Effects and interactions of opioids on plasma exudation induced by cigarette smoke in guinea pig bronchi

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Lei, Yu-Hong, and Duncan F. Rogers. Effects and interactions of opioids on plasma exudation induced by cigarette smoke in guinea pig bronchi. Am. J. Physiol. 276 (Lung Cell. Mol. Physiol. 20): L391–L397, 1999.—The effects of opioids on cigarette smoke-induced plasma exudation were investigated in vivo in the main bronchi of anesthetized guinea pigs, with Evans blue dye as a plasma marker. Acute inhalation of cigarette smoke increased plasma exudation by 216% above air control values. Morphine, 0.1–10 mg/kg but not 30 mg/kg, inhibited the exudation but had no significant effect on substance P–induced exudation. Both 10 and 30 mg/kg of morphine increased exudation in air control animals, an effect inhibited by antihistamines but not by a tachykinin neurokinin type 1-receptor antagonist. Naloxone inhibited all morphine responses. Cigarette smoke–induced plasma exudation was inhibited by a µ-opioid-receptor agonist (DAMGO) but not by agonists at δ (DPDPE) or κ (U-50488H) receptors. None of these agonists affected exudation in air control animals. DAMGO prevented the inhibition by DAMGO of cigarette smoke–induced plasma exudation, and the combination of DAMGO and DPDPE increased exudation in air control animals. Prevention of inhibition and the combination–induced increase were inhibited by antihistamines or the mast cell–stabilizing drug sodium cromoglycate. U-50488H did not alter the response to either DAMGO or DPDPE. We conclude that, in guinea pig main bronchi in vivo, μ-opioid-receptor agonists inhibit cigarette smoke–induced plasma exudation via a prejunctional mechanism. Plasma exudation induced by μ- and δ-receptor interactions is due to endogenous histamine release from mast cells.

PLASMA EXUDATION is part of the acute inflammatory response to noxious stimuli in a variety of organ systems including the skin and airways. Plasma exudation is under humoral and neuronal control. Neural induction of plasma exudation is mediated exclusively via a population of capsaicin-sensitive C fibers that subserve a motor function (termed “sensory-efferent” nerves) (27). Stimulation of these fibers either directly (e.g., by capsaicin) or indirectly (e.g., by noxious stimuli) induces plasma exudation (8). In the airways, inhalation of cigarette smoke triggers reflex plasma exudation via activation of sensory-efferent nerves (24, 26). The sensory neuropeptides substance P and neurokinin (NK) A, released from the nerves, interact with tachykinin NK1 receptors on the bronchial microvasculature to induce exudation (6, 15). A variety of airway responses mediated via activation of sensory-efferent nerves, including plasma exudation, bronchoconstriction, and mucus secretion, are inhibited by drugs acting on specific prejunctional receptors (1). For example, in the context of the present study, morphine inhibits cigarette smoke–induced plasma exudation (24).

Three opioid-receptor types, namely δ (also termed OP1), κ (OP2), and μ (OP3), are recognized in central and peripheral nervous tissues (7). In the airways, opioid inhibition may be response specific, cholinergic- and sensory-efferent-induced guinea pig airway smooth muscle contraction is inhibited by activation of μ-opioid receptors (2, 3), whereas neurogenic mucus secretion in guinea pig ferret tracheae is inhibited by activation of either μ- or δ-receptors (20, 32). It would therefore be of interest to determine whether opioid inhibition of neurogenic airway plasma exudation conformed more to the “contraction” or “secretion” profile of receptor type. In addition to the inhibitory effects of activation of single opioid-receptor types, central opioid receptors interact (31). Opioid interactions are comparatively poorly defined for peripheral tissue and have not been studied in airway neurogenic plasma exudation. The mechanism of any interaction in the airways is unexplored.

In the present study, we determined in guinea pig main bronchi in vivo whether morphine or the opioid receptor–selective agonists DAMGO (μ-receptor) (12), DPDPE (δ-receptor) (28) or U-50488H (κ-receptor) (22) inhibited cigarette smoke–induced plasma exudation and also investigated opioid–receptor interactions. Possible mechanisms of action underlying opioid inhibition and interactions were investigated with the opioid-receptor antagonist naloxone, the tachykinin NK1-receptor antagonist CP-96345 (36), the histamine-receptor antagonists mepyramine and cimetidine, and the mast cell–“stabilizing” drug sodium cromoglycate (5). Plasma exudation was quantified with Evans blue dye as a plasma marker.

MATERIALS AND METHODS

Animal preparation. Male Dunkin-Hartley guinea pigs (Charles River, Margate, UK), 320–370 g body weight, were housed in a temperature-controlled room (21°C) with food (Special Diet Services, Witham, UK) and tap water freely available. They were anesthetized with urethan [2 g/kg as 8 ml/kg of a 25% (wt/vol) solution of urethan in 0.9% (wt/vol) saline] and laid supine on a heated blanket (Homeothermic System, Harvard Apparatus, Edenbridge, UK), which maintained body temperature at 37°C. Both cervical jugular veins were exposed for intravenous injection of drugs. Systemic blood pressure was recorded as a physiological indication of the condition of the animals during the experiment and to monitor drug activity. A cannula containing heparin sodium
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In separate animals, to determine its time course of effect, morphine (1 or 10 mg/kg) was injected 5 min before exposure to cigarette smoke or air. To determine the selectivity of the morphine effect, naloxone (0.4 mg/kg each; DAMGO and DPDPDE, DPDPDE and U-50488H, or DAMGO and U-50488H were injected 2 min before 50 breaths of cigarette smoke or air. The order of injection of the two drugs in combination was alternated.

To examine the involvement of mast cells in the exudative response, mepyramine and cimetidine in combination (1 mg/kg each) or sodium cromoglycate (10 mg/kg) was injected 15 and 10 min, respectively, before inhalation of 50 breaths of cigarette smoke or air [i.e., 13 and 8 min before injection of DAMGO and DPDPDE in combination, 13 min before DPDPDE, or 5 min before morphine (10 or 30 mg/kg) or saline].

Drugs and chemicals. The following drugs and chemicals were used: DAMGO, formamide, and urethan (Sigma, Poole, UK); cimetidine (Tagamet, SmithKline Beecham Laboratories, Welwyn Garden City, UK); DPDPDE (Bachem, Stevenage, UK); Evans blue dye (Nakarai Chemicals, Osaka, Japan); heparin sodium (CP Pharmaceuticals, Wrexham, UK); morphine sulfate (Evans Medical, Horsham, UK); naloxone hydrochloride (DuPont); and saline (0.9% sodium chloride; Travenol Laboratories, Thetford, UK). CP-96345 [the dihydrochloride salt of (25,35)-cis-2-(di phenylmethyl)N-(2-(1-pyrrolidinyl)cyclohexyl)benzene aceticamide methane sul fonate] was a gift from Upjohn (Kalamazoo, MI).

Data analysis and statistics. Data are arithmetic means ± SE, with n the number of animals. Data for tissue content of Evans blue dye were not normally distributed, and the significance of differences between preselected groups was assessed with the Mann-Whitney U-test. Mean blood pressure was calculated as diastolic pressure + 0.33(systolic pressure – diastolic pressure) and was normally distributed. The significance of differences in mean blood pressure between groups or between pre- and postdrug administration in the same animal was analyzed with Student's t-test for unpaired or paired data, respectively. The null hypothesis was rejected at P < 0.05 (twotail).

RESULTS

Effect of morphine on cigarette smoke- or substance P-induced plasma exudation. Inhalation of 50 breaths of cigarette smoke increased the Evans blue dye content of the main bronchi by 216% above air control values (Fig. 1). Morphine, 0.1–10 mg/kg but not 0.001 or 30 mg/kg, inhibited the cigarette smoke-induced increase in dye content by ~60% (Fig. 1). Naloxone reversed the inhibition by morphine by 95% (Fig. 1). Naloxone alone did not significantly affect dye content in either air-exposed or cigarette smoke-exposed animals, with an increase of 14% and a reduction of 5%, respectively (n = 4–6). Pretreatment with morphine (1 or 10 mg/kg) 5, 10, or 30 min before cigarette smoke gave the greatest inhibition at 10 min (averages of 36% at 5 min and 63% at 10 min, P < 0.05 compared with cigarette smoke group; 19% at 30 min; n = 4–5/group). The 10-min time point was used in subsequent experiments. Substance P (1 nmol/kg) increased dye content
by 254% above the saline control value ($P < 0.05$; $n = 5$). Morphine (1 mg/kg) had no significant effect on this increase, which was reduced by 19% ($n = 5$). The combination of morphine (10 mg/kg) and the antihistamines inhibited cigarette smoke-induced plasma exudation by 89% (air control group, $49.5 \pm 5.0$ ng Evans blue dye/mg tissue; cigarette smoke group, $156.3 \pm 20.1$ ng/mg; morphine plus antihistamines plus cigarette smoke group, $60.8 \pm 7.2$ ng/mg; no significant difference between air control and morphine plus antihistamines plus cigarette smoke groups; $n = 5$/group).

Effect of morphine on plasma exudation in air control animals. In saline-pretreated and air-exposed animals, the Evans blue dye content in the main bronchi was $\sim 50$ ng dye/mg tissue (Figs. 1 and 2). Morphine (10 or 30 mg/kg) increased the basal dye content by 51 and 67%, respectively (the latter shown in Fig. 2). The lower doses of morphine did not induce significant exudation. Naloxone and the antihistamines inhibited the morphine-induced exudation by 76 and 109% respectively (i.e., the latter below the basal value; Fig. 2). In contrast, the NK₁ antagonist CP-96345 at a dose that significantly inhibits neurogenic plasma exudation (22) did not significantly affect the morphine-induced increase in dye content (Fig. 2).

Effect of selective opioid-receptor agonists. The μ-receptor agonist DAMGO significantly inhibited cigarette smoke-induced increases in Evans blue dye content in the main bronchi by 57% (Fig. 3). Neither the δ-receptor agonist DPDPE nor the κ-receptor agonist U-50488H had any significant inhibitory effect (Fig. 3). Neither DAMGO, DPDPE, nor U-50488H alone had any significant effect on the dye content in air-exposed control animals.

![Fig. 1. Effect of morphine on cigarette smoke-induced plasma exudation in guinea pig main bronchi in vivo. Air, 50 ventilated breaths (control); Cigarette smoke, 50 breaths of cigarette smoke; Morphine, indicated doses of intravenous morphine; Nal +1, naloxone (0.4 mg/kg iv) plus morphine (1 mg/kg); Evans blue dye, tissue content of Evans blue dye plasma marker. Data are means ± SE from 4–6 animals/group. Nal reversed inhibition by morphine. Significantly different compared with air control group: *$P < 0.05$; **$P < 0.01$. Significantly different compared with cigarette smoke group, +#$P < 0.05$.](http://ajplung.physiology.org/)

![Fig. 2. Effect of morphine on plasma exudation in guinea pig main bronchi in vivo. Data are means ± SE from 5–6 animals/group. Increase in plasma exudation induced by morphine (30 mg/kg iv) was inhibited by Nal (0.4 mg/kg iv) or antihistamines mepyramine and cimetidine in combination (Antih; 1 mg/kg iv each) but not by tachykinin neurokinin type 1-receptor antagonist CP-96345 (CP; 1 μmol/kg iv). Significantly different compared with saline control group, *$P < 0.05$.](http://ajplung.physiology.org/)

![Fig. 3. Effect of opioid-receptor agonists on cigarette smoke-induced plasma exudation in guinea pig main bronchi in vivo. DAMGO, μ-opioid-receptor agonist; DPDPE, δ-opioid-receptor agonist; U-50488H, κ-opioid-receptor agonist (1 mg/kg each iv). Data are means ± SE from 5–6 animals/group. Significantly different ($P < 0.05$) compared with: *air control group; #cigarette smoke group.](http://ajplung.physiology.org/)
animals, which increased by 6, 26, and 19%, respectively (P > 0.2; n = 4–5).

Opioid interactions and cigarette smoke-induced plasma exudation. The inhibition by DAMGO of cigarette smoke-induced plasma exudation was prevented by DPDPE (Fig. 4, left). The preventive effect was inhibited by the antihistamines or sodium cromoglycate by 95 and 91%, respectively (Fig. 4, left). The antihistamines or sodium cromoglycate alone did not affect cigarette smoke-induced plasma exudation (by 43%; n = 4–6/group), whereas DPDPE still failed to inhibit the exudation (reduced by 20%; n = 4–6/group).

Opioid interactions in air control animals. The combination of DAMGO and DPDPE significantly increased the Evans blue dye content in the main bronchi of the air-exposed animals by 76% (Fig. 5, left). The increase in dye content was blocked by the antihistamines or sodium cromoglycate (Fig. 5, left). The combination of U-50488H with either DAMGO or DPDPE did not significantly increase the dye content of the air control animals (Fig. 5, right).

Blood pressure. Mean baseline carotid arterial pressure in the guinea pigs was 39 ± 1 mmHg. Intravenous morphine (1, 10, or 30 mg/kg) dose dependently increased blood pressure by 50 ± 10, 55 ± 11, and 79 ± 15%, respectively (P < 0.05 for all; n = 12–18). Naloxone alone had no significant effect on blood pressure and prevented the hypertension induced by morphine. DAMGO raised blood pressure by 55 ± 12% (P < 0.05; n = 10). U-50488H significantly lowered blood pressure (by 28 ± 2%; P < 0.05; n = 13), whereas DPDPE had no significant effect on blood pressure (lowered by 14 ± 5%; n = 13). After injection of DPDPE, a subsequent injection of DAMGO raised the blood pressure by 39 ± 7% (P < 0.05; n = 5). In the presence of the antihistamines, morphine (30 mg/kg) increased blood pressure by 4 ± 7% (n = 4). Sodium cromoglycate did not affect blood pressure (n = 8).

DISCUSSION

In the present study, cigarette smoke increased the Evans blue dye content of guinea pig main bronchi. In guinea pig airways, exudation of dye correlates with exudation of 125I-labeled human serum albumin (35), an observation that indicates that changes in tissue dye content reflect changes in airway plasma exudation. Morphine (0.1–10 mg/kg) inhibited the cigarette smoke-induced plasma exudation. Inhibition was prevented by the opioid-receptor antagonist naloxone, which indicates that the inhibition by morphine was via activation of opioid receptors. Because cigarette smoke-induced airway plasma exudation in the guinea pig is due to activation of sensory-efferent nerves (24), mor-
phine inhibition should be associated with the inhibition of neurotransmission. The failure of morphine to inhibit substance P-induced plasma exudation indicates that morphine inhibition of cigarette smoke-induced plasma exudation is via activation of prejunctional opioid receptors leading to inhibition of sensory neuropeptide release (Fig. 6). This suggestion is consistent with the observation that morphine inhibits stimulated release of substance P-like immunoreactivity from the rat trachea in vitro (33).

Among the three selective opioid-receptor agonists used herein, inhibition of cigarette smoke-induced plasma exudation by DAMGO was greater than that by DPDPE or U-50488H, which indicates that µ-receptors predominate in mediating the inhibitory effect of opioids on cigarette smoke-induced plasma exudation. The dose of DPDPE used herein has previously been shown to inhibit neurogenic mucus secretion in vivo in guinea pigs (20). The involvement herein of µ-receptors is consistent with previous studies (2, 3) that found that the µ-receptor mediated inhibition of neurogenic airway smooth muscle contraction. In contrast, opioid inhibition of neurogenic airway mucus secretion is mediated by both µ- and δ-receptors (20, 32), whereas inhibition of citric acid-induced cough and reflex bronchoconstriction is mediated by µ- and κ-receptors (19).

Thus, from the above, it would appear that, for opioid inhibition in the airways, the µ-receptor is ubiquitous, whereas the involvement of additional receptor types is response dependent.

In the present study, morphine over an effective dose range did not completely inhibit cigarette smoke-induced plasma exudation. In addition, a dose of 30 mg/kg of morphine did not significantly inhibit the neurogenic exudation. These observations may relate to the finding that 10 or 30 mg/kg of morphine induced airway plasma exudation in air-exposed control animals, with increasing baseline exudation eventually overcoming inhibition of stimulated exudation. Naloxone abolished the exudative response in the air control animals, whereas the tachykinin NK1-receptor antagonist CP-96345 at an effective dose (23) did not block exudation. These two observations indicate that the increased exudation is opioid receptor mediated but is not due to substance P activity (i.e., is not neurogenic). The histamine-receptor antagonists mepyramine and cimetidine abolished morphine-induced plasma exudation, which suggests that the response is due to endogenous histamine release. Furthermore, the combination of morphine and antihistamines reduced cigarette smoke-induced plasma exudation to air control values. This indicates that morphine-induced histamine release accounted for the residual increase in permeability. Consistent with the above suggestions is the observation that histamine induces plasma exudation in guinea pig airways (10). The lack of effect of the antihistamines on cigarette smoke-induced plasma exudation indicates that the histamine release was not associated with neural activity. The inhibitory effect of the mast cell stabilizer sodium cromoglycate (5) indicates that mast cells are the principal source of histamine (Fig. 6). The lack of an inhibitory effect of sodium cromoglycate on cigarette smoke-induced plasma exudation indicates that sodium cromoglycate had no neural effect under the present experimental conditions. This is consistent with the observation that sodium cromoglycate does not inhibit stimulated release of substance P-like immunoreactivity from the rat trachea (33). Release of histamine by morphine in the present study is consistent with previous observations. For example, morphine was considered a histamine liberator in the early part of this century (25), and histamine is found in plasma after intravenous injection of morphine (11, 29). Formal studies (4, 9, 13) demonstrated that opiates release histamine from mast cells of many animal species.

In the present study, the combination of the µ- and δ-opioid-receptor agonists (DAMGO and DPDPE, respectively) induced plasma exudation in air-exposed animals. Neither drug alone induced significant airway plasma exudation. In contrast, combinations of the

![Fig. 6. Effects and interactions of opioids on cigarette smoke-induced plasma exudation in guinea pig airways. Cigarette smoke stimulates sensory-efferent nerves to induce release of tachykinins, for example, substance P (SP), which, in turn, interacts with tachykinin neurokinin type 1 (NK1) receptor on venular endothelial cells to increase plasma exudation (+). Low doses of morphine (<10 mg/kg) or selective opioid agonists such as DAMGO interact with µ-opioid receptors to inhibit neurotransmission (−) and hence reduce plasma exudation. In contrast, activities of agonists at µ- and δ-opioid receptors on mast cells combine to induce release of histamine (+), which, in turn, increases plasma exudation via histamine receptors (H) on endothelial cells (+). High doses of morphine (>10 mg/kg) mimic this response, presumably via an interaction with µ- and δ-opioid receptors. Thus the inhibitory effect of morphine on neurotransmission and plasma exudation is overcome by a direct stimulatory action on exudation.](image-url)
κ-receptor agonist (U-50488H) with either DAMGO or DPDPE did not induce exudation. These observations indicate that interactions between μ- and δ-receptors (14) mediate opioid-induced airway plasma exudation. This suggestion is consistent with the observation that, in the rat, the antitussive effect of DAMGO or U-50488H on capsaicin-induced cough was reversed by coadministration of DPDPE (17, 18). The failure of the higher dose of morphine (30 mg/kg) to inhibit cigarette smoke-induced airway plasma exudation might be because morphine is a multiple opioid-receptor agonist (16). This can be explained if the dose-response curve for the stimulation with morphine of δ-receptors is well to the right of that of μ-receptors; only at high doses would morphine costimulate the μ- and δ-receptors intensely enough to activate mast cells (Fig. 6).

In the present study, DAMGO inhibited cigarette smoke-induced plasma exudation. Inhibition was prevented by DPDPE. This prevention was inhibited by the antihistamines or sodium cromoglycate. The latter three observations indicate that interactions between the μ- and δ-receptors lead to histamine release from mast cells that, in turn, mediate the exudative response (Fig. 6). The lack of inhibition by the antihistamines in the presence of DPDPE indicates that histamine release is not due to δ-receptors alone. Sodium cromoglycate alone did not inhibit cigarette smoke-induced plasma exudation. This indicates that its prevention of the DAMGO-DPDPE-induced exudation was not due to inhibition of sensory nerve activation.

Changes in systemic blood pressure can reflect changes in blood flow that, in turn, may affect plasma exudation. Cigarette smoke may induce vasodilatation. Lei et al. (24) previously found that cigarette smoke did not alter systemic (carotid arterial) blood pressure, and we noted similar results in the present study. This indicates little or no systemic vasodilator effect for cigarette smoke. There was no consistent association between the effects on plasma exudation and the effects on blood pressure. For example, morphine raised blood pressure. Increased blood pressure may affect capillary transit time and drug availability. The lack of an inhibitory effect of morphine on substance P-induced plasma exudation argues against morphine-induced hypertension contributing significantly to its inhibition of cigarette smoke-induced plasma exudation. However, local bronchial vasodilatation would not necessarily influence carotid arterial pressure. Vasodilatation in the bronchial vasculature would increase blood flow and driving pressure, which, in turn, could facilitate downstream venular plasma exudation. Thus the inhibitory effect of morphine observed in the present study could be related to vascular effects rather than to neural inhibition.

The physiological significance of opioids on plasma exudation during cigarette smoking is unclear from the present study. For example, the opioid-receptor antagonist naloxone had no significant effect on either baseline exudation or cigarette smoke-induced exudation. These observations indicate that endogenous opioids do not play a significant part in the regulation of sensory nerve activity, at least under the present experimental conditions. However, the present study provides indirect evidence (i.e., opioid inhibition of cigarette smoke-induced plasma exudation) for the presence of functional opioid receptors on peripheral nerves. This is consistent with the localization of opioid binding sites on rat vagal sensory fibers (21). It is possible that endogenous opioids are relevant in the pathophysiology of the airways. For example, naloxone potentiates capsaicin-induced mucus secretion in vitro in bronchi resected from patients with lung carcinoma (34). This observation indicates that opioids bound to opioid receptors on sensory nerves associated with bronchial mucous cells were regulating the magnitude of the secretory response. The source of the opioids, produced either endogenously in response to chronic pain or airway inflammation or from the premedication for surgery, is unexplored. Interestingly, concentrations of substance P- and endorphin-like immunoreactivities are significantly higher in bronchoalveolar lavage fluid from allergic subjects than in that from nonallergic subjects (30). Allergen challenge elevated bronchoalveolar lavage concentrations of both peptides in the allergic subjects but not in the nonallergic subjects. Thus it would appear from the above that, in pathophysiological conditions of the airways, increases in excitatory neurotransmitters may be associated with increases in inhibitory mechanisms. Formal investigations of opioid/opioid-receptor expression in pathophysiological model(s) are required to address this hypothesis.

In conclusion, the present study has shown that in guinea pig main bronchi in vivo, morphine and opioid-receptor agonists inhibit cigarette smoke-induced plasma exudation via prejunctional actions at μ-opioid receptors and that the plasma exudation induced via μ- and δ-receptor interaction is due to endogenous histamine release from mast cells (Fig. 6).

REFERENCES


