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Differential inhibitory effects of opioids on cigarette smoke, capsaicin and electrically-induced goblet cell secretion in guinea-pig trachea

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1 Goblet cell secretion in guinea-pig airways is under neural control. Opioids have previously been shown to inhibit neurogenic plasma exudation and bronchoconstriction in guinea-pig airways. We have now examined the effects of morphine and opioid peptides on tracheal goblet cell secretion induced by either electrical stimulation of the cervical vagus nerves, exogenous capsaicin, or acute inhalation of cigarette smoke. The degree of goblet cell secretion was determined by a morphometric method and expressed as a mucus score which is inversely related to mucus discharge.

2 Morphine, 1 mg kg^{-1}, completely blocked goblet cell secretion induced by electrical stimulation of the vagus nerves. Morphine also inhibited the response to cigarette smoke given either at a low dose (10 breaths of 1:10 diluted in air), which principally activates cholinergic nerves, or at a high dose (20 breaths of undiluted), which activates capsaicin-sensitive sensory nerves, by 100% and 73% respectively. In contrast, morphine had no significant inhibitory effect on capsaicin-induced goblet cell secretion. The inhibitory effect of morphine was reversed by naloxone.

3 Selective μ- or δ-opioid receptor agonists, [D-Ala^2, NMePhe^4, Glyol^5]enkephalin (DAMGO) or [D-Pen^2, D-Pen^5]enkephalin (DPDPE) respectively, caused a dose-related inhibition of low dose cigarette smoke-induced goblet cell discharge, with DPDPE more potent than DAMGO. A κ-receptor agonist, *trans*-3,4-dichloro-*N*-methyl-*N*-(2-(1-pyrollidinyl)cyclohexyl) benzeneacetamine (U-50,488H), had no inhibitory effect. DPDPE had no inhibitory effect on goblet cell secretion induced by exogenous methacholine.

4 DAMGO dose-dependently blocked the response to high dose cigarette smoke with a maximal inhibition of 95% at 2×10^{-7} mol kg^{-1}. Neither DPDPE nor U-50,488H had any significant inhibitory effect. The increase in goblet cell secretion induced by exogenous substance P was not affected by DAMGO.

5 We conclude that opioids inhibit neurally-mediated goblet cell secretion via actions at prejunctional δ- and μ-receptors on cholinergic nerves and at μ-receptors on sensory nerve endings, and that capsaicin activation of sensory nerves is via a different mechanism from that of electrical or cigarette smoke activation.

Keywords: Morphine; opioid; mucus secretion; goblet cell; cigarette smoke; capsaicin; μ- and δ-opioid receptor

Introduction

Morphine and opioid peptides inhibit neurally-mediated broncho-constriction (Frossard & Barnes, 1987; Bartho *et al.*, 1987; Belvisi *et al.*, 1988) and plasma exudation (Belvisi *et al.*, 1989) in guinea-pig airways, and mucus secretion in human bronchi (Rogers & Barnes, 1989) by blocking the release of tachykinins from capsaicin-sensitive sensory nerves. Opioids have also been shown to inhibit the cholinergic component of airway excitation in guinea-pigs (Johansson *et al.*, 1989; Belvisi *et al.*, 1990). Recently we have demonstrated that airway goblet cells are under neural control. Electrical stimulation of the cervical vagus nerves provokes a significant increase in goblet cell secretion partly via activation of cholinergic nerves and partly via capsaicin-sensitive sensory nerves (Tokuyama *et al.*, 1990). Acute injection of capsaicin, which activates sensory nerve endings to release tachykinins, was also demonstrated to cause goblet cell discharge (Kuo *et al.*, 1990a). Therefore, morphine and opioid peptides may have inhibitory effects on neurally-mediated airway goblet cell secretion.

The inhibitory effect of morphine or opioid peptides is inversely related to the frequency of stimulation used (Paton, 1957; Russell & Simons, 1985; Duckles & Budal, 1990). Because autonomic neurones generally discharge at relatively low frequencies *in vivo*, the inhibitory effect of opioids on neurally-mediated responses to more 'physiological' stimulation intensities might be different from those elicited by experimental stimulation. We recently demonstrated that acute inhalation of cigarette smoke, at concentrations giving plasma nicotine levels in the range reported for human smokers, stimulated neurally-mediated goblet cell discharge: 10 breaths of 1:10 diluted cigarette smoke (reported as low dose) activates predominantly parasympathetic cholinergic nerves (Kuo *et al.*, 1990b), whereas 20 breaths of undiluted cigarette smoke (reported as high dose) selectively activates capsaicin-sensitive sensory nerves (Kuo *et al.*, 1991). Thus, investigation of the modulatory effect of opioids on cigarette smoke-induced goblet cell secretion may provide an understanding of potentially important mechanisms under 'physiological' conditions of stimulation. The purpose of the present study was to examine the possible effect of opioids on goblet cell secretion in response to electrical stimulation of the vagus nerves, acute injection of capsaicin or acute inhalation of cigarette smoke in guinea-pig trachea *in vivo*, using morphine or the opioid agonists [D-Ala^2, NMePhe^4, Gly-ol^5]enkephalin (DAMGO), [D-Pen^2, D-Pen^5]enkephalin (DPDPE) or *trans*-3,4-dichloro-*N*-methyl-*N*-(2-(1-pyrollidinyl)cyclohexyl) benzeneacetamine (U-50,488H), which are selective at μ, δ or κ-opioid receptors respectively.

Methods

Male Dunkin-Hartley outbred guinea-pigs (256–450 g body wt, Charles River, Kent) anaesthetized with urethane (2 g kg^{-1}, i.p.), were laid supine on a heated blanket which maintained body temperature (rectal) at 37°C. The guinea-pigs

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were ventilated with a tracheal cannula and a constant-volume ventilator (Harvard Apparatus, MA, U.S.A.) at a tidal volume of 10 ml kg^{-1} and a frequency of 60 breaths min^{-1}. Pulmonary insufflation pressure, a measure of airway constriction, was determined via a side arm connected to the inspiratory limb of the ventilator tubing and connected to a differential pressure transducer (Franell Electronic Components Ltd., Leeds). To monitor the physiological condition of the animals and to establish drug activity, blood pressure and heart rate in the left carotid artery were recorded on a two-channel recorder (Devices, Ormed Ltd., Welwyn Garden City, U.K.) via an indwelling Portex cannula filled with heparin-saline (10 u ml^{-1}) linked to a pressure transducer (Bell and Howell, Basingstoke, Hants.).

Vagal nerve stimulation

Both cervical vagus nerves were carefully dissected free and sectioned (to avoid stimulating the central nervous system) at the level of the fifth tracheal cartilage ring. Their caudal ends were placed across bipolar platinum electrodes and were electrically stimulated (10 Hz, 5 ms, 5 V, for 3 min) by square-wave pulses (model S88 stimulator, Grass Instruments, Quincy, MA, U.S.A.). The effect of dissection and manipulation on goblet cell discharge was determined by repeating the above but without electrical stimulation (sham stimulation).

Capsaicin pretreatment

Aminophylline (25 mg kg^{-1}, i.p.) and terbutaline (0.1 mg kg^{-1}, s.c.) were injected 30 min before capsaicin administration to alleviate the bronchoconstriction. Guinea-pigs were anaesthetized with ketamine (50 mg kg^{-1}, i.m.) and xylazine (0.1 mg kg^{-1}, i.m.). Capsaicin (50 mg kg^{-1}, s.c.) was injected, and animals were studied 1 week later (Lundberg *et al.*, 1983). In these animals, the completeness of sensory denervation was determined by measuring bronchoconstriction in the presence of hexamethonium during experimentation. In vehicle pretreated animals, vagal stimulation increased airway pressure by $130 \pm 46\%$ ($n = 5$), whereas in capsaicin pretreated animals, the increase was only $2 \pm 2\%$ ($n = 5$), which indicates that capsaicin-pretreatment was completely effective.

Cigarette smoke exposure

Cigarettes were lit in a fume cupboard where a constant laminar flow prevented smoke accumulation. After the first quarter of the cigarette had burned, cigarette smoke was drawn through the cigarette into a 60 ml polypropylene syringe in less than 2 s. The cigarettes used were U.K. government category 'Middle Tar' (nicotine content 1.2 mg per cigarette; carbon monoxide content 11 mg per cigarette) which were commercially available and unfiltered. Two doses of cigarette smoke were used: 10 breaths diluted 1:10 in air (low dose) or 20 breaths of undiluted smoke (high dose). Diluted smoke was generated by expelling all but 6 ml smoke from the syringe and diluting to 60 ml in room air. Cigarette smoke was introduced into the animal's trachea and lungs via a 3-way tap just rostral to the endotracheal tube. Five or six ventilated breaths of room air were given after each tidal volume cigarette smoke. Control animals underwent the same procedure except air drawn through an unlit cigarette was used.

Protocol

To confirm that cholinergic nerves and capsaicin-sensitive sensory nerves were activated by electrical stimulation of the vagus nerves in the present batches of animals, hexamethonium, 5 mg kg^{-1}, was given 10 min before stimulation to establish that blocking ganglionic transmission to leave antidromic transmission had a significant but partial inhibitory effect on vagally-induced goblet cell secretion. The effectiveness of hexamethonium blockade was checked by determining its effect on nerve stimulation-induced changes in heart rate, blood pressure and airway pressure. Without hexamethonium, vagal stimulation caused mean heart rate to fall from 168 ± 8 ($n = 5$) to 82 ± 7 beats min^{-1}, decreased mean blood pressure by $55 \pm 5\%$ ($n = 5$) and increased airway pressure by $196 \pm 27\%$ ($n = 5$). Hexamethonium pretreatment significantly ($P < 0.05$) inhibited the reduction in heart rate (146 ± 7, $n = 5$, beats min^{-1} before stimulation; 134 ± 6 after stimulation), reduced the decrease in blood pressure to $6 \pm 4\%$ ($n = 5$) and the increase in airway pressure to $122 \pm 9\%$ ($n = 5$). The latter response was totally blocked by capsaicin pretreatment, increasing by $2 \pm 2\%$ ($n = 5$). This indicates that ganglionic blockade was virtually complete.

Morphine (1 mg kg^{-1}, i.v.) was administered 2 min before vagal stimulation, cigarette smoke exposure or exogenous capsaicin (1 $\mu g\,kg^{-1}$). The selective opioid receptor agonists, DAMGO (10^{-9}–2×10^{-7} mol kg^{-1}), DPDPE (10^{-9}–2×10^{-7} mol kg^{-1}) or U-50,488H (2×10^{-7} mol kg^{-1}), were studied in animals given cigarette smoke and were administered 2 min before exposure. The effect of the selective opioid receptor agonists on secretion induced by exogenous substance P (SP, 10^{-11} mol kg^{-1}, i.v.) or methacholine (10^{-13} mol kg^{-1}, i.v.) was also determined in two groups of animals. The dose of methacholine chosen gave an increase in goblet cell discharge which matched the response to low dose cigarette smoke. Naloxone (1 mg kg^{-1}) was injected 8 min before morphine in some separate studies to see whether reversal of the opioid effect was possible. Naloxone was also injected alone in other experiments to determine whether endogenous opioids had an inhibitory effect on goblet cell secretion in response to cigarette smoke exposure. The vehicle for these drugs (saline) was given at the same time points in control animals.

Tissue preparation

The methods used for tissue preparation and quantification of goblet cell discharge have been described in detail previously (Tokuyama *et al.*, 1990; Kuo *et al.*, 1990) and are outlined here. One minute after vagal nerve stimulation, or 15 min after cigarette smoke exposure or drug administration, the lungs were inflated by injecting 10% formal saline (approximately 10 ml) through the upper trachea until the lungs were fully expanded. The systemic circulation was perfused with 10% formal saline by incising the left ventricle, inserting a blunt-ended needle into the aorta, cross clamping the ventricles, and expelling blood via the incised right atrium at 100 mmHg pressure until the perfusate was clear. The trachea and lungs were removed, attached with thread to card to preserve their shape and orientation and fixed for at least 24 h in 10% formalin. Three micrometer thick sections of the trachea were cut in the coronal plane and stained with Alcian blue and periodic acid-Schiff (AB pH 2.5/PAS) in sequence to demonstrate the acidic and neutral intracellular glycoprotein of the secretory cells. Slides were coded to avoid observer bias during quantification and observed at a magnification of $\times 400$ with an Axioplan microscope (Carl Zeiss, Oberkochen, Germany) equipped with a Plan-neofluar 40–0.75 objective lens. Sections with unequally sized cartilage in the two walls of the trachea or apparent thickening of the epithelium indicated oblique sectioning and were discarded before analysis.

Quantification of goblet cell discharge

The intracellular mucin of the epithelial secretory cells appeared as purple coloured oval discs which were graded according to size: Grade 1, the vertical distance of the stained area was within 1/3 of the epithelial layer, measured from basement membrane to cell apices. Grade 2: the vertical distance of the stained area exceeded 1/3 of the epithelial layer. Stained areas were graded in 20 consecutive high power fields

(HPF) along both sides of the lower airways (a total of 40 HPF), starting at the carina and moving cranially. A mucus score (MS) was calculated for each animal as $n_1 + 2n_2$, where n_1 and n_2 were the number of cells in each grade respectively. Thus, MS was inversely related to degree of goblet cell discharge: the lower the score, the greater the degree of discharge and *vice versa*. Stained areas which were agranular or had ill-defined boundaries were not included in the counts.

Drugs

Drugs and chemicals were obtained from the following sources: SP, capsaicin, Tween 80, urethane, methacholine chloride, and hexamethonium chloride (Sigma Chemical Co., Poole, Dorset); atropine sulphate BP (Phoenix Pharmaceuticals Ltd., Oxford); naloxone hydrochloride (DuPont U.K., Hertfordshire); morphine sulphate (May and Baker, Dagenham, Essex); DAMGO, DPDPE (Bachem Feincheikalien AG, Bubendorf, Switzerland); U-50,488 (The Upjohn Company, Kalamazoo, U.S.A.); heparin injection BP (CP Pharmaceuticals Ltd., Wrexham, Wales); aminophylline (Antigen, Roscrea, Ireland); terbutaline (Astra Pharmaceuticals, Kings Langley, U.K.); xylazine (Bayer, Bury St Edmunds, U.K.). Capsaicin for pretreatment was prepared at a concentration of $50\,mg\,kg^{-1}$ in 10% ethanol and 10% Tween 80 vol/vol in saline. Capsaicin for acute injection was dissolved in absolute ethanol at a concentration of $1\,g\,ml^{-1}$, then diluted to $1\,mg\,ml^{-1}$ in saline before experimentation. All other drug solutions were freshly prepared on each day of experimentation in saline or vehicle.

Statistical analysis

Data did not approximate a Gaussian distribution and the probability of differences between groups was initially assessed by Kruskal-Wallace analysis followed by the Mann-Whitney U-test (two-tailed) to determine the significance of differences in MS between groups. Data in Results are mean ± s.e.mean. The null hypothesis was rejected at $P < 0.05$.

Results

Effect of morphine on goblet cell secretion induced by electrical stimulation of the vagus nerves

Bilateral cervical vagus nerve stimulation produced a significant ($P < 0.01$) decrease of 45% in MS (indicative of secretion) compared with sham stimulated controls (Figure 1). Pretreatment with hexamethonium ($5\,mg\,kg^{-1}$) partially and significantly inhibited the response to vagal stimulation (Figure 1). The hexamethonium-resistant part of the response was significantly ($P < 0.02$) different both from hexamethonium with sham stimulation and from vagal stimulation alone (Figure 1). Capsaisin pretreatment significantly blocked the hexamethonium-resistant part of the response compared with vehicle pretreated controls (Figure 1). Morphine, $1\,mg\,kg^{-1}$, completely ($P < 0.01$) inhibited the response to vagal stimulation (Figure 1). The inhibitory effect of morphine was significantly ($P < 0.05$) reversed by naloxone ($1\,mg\,kg^{-1}$) (Figure 1).

Effect of morphine on responses to cigarette smoke-induced goblet cell secretion

Acute inhalation of either low dose of cigarette smoke or high dose cigarette smoke caused a significant ($P < 0.01$) reduction in MS of 55% and 44% respectively compared with corresponding sham air controls (Figure 2). Morphine significantly ($P < 0.01$) inhibited the response to either low or high dose cigarette smoke by 100% and 73% respectively (Figure 2).

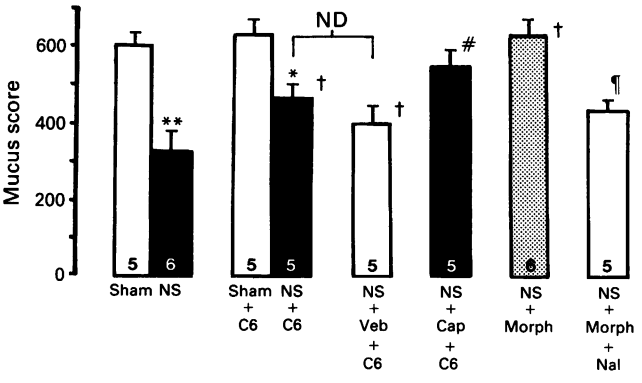

Figure 1 Effects of hexamethonium (C6, $5\,mg\,kg^{-1}$, i.v.), alone or after pretreatment with capsaicin (Cap.) or its vehicle (Veh.), as well as morphine (Morph., $1\,mg\,kg^{-1}$ i.v.) in the presence or absence of naloxone (Nal, $1\,mg\,kg^{-1}$, i.v.) on electrical stimulation of the vagus nerves (NS)-induced decrease in mucus score in guinea-pig trachea. Data are mean mucus score (bar = s.e.mean) for numbers of animals indicated in the histograms. * $P < 0.05$, ** $P < 0.01$ compared with corresponding sham stimulation (Sham). † $P < 0.05$ compared with NS alone; # $P < 0.05$ compared with vehicle control; ¶ $P < 0.05$ compared with NS + Morph. ND: no significant difference.

Naloxone reversed both inhibitory effects of morphine (Figure 2). Naloxone alone given 10 min before either dose of cigarette smoke exposure failed to influence the responses without naloxone (Figure 2).

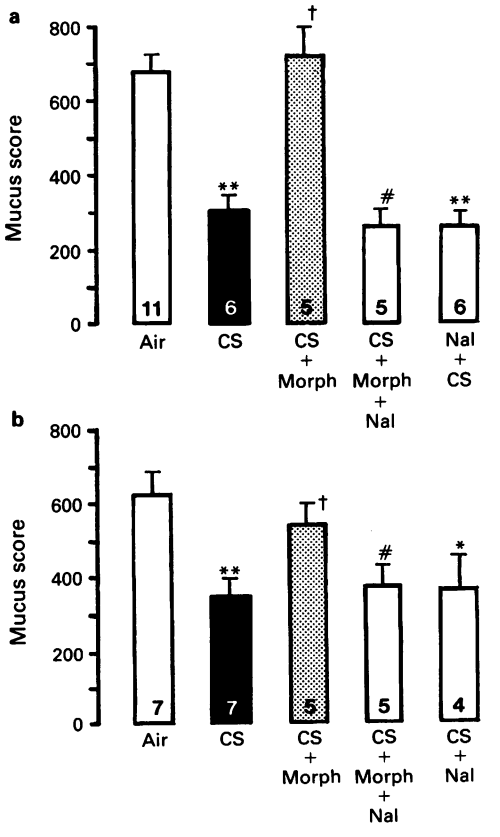

Figure 2 Effect of morphine (Morph, $1\,mg\,kg^{-1}$, i.v.) on (a) low dose or (b) high dose cigarette smoke (CS)-induced decrease in mucus score in guinea-pig trachea in presence or absence of naloxone (Nal, $1\,mg\,kg^{-1}$, i.v.). Data are mean mucus scores (bars = s.e.mean) for numbers of animals indicated in the histograms. ** $P < 0.01$, * $P < 0.05$ compared with air control (Air); † $P < 0.05$ compared with CS alone; # $P < 0.05$ compared with morphine-treated group.

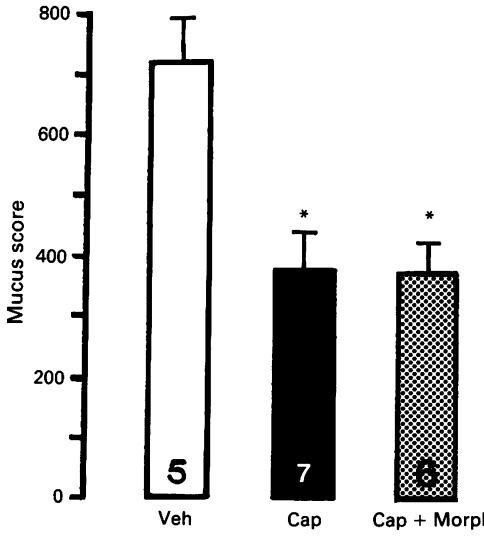

Figure 3 Effect of morphine (Morph, 1 mg kg^{-1}, i.v.) on capsaicin (Cap, 1 μg kg^{-1})-induced goblet cell secretion in guinea-pig trachea. Data are mean mucus scores (bars = s.e.mean) for numbers of animals indicated in the histograms. * $P < 0.05$ compared with vehicle control (Veh).

Effect of morphine on exogenous capsaicin-induced goblet cell secretion

Acute injection of capsaicin (1 μg kg^{-1}, i.v.) caused a significant ($P < 0.01$) decrease in MS of 48% compared with vehicle controls (Figure 3). Morphine did not alter the response to capsaicin (Figure 3).

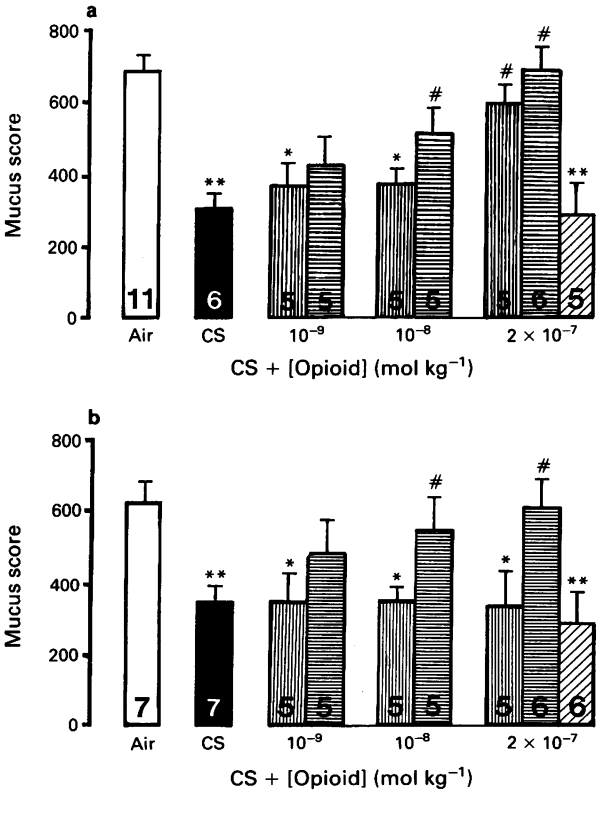

Figure 4 Effect of DPDPE (▥), DAMGO (▤) or U-50,488H (▨) on goblet cell secretion induced by (a) low dose or (b) high dose cigarette smoke (CS) in guinea-pig trachea. Data are mean mucus scores (bars = s.e.mean) for numbers of animals indicated in the histograms. ** $P < 0.01$, * $P < 0.05$ compared with air control (Air); # $P < 0.05$ compared with CS alone.

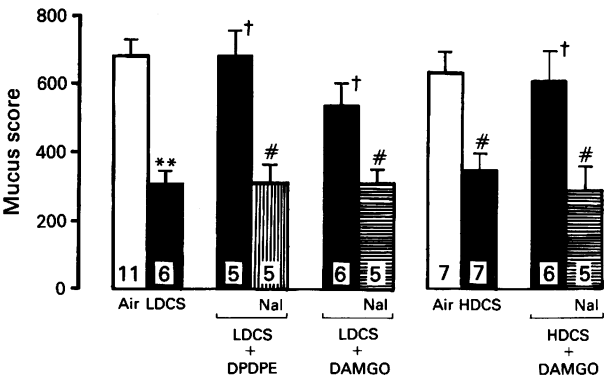

Figure 5 Effect of naloxone (Nal, 1 mg kg^{-1}) on the inhibitory effects of DPDPE or DAMGO (2 × 10^{-7} mol kg^{-1} respectively) on goblet cell secretion induced by low dose cigarette smoke (LDCS), or the effect of DAMGO on the response to high dose cigarette smoke (HDCS). Data are mean mucus scores (bars = s.e.mean) for numbers of animals indicated in the histograms. ** $P < 0.01$ compared with corresponding air controls (Air). † $P < 0.05$ compared with corresponding cigarette smoke alone groups. # $P < 0.05$ compared with corresponding DPDPE or DAMGO-treated groups.

Morphine and naloxone alone had no significant effect on mucus score in vehicle controls (635 ± 71, $n = 5$; 542 ± 30, $n = 6$, respectively).

Effect of selective opioid receptor agonists on responses to cigarette smoke

The δ-opioid receptor agonist, DPDPE (10^{-9}–2 × 10^{-7} mol kg^{-1}, $n = 5$–6) or the μ-selective agonist DAMGO (10^{-9}–2 × 10^{-7} mol kg^{-1}, $n = 5$–6) produced dose-related inhibitions of the secretory response to low dose cigarette smoke (Figure 4a) with maximal inhibitions of 100% and 75% at 2 × 10^{-7} mol kg^{-1} of each agonist respectively. The κ-selective agonist, U-50,488H, had no significant inhibitory effect on the response (Figure 4a). DAMGO also caused a dose-dependent inhibition of the response to high dose cigarette smoke with a maximal inhibition of 95% at 2 × 10^{-7} mol kg^{-1} (Figure 4b). In contrast, DPDPE had no significant effect on the response to high dose cigarette smoke (Figure 4b) neither did U-50,488H have any significant effect (Figure 4b). The inhibitory effects of DAMGO or DPDPE on the responses to either dose of cigarette smoke were completely reversed by naloxone (Figure 5).

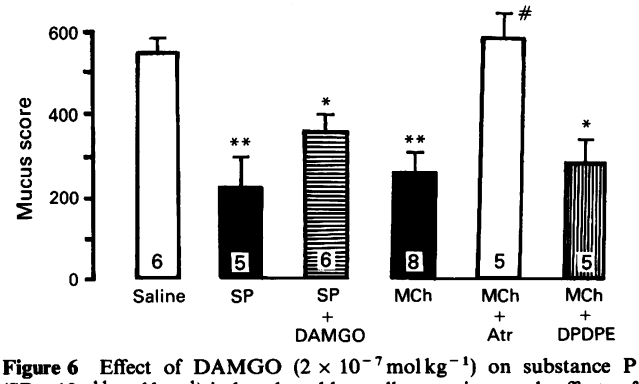

Figure 6 Effect of DAMGO (2 × 10^{-7} mol kg^{-1}) on substance P (SP; 10^{-11} mol kg^{-1})-induced goblet cell secretion and effect of DPDPE (2 × 10^{-7} mol kg^{-1}) or atropine (Atr, 0.1 mg kg^{-1}) on methacholine (MCh, 10^{-13} mol kg^{-1})-induced goblet cell discharge in guinea-pig trachea. Data are mean mucus scores (bars = s.e.mean) for numbers of animals indicated in the histograms. ** $P < 0.01$, * $P < 0.05$ compared with saline control (saline); # $P < 0.05$ compared with MCh alone.

Effect of opioids on the response to exogenous methacholine and substance P

Exogenous methacholine (10^{-13} mol kg^{-1}) caused a similar degree of goblet cell discharge as low dose cigarette smoke in decreasing MS by 51% compared with saline controls. The effect of methacholine was inhibited completely by atropine (0.1 mg kg^{-1}, MS = 585 ± 56, $n = 5$, $P < 0.01$). Substance P (10^{-11} mol kg^{-1}) also caused a significant ($P < 0.05$) reduction in MS of 59% compared with saline controls. The response to methacholine was not significantly affected by DPDPE 2×10^{-7} mol kg^{-1} (Figure 6) neither was the response to SP inhibited by DAMGO 2×10^{-7} mol kg^{-1} (Figure 6).

Discussion

We have demonstrated in the present study that morphine inhibits the secretory responses of tracheal goblet cells to vagal stimulation and acute inhalation of cigarette smoke in guinea-pigs. The inhibitory effects of morphine were significantly reversed by naloxone suggesting that the inhibitory effects were mediated via opioid receptors. Our present study showed that vagal stimulation-induced goblet cell secretion was partly inhibited by the ganglionic blocker hexamethonium with the remaining hexamethonium-resistant part of the response inhibited by capsaicin pretreatment, which suggests that capsaicin-sensitive sensory nerves as well as cholinergic or adrenergic nerves were involved in the secretory response. These results are consistent with our previous study which showed that electrical stimulation of the cervical vagus nerves provokes airway goblet cell discharge by activating cholinergic and capsaicin-sensitive sensory nerves (Tokuyama *et al.*, 1990). Therefore, morphine might have inhibitory effect on both components.

The failure of morphine to block the acute effect of capsaicin on goblet cell discharge suggests that the mechanism underlying capsaicin-induced activation of sensory nerves might be different from that of vagal stimulation or acute inhalation of cigarette smoke. Acute administration of capsaicin induces release of tachykinins including substance P from sensory nerve endings via a Ca^{2+}-dependent but tetrodotoxin-resistant mechanism, suggesting that capsaicin has a direct action on nerve endings (Szolcsanyi, 1983; Maggi *et al.*, 1987). It has been reported that capsaicin-induced release of tachykinins (Maggi *et al.*, 1988) or depolarization of sensory neurones (Rang *et al.*, 1987) is resistant to ω-conotoxin, which blocks neuronal depolarization-coupled transmitter secretion (Augustine *et al.*, 1987; Miller, 1987), which suggests that capsaicin-induced release of tachykinins is different from that induced by antidromic stimulation of nerves and independent of action potential propagation. Morphine and opioid peptides inhibit nerve cell discharge by postsynaptic inhibition of cell firing, or a presynaptic reduction in the release of neurotransmitters by blocking the propagation of action potentials and by reducing the entry of calcium (North & Williams, 1983). Morphine and opioid peptides might, therefore, be expected to have no inhibitory effect on the direct effect of capsaicin on sensory nerves. This suggestion is consistent with the inability of morphine to inhibit capsaicin-induced release of CGRP from guinea-pig isolated heart (Franco-Cereceda *et al.*, 1989) and the resistance of capsaicin-induced bronchoconstriction in the guinea-pig to [D-Met^2, Pro^5]enkephalin (Bartho *et al.*, 1987).

The inhibitory effect of morphine on both low and high dose cigarette smoke-induced goblet cell discharge indicated that morphine might inhibit both cholinergic and capsaicin-sensitive mechanisms. That is consistent with a previous report in which morphine was shown to inhibit both cholinergic and non-cholinergic excitatory constrictor responses in guinea-pig airways (Johansson *et al.*, 1989). With low dose cigarette smoke, both the δ-selective opioid receptor agonist, DPDPE and the μ-opioid agonist, DAMGO inhibited cigarette smoke-induced goblet cell secretion. DPDPE was more potent than DAMGO which suggests a predominance of δ-opioid receptors. The inhibitory effects of DPDPE and DAMGO were completely reversed by naloxone, confirming that these effects were mediated via opioid receptors. The site of action of the inhibitory opioid receptors in the airways is not conclusively determined in this study. Because DPDPE did not significantly affect methacholine-induced goblet cell secretion, a presynaptic site of action seems likely. Low dose cigarette smoke principally activates parasympathetic ganglia to activate cholinergic systems (Kuo *et al.*, 1990b). Enkephalins have been reported to inhibit presynaptically cholinergic neurotransmission at autonomic ganglia (Konishi *et al.*, 1979). Morphine and enkephalins also inhibit the nicotinic receptor-mediated release of catecholamines in the adrenal medulla (Kumakura *et al.*, 1980). Therefore, it is possible that opioids inhibit goblet cell discharge at the level of the ganglion. However, it is also possible that opioids block neuroeffector transmission (Cowie *et al.*, 1968; Oka, 1980).

Only DAMGO significantly inhibited the response to high dose cigarette smoke, which selectively stimulates capsaicin-sensitive sensory nerves (Kuo *et al.*, 1991), and was reversed by naloxone indicating that inhibition was via an action on μ-opioid receptors. μ-Opioid receptors have been shown to be present on a capsaicin-sensitive population of sensory nerves (Laduron, 1984) which contain neuropeptides including the tachykinins substance P and neurokinin A as well as calcitonin gene-related peptide. Opioid agonists have been demonstrated to inhibit stimulus-evoked release of substance P from the rat trigeminal nucleus *in vitro* (Jessell & Iversen, 1977). Substance P has been shown to be the most potent of a number of neuropeptides, including those mentioned above, in causing goblet cell discharge (Kuo *et al.*, 1990a). Therefore, μ-opioid receptor inhibition of high dose cigarette smoke-induced goblet cell secretion is probably mediated via inhibition of the release of substance P. DAMGO had no significant effect on SP-induced goblet cell secretion which further suggests that inhibition is at a presynaptic site. Neither the δ-opioid receptor agonist, DPDPE, nor the κ-opioid receptor agonist, U-50,488, had significant inhibitory effect on high dose cigarette smoke-induced goblet cell secretion. Pretreatment with naloxone did not significantly affect the response to either high or low dose cigarette smoke, indicating that the role of endogenous opioids in this response is minimal.

Our present results extend the spectrum of inhibitory effects of morphine and opioid peptides on neurally-mediated airway responses. Opioid inhibition of cigarette smoke-induced goblet cell secretion may have interesting clinical implications. In rodent airways, cigarette smoke triggers capsaicin-sensitive nerves to induce microvascular leakage (Lundberg *et al.*, 1983) and mucus secretion from goblet cells (Kuo *et al.*, 1991). In addition, cigarette smoke inhalation has been shown to stimulate cholinergic nerves to induce secretion from submucosal glands (Peatfield *et al.*, 1986) and goblet cells (Kuo *et al.*, 1990b). Similar neurally-mediated responses may contribute to the hypersecretory state of cigarette smokers and patients with chronic bronchitis, which is a cigarette smoke-related condition. The hyperfunction of goblet cells in the distal airways may adversely affect small airway function in smokers (Lumsden *et al.*, 1984). Since the response of goblet cells can be effectively blocked by opioids acting on prejunctional receptors on cholinergic or sensory nerves, opioids may be useful in blocking airway hypersecretory states in bronchial diseases associated with cigarette smoking. The mucus hypersecretion of asthma may also be susceptible to this treatment, since activation of cholinergic nerves and axon reflexes by inflammatory mediators have been suggested as important in the pathophysiology of asthma (Barnes, 1986).

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