Br. J. Pharmacol. (1991), **102**, 79–84

The effects of calcitonin gene-related peptide on submucosal gland secretion and epithelial albumin transport in the ferret trachea *in vitro*

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1 We have examined the effect of calcitonin gene-related peptide (CGRP) on basal mucus volume, lysozyme and albumin outputs from the ferret whole trachea *in vitro*, and on the outputs produced by methacholine and substance P (SP). We have also examined the effect of inhibiting neutral enkephalinase with thiorphan on the responses to CGRP.

2 CGRP (1–100 nM) produced small concentration-dependent increases in basal mucus volume, lysozyme and albumin outputs. These effects of CGRP were enhanced by thiorphan. The increases in basal outputs with CGRP and the potentiation by thiorphan were considerably less than previously observed with SP and neurokinin A (NKA). CGRP had no significant effect on potential difference (PD) across the trachea.

3 CGRP produced a concentration-dependent inhibition of methacholine- and SP-induced lysozyme output but a concentration-dependent increase in methacholine- and SP-induced albumin output. The effects of CGRP on methacholine-induced lysozyme and albumin outputs were enhanced by thiorphan. CGRP weakly inhibited methacholine-induced mucus volume output and weakly enhanced SP-induced mucus volume output.

4 Thus, CGRP weakly stimulates basal serous cell secretion and epithelial albumin transport, but does not alter epithelial integrity. CGRP inhibits the serous cell secretion due to methacholine or SP, but potentiates the epithelial albumin transport produced by these agents. The interaction between CGRP and other sensory neuropeptides or muscarinic agonists on airway submucosal glands and epithelium may be important in the normal airway and in inflammatory airway diseases where release of sensory neuropeptides is enhanced.

Introduction

Calcitonin gene-related peptide (CGRP) is a 37 amino acid peptide which is derived from alternative processing of the calcitonin gene mRNA transcript, and which was first characterized in neural tissue (Rosenfeld *et al.*, 1983). CGRP has been found in numerous species including man and has actions in many different organs e.g. neural tissue, striated muscle, cardiac muscle, vasculature (including that of the airways) and bone (Breimer *et al.*, 1988).

CGRP has been localized to the airways of a number of species including guinea-pig, rat, ferret and man (Palmer *et al.*, 1987). High affinity binding sites for CGRP have been demonstrated in rat visceral organs including lung (Nakamuta *et al.*, 1986). In human lung preparations CGRP was detected by radioimmunoassay, with the highest concentrations found in the cartilaginous airways. Autoradiographic studies have shown CGRP binding sites in the human lung which are particularly densely distributed over smooth muscle with some labelling over seromucous glands but no apparent binding over epithelium or smooth muscle (Mak & Barnes, 1988). CGRP has been localized to nerves and ganglia in human airways, by use of immunocytochemistry, and particularly in association with sensory nerves. In the rat, CGRP immunoreactive fibres have been localized to smooth muscle, seromucous glands, beneath and within the epithelium and in association with blood vessels (Cadieux *et al.*, 1986). From immunohistochemical distribution and capsaicin depletion studies, it is thought likely that CGRP co-exists with a number of other peptides, especially substance P (SP) and neurokinin A (NKA), in these primary sensory nerves (Lundberg *et al.*, 1985).

When released CGRP has potent effects on the airways. It produces a concentration-dependent contraction of human bronchi *in vitro*, and is more potent than either SP or carbachol (Palmer *et al.*, 1987). CGRP is also a potent and long-lasting vasodilator of tracheobronchial blood vessels *in vitro* (McCormack *et al.*, 1989) and *in vivo* (Salonen *et al.*, 1988) and can increase airway microvascular permeability (Aursudkij *et al.*, 1988). There is also evidence that CGRP can interact with other sensory nerve peptides such as SP in the skin (Gamse & Saria, 1985) and airways (Gatto *et al.*, 1989).

Thus, there is evidence that CGRP is localized to airway submucosal glands and epithelium, has potent effects on some airway tissues and can interact with other sensory transmitters such as SP. However, the effects of CGRP on submucosal gland secretion and epithelial transport mechanisms, and the interaction with other pharmacological agents including SP on these tissues have not yet been studied. It is also not known if inhibiting neutral enkephalinase enhances any action of CGRP on airway tissues. Therefore we have used the ferret whole trachea *in vitro* (Webber & Widdicombe, 1987) to examine the effect of CGRP on submucosal gland secretion including lysozyme secretion from serous cells, and on epithelial albumin transport (Webber & Widdicombe, 1989) in the presence and absence of thiorphan. We have also examined the interaction of CGRP with SP and methacholine on these parameters.

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 (Webber & Widdicombe, 1987). The ferret was then killed with an overdose of sodium pentobarbitone 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 with 0.5 ml distilled H_2O. The vials were frozen and stored for use in the albumin and lysozyme assays. Preliminary experiments had shown that frozen storage for up to 6 months does not affect the enzymatic activity of lysozyme or the albumin content. 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 $\mu l\,min^{-1}$ (assuming 1 g of secretion is equivalent to 1 ml).

The electrical potential difference (PD) across the tracheal wall was measured with two calomel reference electrodes. These were filled with 3.8 M KCl and placed in separate beakers of the same solution. Electrical contact was made with the preparation by use of two agar bridges. These were constructed from polyethylene tubing (0.5 mm internal diameter) filled with 3.8 M KCl in 2.5% w/v agar solution. One bridge was placed in the buffer on the submucosal side of the trachea and the second inserted into a second hole in the perspex cannula used to collect the mucus. Electrical contact between this bridge and the tracheal luminal wall was maintained by the mucus collecting in the perspex cannula. Output from the two electrodes was into a high input impedance buffer amplifier and then displayed on a digital voltmeter. The two agar bridges were initially placed together in 0.15 M NaCl to confirm that this produced a stable potential difference close to 0 V. Any residual voltage measured here was subtracted from subsequent measurements of potential difference made in the preparation.

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.

Assay for lysozyme

The lysozyme concentrations of the mucus samples were measured by 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 albumin (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 was incubated in 1.5 ml potassium phosphate buffer (50 mM, pH 7.4), 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 then calculated by dividing the total amount of lysozyme in a mucus sample by the time over which the sample accumulated.

Albumin transport

To examine the effect of CGRP on methacholine-induced transport of albumin across the ferret trachea, BSA was added to the buffer bathing the submucosal surface of the trachea in a concentration of $4\,mg\,ml^{-1}$. Fluorescent BSA ($0.02–0.03\,mg\,ml^{-1}$) was also added to the buffer as a marker and enabled an estimate to be made of the total amount of albumin which appeared in the mucus samples.

The fluorescence of the mucus samples was measured with a fluorimeter, using an excitation wavelength of 550 nm and an emission wavelength of 490 nm. The fluorescent albumin concentration of the mucus samples was estimated from a standard curve relating fluorescence (arbitrary units) to the concentration of fluorescent albumin (range $25\,ng\,ml^{-1}$ to $3\,\mu g\,ml^{-1}$). The total concentration of albumin in the mucus samples was obtained by multiplying the fluorescent albumin concentration (estimated from the standard curve) by the ratio of non-fluorescent to fluorescent albumin used in the experiment. The rate of output of albumin was determined by dividing the total amount of albumin in a mucus sample by the time over which that sample accumulated.

Experimental protocol

Effect of CGRP on baseline secretion After a 30 min control period, three concentrations (1–100 nM) of CGRP were added to the buffer surrounding the trachea in a random sequence. Each concentration of CGRP was left in contact with the trachea for 30 min. After each 30 min, any secretion produced was withdrawn and processed as described above. One or two control periods of 30 min were allowed between each addition of peptide. After three concentrations of CGRP had been added to the trachea, the buffer surrounding the trachea was replaced with buffer containing thiorphan. This buffer was left in contact with the trachea for 30 min and any mucus produced was withdrawn and processed. The same three concentrations of CGRP were then added to the trachea in the same sequence as above, with the same number of control periods between peptide additions. The CGRP was always diluted and added to the trachea in buffer containing thiorphan. Changes in mucus volume output produced by CGRP were calculated as the difference in the mucus volume output obtained between the control period immediately before the peptide was added and the period when the peptide was in the organ bath. All mucus samples obtained in these experiments were assayed for lysozyme and albumin.

Effect of CGRP on methacholine- and SP-induced secretion Previous studies have shown that both methacholine and SP produce concentration-dependent increases in mucus volume, lysozyme and albumin outputs from the ferret trachea (Webber & Widdicombe, 1987; Webber, 1989). After a 30 min control period either methacholine (20 μM) or SP (0.1 μM) was added to the buffer bathing the trachea. These concentrations of methacholine and SP produce 70–80% of their respective maximum responses. Mucus was withdrawn every 30 min until a steady 'maintained' mucus volume output had been obtained. After each 30 min period the buffer surrounding the trachea was replaced with fresh buffer containing either methacholine or SP. When a maintained mucus volume output had been obtained, three concentrations of

Table 1 Mucus volume, lysozyme and albumin outputs in control periods before the addition of any drugs, immediately after addition of methacholine or substance P (SP) and the maintained outputs to methacholine and SP immediately before the addition of calcitonin gene-related peptide (CGRP, 2.5–3.5 h after first addition of methacholine or SP)

	Methacholine						*SP*	
	Control		*After methacholine*		*Before CGRP*		*Control*	*After SP*	*Before CGRP*
	− Thiorphan	+ Thiorphan	− Thiorphan	+ Thiorphan	− Thiorphan	+ Thiorphan			
Mucus volume ($\mu l\,min^{-1}$)	0.03 ± 0.02	0.04 ± 0.03	2.40 ± 0.24*	2.62 ± 0.50*	0.79 ± 0.05*	0.88 ± 0.19*	0.04 ± 0.02	0.80 ± 0.17*	0.54 ± 0.14*
Lysozyme ($ng\,min^{-1}$)	19 ± 6	21 ± 6	700 ± 84*	663 ± 109*	126 ± 30*	164 ± 21*	23 ± 5	381 ± 104*	168 ± 46*
Albumin ($\mu g\,min^{-1}$)	0.27 ± 0.11	0.30 ± 0.14	6.1 ± 1.0*	5.8 ± 1.4*	1.9 ± 0.4*	2.1 ± 0.4*	0.3 ± 0.2	2.2 ± 0.5*	1.6 ± 0.4*

Values shown are the means (± s.e.mean) of 6 determinations. * Significantly different ($P < 0.05$) from control value by Student's paired t test.

CGRP (1–100 nM) were added in ascending order to the secretagogue in the buffer surrounding the trachea. Each concentration of CGRP was left in contact with the trachea for 30 min. After 30 min the secretion produced was withdrawn and processed. The buffer surrounding the trachea was then replaced with fresh buffer containing the secretagogue and the next concentration of CGRP. After three concentrations of CGRP had been added, the buffer was replaced with buffer containing only methacholine or SP and the mucus volume output determined for two further periods of 30 min. The change in mucus volume output produced by CGRP was calculated as the difference in mucus volume output obtained between the period immediately before the peptide was added and the period when the peptide was in the organ bath, expressed as a percentage. SP only produced a satisfactory maintained secretion in the presence of thiorphan; therefore in all experiments with SP, thiorphan was present in the buffer throughout. With methacholine, thiorphan was present in the buffer in half and absent in half of experiments. All mucus samples obtained in these experiments were assayed for lysozyme and albumin.

Analysis of results

The effects of CGRP on baseline mucus volume, lysozyme and albumin outputs, and on the maintained outputs produced by methacholine and SP were analysed for statistical significance by one-way analysis of variance followed by Student's paired t tests. Significance was accepted for $P < 0.05$. Values shown are means ± s.e.mean.

Results

Effects of thiorphan

In experiments with methacholine, the mean mucus volume, lysozyme and albumin outputs in control periods before the addition of any drugs and in the absence and presence of thiorphan are shown in Table 1. There were no significant differences between the control values in the absence and presence of thiorphan suggesting that thiorphan has no effect on baseline mucus volume, lysozyme or albumin output. Similarly the control PD's across the trachea in the absence and presence of thiorphan were -8.6 ± 0.6 ($n = 18$) and -8.1 ± 0.5 mV ($n = 24$) respectively. These values are also not significantly different, suggesting thiorphan has no effect on PD.

Effects of CGRP on baseline parameters

In the absence of thiorphan, CGRP (1–100 nM) produced small, concentration-dependent increases in mucus volume, lysozyme and albumin outputs (Figure 1). In the presence of thiorphan, the concentration-response curves for CGRP-induced mucus volume, lysozyme and albumin outputs were all shifted upwards with significantly increased responsiveness at 1, 10 and 100 nM (Figure 1).

CGRP (1–100 nM) had no significant effect on PD across the trachea, in the presence or in the absence of thiorphan (Table 2).

Effects of CGRP on methacholine-induced responses

In the 30 min period immediately after addition of methacholine the mucus volume, lysozyme and albumin outputs increased significantly from preceding control values (Table 1). There were no significant differences between the outputs obtained in the presence and absence of thiorphan, suggesting thiorphan does not affect the responses to methacholine. On continued application of methacholine the mucus volume, lysozyme and albumin outputs declined but reached a steady 'maintained' level after 2.5–3.5 h (Table 1). There were no sig-

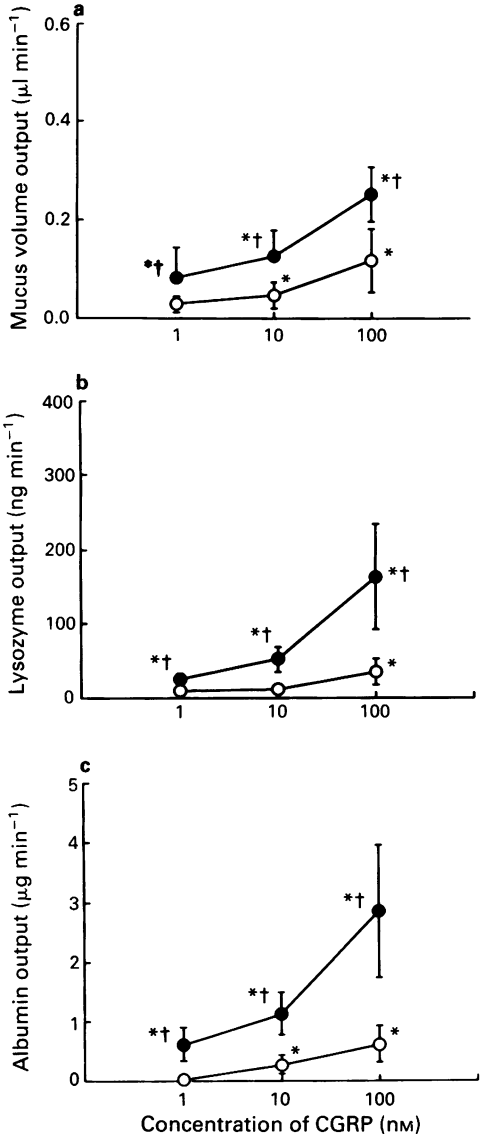

Figure 1 Concentration-response curves for the effect of calcitonin gene-related peptide (CGRP) on (a) mucus volume, (b) lysozyme and (c) albumin outputs from the ferret trachea *in vitro*. Responses to CGRP in the absence (○) and presence (●) of thiorphan (10 μM). Points are means of 4–6 determinations with s.e.means shown as vertical lines. * Response significantly ($P < 0.05$) different from zero. † Significantly different from response in the absence of thiorphan.

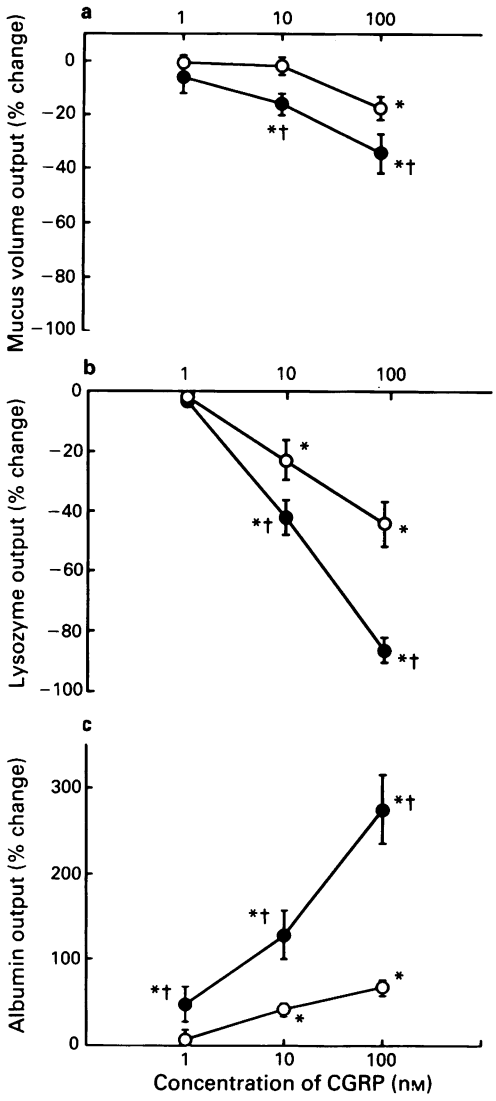

Figure 2 Concentration-response curves for the effect of calcitonin gene-related peptide (CGRP) on the maintained (a) mucus volume, (b) lysozyme and (c) albumin outputs produced by methacholine (20 μM). Responses to CGRP in the absence (○) and presence (●) of thiorphan. Points are means of 6 determinations with s.e.means shown as vertical lines. * Response significantly different from zero. † Significantly different from response in the absence of thiorphan.

nificant differences in the maintained outputs with or without thiorphan (Table 1).

In the absence of thiorphan, CGRP produced concentration-dependent reductions in the maintained methacholine-induced mucus volume and lysozyme outputs, but a concentration-dependent increase in maintained methacholine-induced albumin output (Figure 2). The effects of CGRP at 10 and 100 nM were all significantly enhanced in the presence of thiorphan (Figure 2).

Effects of CGRP on substance P-induced responses

SP significantly increased mucus volume, lysozyme and albumin outputs from preceding control values (Table 1). On continued application of SP the outputs fell slightly but reached a steady 'maintained' level after 2.5–3.5 h (Table 1). CGRP (1–100 nM) had no significant effect on SP-induced mucus volume output, but produced a concentration-dependent reduction in SP-induced lysozyme output and a concentration-dependent increase in SP-induced albumin output (Figure 3).

Table 2 The effect of calcitonin gene-related peptide (CGRP) on potential difference (PD) across the ferret trachea in the absence and presence of thiorphan

	Absence of thiorphan		*Presence of thiorphan*	
Concentration of CGRP (nM)	*Control*	*+ CGRP*	*Control*	*+ CGRP*
	PD (mV)			
1	−7.2 ± 0.8	−7.2 ± 0.8	−8.1 ± 0.6	−8.3 ± 0.7
10	−7.7 ± 0.8	−8.2 ± 0.9	−8.0 ± 0.8	−7.7 ± 0.7
100	−8.2 ± 0.8	−8.3 ± 0.8	−8.0 ± 0.6	−8.0 ± 0.5

Values shown are means ± s.e.mean. $n = 4$–6.

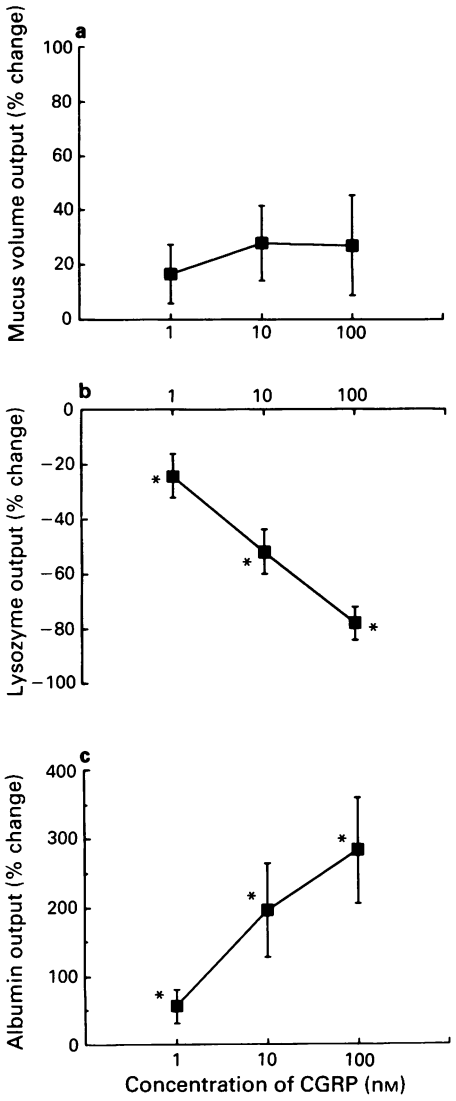

Figure 3 Concentration-response curves for the effect of calcitonin gene-related peptide (CGRP) on the maintained (a) mucus volume, (b) lysozyme and (c) albumin outputs produced by substance P (0.1 μM). All responses to CGRP were obtained in the presence of thiorphan. Points are the means of 4–6 determinations with s.e.means shown as vertical lines. * Response significantly different from zero.

Discussion

The ferret trachea has numerous submucosal glands, possibly in compensation for the notable paucity of goblet cells, the mucus secreting epithelial cells usually found in greatest density at the caudal end of the trachea and in the main bronchi of larger animals such as the cat and rabbit (Richardson & Somerville, 1988). There are two main secretory cells in the submucosal glands of the ferret trachea, mucous and serous cells. Mucous cells produce a thick viscous secretion rich in acidic glycoprotein. Unfortunately there is no specific marker for secretion from these cells. Serous cells produce a much thinner watery secretion which, as well as containing neutral glycoprotein, also contains the antibacterial enzymes lysozyme and lactoferrin. Lysozyme can be easily assayed turbidimetrically (Selsted & Martinez, 1980) and is therefore a useful specific marker for secretion from these cells. Additionally albumin is actively and specifically transported across the ferret trachea from the submucosa into the lumen (Webber & Widdicombe, 1989) and this transport is thought to occur across the epithelium (Price *et al.*, 1990).

In the present study, CGRP produced small concentration-dependent increases in baseline mucus volume, lysozyme and albumin outputs. The increase in basal lysozyme output with CGRP suggests stimulation of epithelial albumin transport. increase in basal mucus volume output may be due entirely to this stimulation but it may also stimulate secretion from mucous cells; however, it is not possible to determine this because of the lack of a specific marker for secretion from these cells. The increase in baseline albumin output with CGRP suggests a stimulation of epithelial albumin transport. Thus, CGRP has similar stimulatory actions on serous cell secretion and albumin transport as the other sensory peptides SP and NKA (Webber, 1989). However, it should be emphasized that the responses to CGRP at a particular concentration are considerably smaller than those produced by SP and NKA at the same concentration, although it was not possible to obtain a maximum response with CGRP due to the cost of this peptide. Thiorphan had no significant effect on any of the baseline outputs suggesting that there is no effective basal release of sensory neuropeptides from the ferret trachea *in vitro*, and that thiorphan has no direct effect on submucosal gland secretion or albumin transport. The effects of CGRP on all the basal outputs were enhanced in the presence of thiorphan (as would be expected if thiorphan prevented the degradation of CGRP by neutral enkephalinase). However, the enhancement of CGRP-induced effects was again much less than the enhancement of SP and NKA-induced outputs by thiorphan (Webber, 1989). For instance at 100 nM, CGRP-induced lysozyme and albumin outputs were increased 8 fold in the presence of thiorphan, whereas those due to SP (100 nM) were increased about 25 fold. Thus, it is likely that CGRP is a more stable peptide than SP or that there are more important enzymes than neutral enkephalinase responsible for the breakdown of CGRP, whereas this is the major enzyme responsible for the degradation of SP and NKA. CGRP had no effect on the potential difference across the trachea either in the absence or presence of thiorphan, suggesting it was not affecting mucosal integrity.

Methacholine- and SP-induced lysozyme outputs were inhibited by CGRP suggesting that CGRP inhibits the serous cell secretion produced by these two secretagogues. It is not clear why CGRP enhances baseline serous cell secretion whilst inhibiting the stimulated secretion due to methacholine or SP. It is possible that there are two different receptors for CGRP; the first is excitatory and leads to an increase in serous cell secretion and the second is inhibitory and is only activated by CGRP when secretion has already been stimulated for instance by SP or methacholine. Methacholine-induced mucus volume output was slightly reduced by CGRP, whilst that due to SP was slightly enhanced. These results, particularly those with SP, might be explained by CGRP exerting a stimulatory effect on mucous cell secretion whilst inhibiting secretion from serous cells (as indicated by the reduction in lysozyme output), the mucus volume output reflecting the net effect of these changes. However, it is difficult to gauge the nature of CGRP-induced effects on mucous cell secretion without a specific marker for secretion from these cells. Methacholine- and SP-induced albumin outputs were increased by CGRP suggesting enhancement of epithelial albumin transport produced by these agents. The effects of CGRP on methacholine-induced lysozyme, mucus volume and albumin outputs were enhanced by thiorphan, again suggesting that CGRP is at least partly being degraded by neutral enkephalinase in the ferret trachea.

CGRP interacts with SP on other tissues including some from the airways. It potentiates SP-induced microvascular leakage in rat skin (Gamse & Saria, 1985; Brain & Williams, 1985), which is consistent with its potentiation of SP-induced epithelial albumin transport in the present study. This increased responsiveness to SP may be due to CGRP preventing the breakdown of SP (Le Greves *et al.*, 1985); however, this seems unlikely in the present study as thiorphan was present throughout the experiments with CGRP and SP. In contrast CGRP had no significant effect on the increased

airway microvascular leakage produced by SP in guinea-pigs (Rogers *et al.*, 1988). In contrast to enhancing the action of SP, CGRP blocks the increased airway resistance produced by SP in anaesthetized guinea-pigs (Gatto *et al.*, 1989); this is consistent with the inhibitory action of CGRP on SP-induced serous cell secretion shown in the present study. The mechanism of action of CGRP in inhibiting SP-induced airway responses is not known. CGRP may bind to its own receptors and elicit responses which are opposite to those produced by SP, or may bind to SP receptors themselves preventing the access of SP. Clearly CGRP can either enhance, inhibit or have no effect on SP-induced airway responses. The type of response observed *in vivo* when the peptides are released together will depend on the amount of each peptide released and the tissue they are acting on.

Thus, CGRP has a weak stimulatory action on submucosal gland serous cell secretion and epithelial albumin transport in the ferret trachea. Even after inhibition of enkephalinase the effects of CGRP are considerably weaker than those produced by similar concentrations of other sensory neuropeptides such as SP or NKA, or by muscarinic agonists such as methacholine. Therefore, it is unlikely that CGRP is an important mediator of mucus secretion and epithelial transport mechanisms *in vivo*. However, in view of its potent modulatory actions demonstrated in this study it is likely that CGRP, when released *in vivo* with SP or at the same time as muscarinic receptor stimulation by acetylcholine, will modulate the serous cell secretion and epithelial albumin transport produced by these mediators. This may lead to considerable changes in the composition of airway surface liquid which could have important implications in health and inflammatory airway disease.

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(Received June 21, 1990
Revised September 3, 1990
Accepted September 5, 1990)