried without secretions, and the secretion rates were expressed as μl min^{-1} (assuming 1 g of secretion is equivalent to 1 ml).

During an experiment the carinal cannula was attached to a pressure transducer which was connected to a pen-recorder. Changes in smooth muscle tone produced changes in tracheal pressure which were registered by the pressure transducer and recorded on the pen-recorder, thus enabling assessment of changes in smooth muscle tone of the trachea during mucus collection.

Before the start of an experiment each trachea was allowed to equilibrate for 20 min, and during this time changes of bathing medium were made every 5 min.

Cumulative addition of VIP to the precontracted trachea

No secretion rates were measured during these experiments. Concentrations of VIP were added cumulatively to a trachea that had been precontracted with methacholine (1 μM) or phenylephrine (10 μM). These concentrations of methacholine and phenylephrine produce approximately 70–75% of the maximum contractile responses for these agonists with this preparation (H. Kyle, J.G. Widdicombe & B. Wilffert, unpublished observations). This level of response was chosen to avoid working at the top of the concentration-response curves for methacholine and phenylephrine, but gave adequate levels of contraction for the study of the relaxant effects of VIP. A concentration increment of VIP was made when no further relaxation to the previous concentration was observed and a new steady level of tone had been achieved; this took between 5 and 15 min depending on the size of the response. Cumulative addition of VIP was continued until a total concentration of VIP of 800 nM had been achieved. VIP was too expensive to use at higher concentration. All relaxations to VIP have been expressed as percentage reductions of the contractions produced either by methacholine or phenylephrine, depending on which drug was used to contract the trachea.

Addition of VIP during maintained secretion

After a 30 min control period, either methacholine (5 μM) or phenylephrine (100 μM) was added to the buffer bathing the trachea. Secretions were then withdrawn every 15 min for the first 30 min and at 30 min intervals thereafter until a steady rate of secretion had been obtained. After each 30 min period

the buffer surrounding the trachea was replaced with fresh buffer containing either methacholine or phenylephrine. When a steady rate of secretion had been obtained (maintained secretion), VIP (1 nM to 1 μM) was added to the secretagogue in the buffer surrounding the trachea. After the secretion rate had been determined for a further 30 min period, the organ bath was drained and replenished with buffer containing the secretagogue but no further VIP. The secretion rate was then measured for two further periods of 30 min. The change in secretion rate produced by VIP was calculated as the difference in the secretion rates obtained between the period immediately before the VIP was added and the period when the VIP was in the organ bath, expressed as a percentage.

Effect of VIP on methacholine and phenylephrine concentration-response curves

In each of these experiments three different concentrations of methacholine or phenylephrine, covering at least a ten fold concentration range, were added to the ferret trachea in the presence and absence of VIP (0.1 μM), thus making a total of six drug additions altogether. The different spasmogen concentrations were tested in a random sequence. Each concentration of spasmogen was left in contact with the trachea for 30 min and during this time any increase in smooth muscle tone was recorded. After 30 min the secretion produced was withdrawn and processed, as described above. The trachea was then washed twice and fresh buffer containing no drugs was placed in the organ bath. Between two and four control periods of 30 min were allowed between each drug addition, depending on how quickly the secretion rate returned to its basal level.

At the end of each experiment, either methacholine (0.1 mM) or phenylephrine (1 mM) was added to the organ bath. The increase in smooth muscle tone was recorded and the secretion rate determined for a 30 min period. These concentrations of methacholine and phenylephrine have a maximum effect on secretion rate and smooth muscle tone (H. Kyle, J.G. Widdicombe & B. Wilffert, unpublished observations). All other changes in smooth muscle tone and secretion rate produced by methacholine and phenylephrine have been expressed as percentages of the responses produced by these maximally effective concentrations. All mucus secretion samples obtained in these experiments were assayed for lysozyme.

All the log concentration-response curves shown were plotted by hand. However, the pD_2 values ($pD_2 = -\log EC_{50}$) were estimated from log concentration-response curves fitted to the data points by a computerised, non-linear, least squares estimate (Marquardt, 1963).

Assay for lysozyme

The lysozyme concentrations of the mucus samples obtained from the experiments described above were measured using a turbidimetric assay which relies on the ability of lysozyme to break down the cell wall of the bacterium *Micrococcus lysodeikticus*. Addition of lysozyme to a solution of the bacteria reduces the turbidity of the solution, thereby leading to a fall in optical density (OD) measured at 450 nm.

A stock suspension of *M. lysodeikticus* of 3 mg ml^{-1} was prepared. When diluted 10 fold (the dilution in the assay) this suspension gives an OD of approximately 0.6 at 450 nm. To produce a standard curve, various concentrations of hen egg white lysozyme (0.5 to 100 ng ml^{-1}) were incubated in duplicate in 1.5 ml potassium phosphate buffer (50 mM, pH 7.4) containing *M. lysodeikticus* (0.3 mg ml^{-1}), sodium azide (1 mg ml^{-1}) and bovine serum albumen (BSA, 1 mg ml^{-1}). The BSA was included in the assay for its protein-stabilizing effects and the sodium azide was added to prevent the growth of bacteria in the incubating solutions. The reaction mixtures were incubated for 18 h at 37°C. After incubation the OD of each solution was measured at a wavelength of 450 nm with potassium phosphate buffer pH 7.4 containing BSA (1 mg ml^{-1}) as a blank. The standard curve was constructed by plotting the fall in OD (reduction in turbidity) against the concentration of lysozyme in the solution.

To estimate the concentration of lysozyme in a mucus sample, 20 μl of sample were incubated in 1.5 ml potassium phosphate buffer (50 mM, pH 7.4), exactly as described above for the known concentrations of lysozyme used in the preparation of the standard curve. The lysozyme concentrations (equivalent to hen egg white lysozyme) of the 20 μl samples, and hence of the original mucus samples, were estimated from the standard curve. The rate of output of lysozyme was obtained by dividing the total amount of lysozyme in a mucus sample by the time period over which the sample accumulated.

Materials

The materials used were acetyl-β-methylcholine chloride (methacholine), phenylephrine hydrochloride, vasoactive intestinal peptide, bovine serum albumin, lysozyme (hen egg white), *Micrococcus lysodekticus* (all from Sigma), and sodium azide (BDH).

Analysis of results

Responses to identical concentrations on concentration-response curves were analysed for statistical significance by Student's *t* test for paired values.

Results

Effect of VIP on the smooth muscle tone of the pre-contracted trachea

Contractions of the ferret trachea produced by methacholine (1 μM) and phenylephrine (10 μM) were sustained for at least 1 h. For methacholine, taking the contraction at 5 min after addition as unity, the contraction recorded after 60 min was 1.1 ± 0.2 (mean ± s.e.mean, $n = 4$) times this value. Similarly, for phenylephrine after 60 min, the contraction was 1.0 ± 0.2 ($n = 4$) times the 5 min value. The mean pressure change due to the increase in smooth muscle tone produced by methacholine was 10.7 ± 0.7 cmH_2O ($n = 4$) and by phenylephrine was 8.9 ± 0.4 cmH_2O ($n = 4$).

Cumulative addition of VIP to give concentrations within the range 0.5 to 800 nM produced a concentration-dependent relaxation of the ferret trachea which had been precontracted with either methacholine or phenylephrine. Log concentration-response curves for VIP-induced relaxation of the trachea contracted by methacholine or phenylephrine have been constructed from the results pooled from four experiments (Figure 1). The pD_2 values (mean ± s.e.mean) for VIP-induced relaxation of the trachea pre-contracted with methacholine or phenylephrine are 7.6 ± 0.2 and 7.7 ± 0.2, respectively.

The relaxant responses produced by VIP (800 nm) were equivalent to 101% and 103% suppression of the contractions produced by methacholine (1 μM) and phenylephrine (10 μM) respectively.

Effect of VIP on maintained secretion rate

Addition of either methacholine (5 μM) or phenylephrine (100 μM) to the trachea significantly increased the rate of mucus secretion compared to the baseline level which was not significantly different from zero. The mean increase due to methacholine was 2.77 ± 0.38 μl min^{-1} ($n = 16$) and that to phenylephrine was 1.82 ± 0.41 μl min^{-1} ($n = 16$). On continued application of either methacholine or phenylephrine the secretion rate declined but reached a steady level (maintained secretion rate) which was still significantly higher than the secretion rate before the drug had been added; the mean rates of secretion in the 30 min period immediately before addition of VIP were 0.32 ± 0.05 and 0.36 ± 0.03 μl min^{-1} ($n = 16$) for methacholine and phenylephrine, respectively.

Addition of VIP (1 nM to 1 μM) to the organ bath produced a concentration-dependent inhibition of the maintained methacholine-induced secretion (Figure 2a); the reduction was 50% at a concentration of VIP

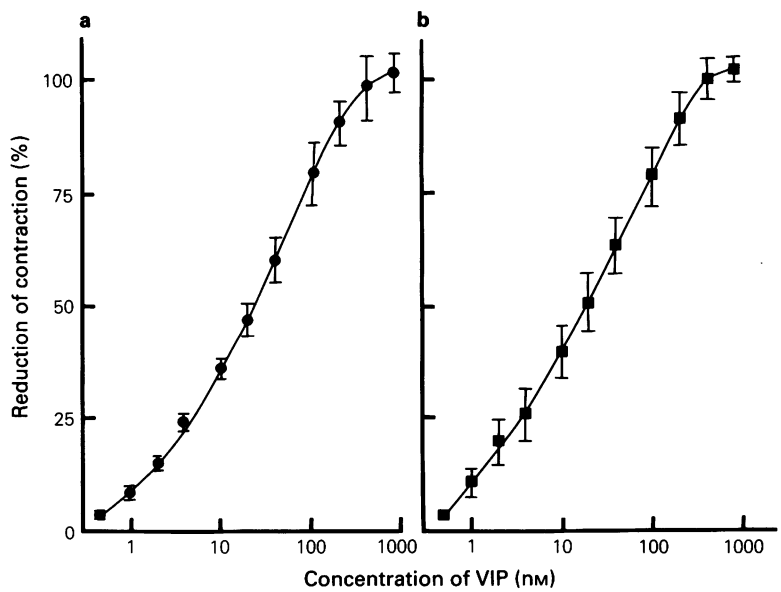

Figure 1 Concentration-response curves showing the effect of vasoactive intestinal peptide (VIP) on internal pressure of the ferret trachea, the muscle of which had been contracted by (a) 1 μM methacholine and (b) 10 μM phenylephrine. VIP concentrations are on log scales. Points are means of four determinations and the vertical lines represent s.e.means.

of approximately 800 nM. However, VIP (1 nM to 1 μM) produced a concentration-dependent increase in the maintained secretion due to phenylephrine (Figure 2b); the increase was 100% at a concentration of VIP of approximately 800 nM.

Effect of VIP on methacholine and phenylephrine concentration-response curves

The effects of VIP on the concentration-response curves for methacholine- and phenylephrine-induced secretions and smooth muscle contractions were studied. A concentration of VIP of 0.1 μM was chosen because it reduced the contractions to methacholine (1 μM) and phenylephrine (10 μM) by approximately 75%. It also yielded a 70% increase in the maintained secretion rate produced by phenylephrine (100 μM) and a 40% inhibition of that due to methacholine (5 μM).

VIP (0.1 μM) shifted to the right the concentration-response curves for contraction of the smooth muscle produced by methacholine and phenylephrine (Figure 3). The pD_2 values (± 95% confidence limits) for methacholine in the absence and presence of VIP were 6.5 ± 0.5 and 5.8 ± 0.4, respectively (concentration-ratio of 5). The pD_2 values for phenylephrine in the same conditions were 5.4 ± 0.4 and 5.0 ± 0.6, respectively (concentration-ratio of 2.7).

VIP (0.1 μM) shifted the concentration-response curve for methacholine-induced secretion to the right and that for phenylephrine to the left (Figure 4). The pD_2 values for methacholine in the absence and presence of VIP were 5.9 ± 0.4 and 5.5 ± 0.6, respectively (concentration-ratio of 2.3) and those for phenylephrine were 4.8 ± 0.7 and 5.5 ± 0.4 (concentration-ratio of 0.2).

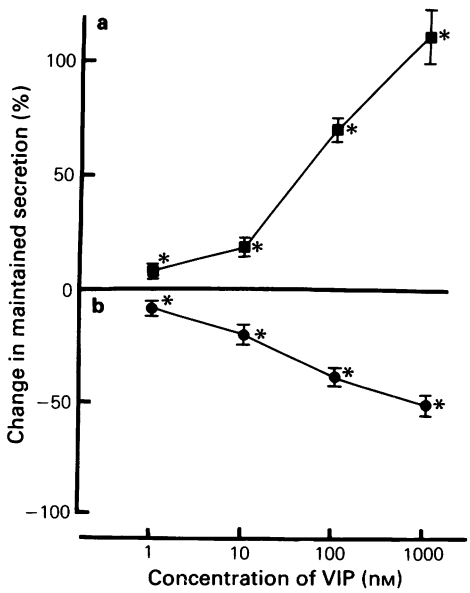

Figure 2 Concentration-response curves showing the effect of vasoactive intestinal peptide (VIP) on maintained mucus secretion from the ferret trachea produced by (a) 5 μM methacholine and (b) 100 μM phenylephrine. VIP concentration is on log scale. Points are means of four determinations and the vertical lines represent s.e.means.
*Significant ($P < 0.05$, paired t test) inhibition of maintained secretion rate produced by methacholine, or significant increase due to phenylephrine.

Effects of VIP on lysozyme output and concentration in mucus produced by methacholine and phenylephrine

In experiments where the effect of VIP on methacholine-induced lysozyme output was to be examined, the mean rate of output of lysozyme during control periods was 12 ± 4 ng min^{-1} and the mean concentration was 0.41 ± 0.12 g l^{-1}, whereas in controls before phenylephrine the mean output of lysozyme was 7 ± 2 ng min^{-1} and the mean concentration was 0.26 ± 0.14 g l^{-1}.

Methacholine (0.1 to 30 μM) produced a concentration-dependent increase in the rate of output of lysozyme (Figure 5a). However, the lysozyme concentration remained nearly constant with increasing concentrations of methacholine after an initial rise between 0.1 and 0.3 μM (Figure 6a). The lysozyme output produced by each concentration of methacholine was not significantly changed ($P > 0.05$) by VIP (0.1 μM) (Figure 5a), but at each concentration of methacholine the concentration of lysozyme in the mucus samples was significantly increased ($P < 0.05$) (Figure 6a).

Phenylephrine (0.1 to 100 μM) produced a concentration-dependent increase in the rate of output of lysozyme (Figure 5b). The concentration of lysozyme increased as the concentration of phenylephrine increased up to 10 μM, but there was no further increase in lysozyme concentration with higher concentrations of phenylephrine (Figure 5b). VIP (0.1 μM) caused a significant increase ($P < 0.05$) in the rate of output of lysozyme produced by each concentration of phenylephrine (Figure 5b). However, there was no corresponding increase in the concentration of lysozyme (Figure 6b).

Discussion

In this study VIP produced a concentration-dependent relaxation of the ferret trachea smooth muscle *in vitro* preparation, which had been precontracted by methacholine or phenylephrine, with similar pD_2 values of

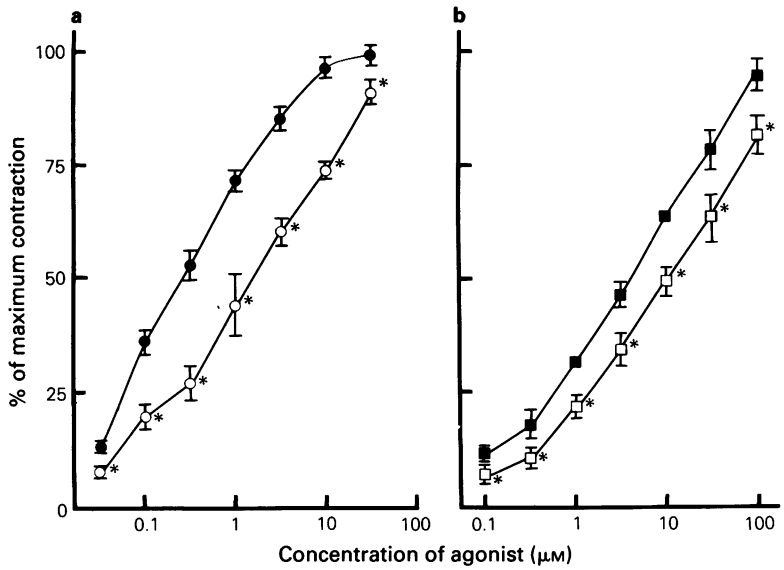

Figure 3 The effect of vasoactive intestinal peptide (VIP) on methacholine- and phenylephrine-induced contractions of muscle of the ferret trachea (internal pressure). Concentration-response curves are (a) for methacholine alone (●) and methacholine in the presence of 0.1 μM VIP (○); (b) phenylephrine alone (■) and phenylephrine in the presence of 0.1 μM VIP (□). All points are means of four determinations and the vertical lines represent s.e.means.
*Significantly different ($P<0.05$, paired t test) response with VIP present compared to that with the same concentration of methacholine or phenylephrine in the absence of VIP.

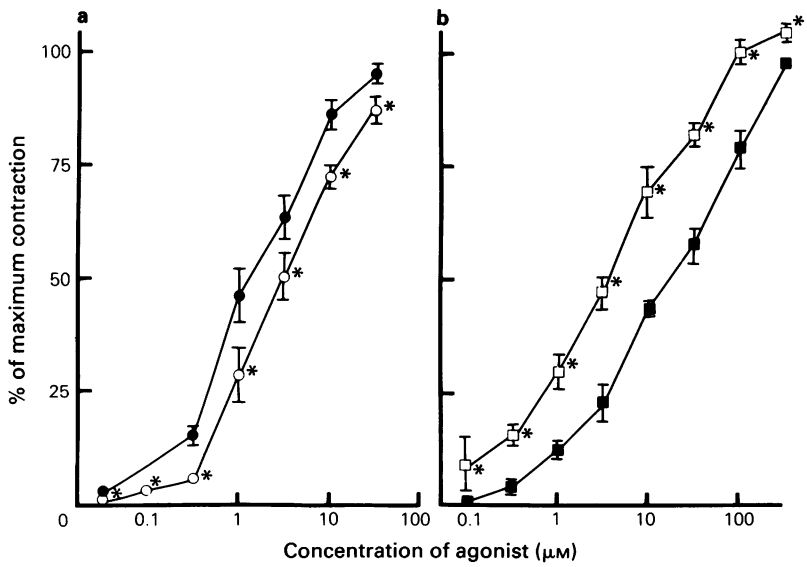

Figure 4 The effects of vasoactive intestinal peptide (VIP) on the secretion of mucus from the ferret trachea produced by methacholine and phenylephrine. Concentration-response curves are for (a) methacholine alone (●) and methacholine in the presence of 0.1 μM VIP (○), and (b) for phenylephrine alone (■) and phenylephrine in the presence of 0.1 μM VIP (□). Methacholine and phenylephrine concentrations are on log scales. All points are means of four determinations and the vertical lines represent s.e.means.
*Significantly different ($P<0.05$, paired t test) response with VIP present compared to that with the same concentration of methacholine and phenylephrine in the absence of VIP.

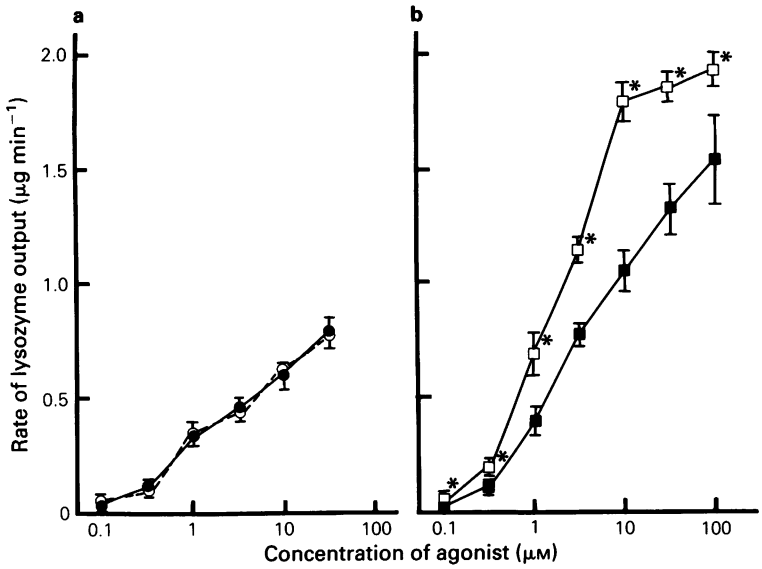

Figure 5 The effect of vasoactive intestinal peptide (VIP) on the rate of output of lysozyme produced by (a) methacholine and (b) phenylephrine. Concentration-response curves in (a) are for methacholine alone (●) and methacholine in the presence of 0.1 μM VIP (○), and in (b) are for phenylephrine alone (■) and phenylephrine in the presence of 0.1 μM VIP (□). Concentrations of methacholine and phenylephrine are on a log scale. All points are means of four determinations and the vertical lines represent s.e.means.
*Significantly different ($P < 0.05$, paired t test) response with VIP present compared to the response with the same concentrations of phenylephrine in the absence of VIP.

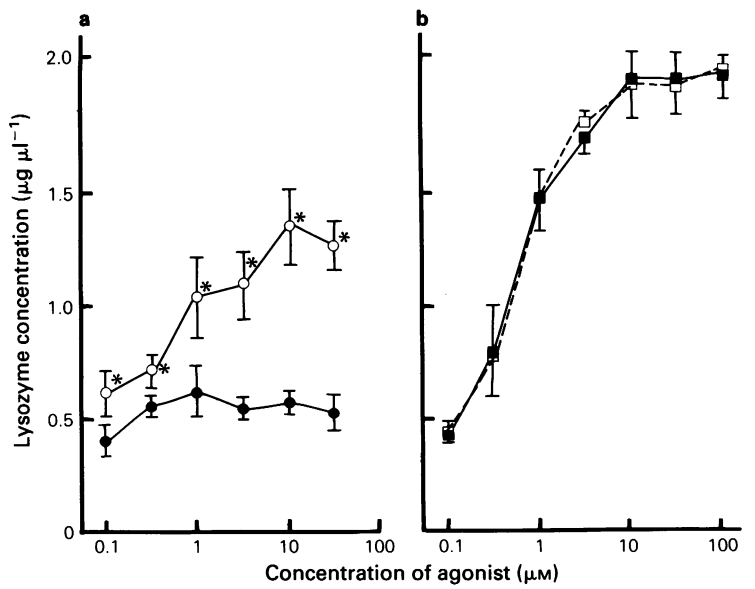

Figure 6 The effect of vasoactive intestinal peptide (VIP) on the concentration of lysozyme produced by (a) methacholine and (b) phenylephrine. Concentration-response curves in (a) are for methacholine alone (●) and methacholine in the presence of 0.1 μM VIP (○), and in (b) are for phenylephrine alone (■) and phenylephrine in the presence of 0.1 μM VIP (□). Concentrations of methacholine and phenylephrine are on a log scale. In both cases points are the means of four determinations and the vertical lines represent s.e.means.
*Significantly different ($P < 0.05$, paired t test) response with VIP present compared to the response with the same concentration of methacholine in the absence of VIP.

7.6 ± 0.2 and 7.7 ± 0.2, (corresponding to mean EC_{50} values of 25 and 22 nM) and maximum responses (101% and 103%). VIP also shifted to the right the concentration-response curves for methacholine and phenylephrine-induced contractions (Figure 3a and b). These results suggest that VIP produces relaxation independently of the contractile agonist used, probably by an interaction with its own receptors on the smooth muscle surface. Similar *in vitro* relaxation responses to VIP, have previously been demonstrated in airway smooth muscle from several species including the cat (Altiere & Diamond, 1984a,b), guinea-pig (Matsuzaki *et al.*, 1980) and man (Davies *et al.*, 1982). Since the EC_{50} values calculated for VIP in our study are similar to those found previously in the cat precontracted trachea (Altiere & Diamond, 1984a,b), the same receptors probably mediate VIP-induced relaxations in the cat and ferret tracheas. The study of VIP receptors on chief cells from guinea-pig gastric mucosa suggests a complex system of subtypes which have not been well defined (Sutcliff *et al.*, 1986).

The ferret trachea has very few epithelial goblet cells (Robinson *et al.*, 1986) and so secretion of mucus is almost entirely from mucous and serous cells in the submucosal glands. This secretion is potently stimulated by drugs acting on muscarinic cholinoceptors and α-adrenoceptors (Basbaum *et al.*, 1981; H. Kyle, J.G. Widdicombe & B. Wilffert, unpublished observations). In the present study both methacholine (a muscarinic agonist) and phenylephrine (a relatively selective α-adrenoceptor agonist) produced concentration-dependent increases in the rate of mucus secretion from the ferret trachea (Figure 4a and b). These increases in secretion rate are not likely to have occurred as a direct consequence of the increase in smooth muscle tone produced by methacholine and phenylephrine, since bradykinin and kallidin produce large increases in intraluminal pressure without significantly affecting the baseline secretion rate (Kyle *et al.*, 1985), as do eledoisin-related peptide (ERP) and neurokinin A (H. Kyle, S.E. Webber & J.G. Widdicombe, unpublished observations).

VIP inhibited the maintained methacholine-induced secretion with a two fold shift to the right of the concentration-response curve. These results are consistent with the work of Coles *et al.* (1981) who found that VIP inhibited methacholine-stimulated release of radiolabelled mucus glycoprotein from explants of human bronchial mucosa. In contrast to its effect on methacholine-stimulated secretion, VIP enhanced the maintained phenylephrine-induced secretion with a five fold shift to the left of the dose-response curve. A similar stimulatory action of VIP on mucus secretion was demonstrated by Peatfield *et al.* (1983), who found that VIP increased the output of sulphated macromolecules from explants of ferret trachea, and by Coles *et al.* (1984), who demonstrated that VIP is a weak partial-secretory agonist in the canine trachea *in vitro*. However, in both these studies the effect of VIP was studied on a considerable baseline output of radiolabelled macromolecules and not on a pharmacologically-stimulated secretion as in our experiments. It should also be noted that the other studies mentioned used the output of radiolabelled macromolecules as an indication of secretion; the cellular source of these macromolecules was not determined, although histological studies suggest that some at least come from glandular serous cells (Gashi *et al.*, 1986). We measured the actual volume of secretion produced, baseline output being small or absent. Since it has been shown that a raised output of radiolabelled macromolecules is not necessarily consistent with a similar increase in the volume of secretion (H. Kyle & J.G. Widdicombe, unpublished observations), our results and those of other investigators may not be directly comparable.

It has been suggested that the inhibitory and enhancing effects of VIP on secretion could be explained by species variation or differences in the techniques used to measure secretion (Coles *et al.*, 1984). However, the dual action of VIP in our study cannot be explained by either of these possibilities, since the same species and the same method were used throughout the study. Nevertheless it is possible that VIP may have different actions on the mucous and serous cells of the submucosal glands. The histological studies of Gashi *et al.* (1986) suggest that serous cells respond primarily to VIP in the ferret. However, the overall effect of VIP would depend on which cell type is more prevalent in a certain species, whether baseline secretion or an enhanced secretion is examined and, most importantly, on which agonist is used to enhance secretion.

Lysozyme is a bactericidal enzyme found in many body tissues and fluids including respiratory tract fluid (Konstan *et al.*, 1981). Immunocytochemistry has shown that lysozyme is located in the secretory granules of serous but not mucous cells in the tracheal submucosal glands of humans (Bowes & Corrin, 1977; Puchelle *et al.*, 1986) and ferrets (Tom-Moy *et al.*, 1983) and is therefore a useful marker for serous but not mucous cell secretion. Therefore, to examine further the possible actions of VIP on mucous and serous cell secretion, the mucus samples obtained in the concentration-response studies were assayed for lysozyme.

Methacholine produced a concentration-dependent stimulation of the rate of output of lysozyme (Figure 5a). This result suggests that methacholine acts on serous cells. Since the concentration of lysozyme remained constant with increasing concentrations of methacholine whilst the secretion rate increased, methacholine probably produced secretion from mucous cells as well as from serous cells, and the

magnitude of the secretory action is similar on both cell types. These conclusions agree with previous histological observations (Basbaum *et al.*, 1981; Tom-Moy *et al.*, 1983; Gashi *et al.*, 1984; Basbaum, 1984). Phenylephrine produced a concentration-dependent increase in the rate of output of lysozyme, suggesting a stimulatory action on serous cells, in accordance with previous work (Basbaum *et al.*, 1981; Tom-Moy *et al.*, 1983; Basbaum, 1984). However, phenylephrine also produced a concentration-dependent increase in the concentration of lysozyme. The mechanism of this effect is not clear. The release of lysozyme from serous cells may not be proportional to the increase in the output of water, thus leading to an increase in lysozyme concentration, but if this were true methacholine should also produce an increase in lysozyme concentration by its action on serous cells. It is possible that phenylephrine stimulates a fluid reabsorptive mechanism in the tracheal epithelium or the gland ducts, leading to a reduced output of water and an increase in the concentration of lysozyme. However, Phipps *et al.* (1980) demonstrated that phenylephrine produced an increase in the ion-mediated movement of water into the lumen of the cat trachea.

VIP had no effect on the output of lysozyme due to each concentration of methacholine, but produced a significant increase in the concentration of lysozyme in the mucus samples. These results suggest that VIP has no effect on the serous cell secretion produced by methacholine but inhibits the secretion from mucous cells, resulting in a decreased volume flow and increased lysozyme concentration. In contrast, VIP inhibits methacholine-induced lysozyme release from explants of human bronchial mucosa (Coles *et al.*, 1984), indicating an inhibitory action on serous cell secretion in this preparation.

VIP significantly increased the rate of output of lysozyme produced by phenylephrine, which suggests that VIP enhances the secretory action of phenylephrine on serous cells. A similar conclusion was reached in a recent study by Gashi *et al.* (1986), who demonstrated that VIP increases spontaneous release of $^{35}SO_4$-labelled macromolecules from ferret tracheal explants. This effect was accompanied by a marked degranulation of serous cells. However, this action of VIP was on baseline release and not on a stimulated preparation as in our study. Although the phenylephrine-induced output of lysozyme was increased by VIP, there was no corresponding increase in lysozyme concentration. Therefore if phenylephrine is causing fluid reabsorption, VIP may inhibit this effect to produce an increased output of water but no increase in lysozyme concentration. Indeed, Nathanson *et al.* (1983) found that VIP stimulated active secretion of Cl^- across sheets of dog tracheal epithelium, a process which is likely to increase the output of water (Widdicombe & Welsh, 1980).

In conclusion, VIP inhibits the contractile actions of both methacholine and phenylephrine on ferret tracheal smooth muscle. However, its effects on enhanced mucus secretion are complex and depend on the agonist used to produce secretion and, therefore, the cell type from which the secretion comes. In general it seems that VIP inhibits the secretion from mucous cells produced by methacholine and enhances the secretion from serous cells produced by phenylephrine, but the receptor mechanisms underlying these effects on smooth muscle and secretory cells are unknown.

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