Bradykinin-induced airflow obstruction and airway plasma exudation: effects of drugs that inhibit acetylcholine, thromboxane A₂ or leukotrienes

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1 The mechanisms behind bradykinin-induced effects in the airways are considered to be largely indirect. The role of cholinergic nerves and eicosanoids, and their relationship in these mechanisms were investigated in guinea-pigs.

2 The role of cholinergic nerves was studied in animals given atropine (1 mg kg⁻¹, i.v.), hexamethonium (2 mg kg⁻¹, i.v.), or vagotomized. To study the role of eicosanoids, animals were pretreated with a thromboxane A₂ (TxA₂) receptor antagonist (ICI 192,605; 10⁻⁶ mol kg⁻¹, i.v.) or with a leukotriene (LT) receptor C₄/D₄/E₄ antagonist (ICI 198,615; 10⁻⁶ mol kg⁻¹, i.v.).

3 After pretreatment with a drug, bradykinin (150 nmol) was instilled into the tracheal lumen. We measured both airway insufflation pressure (Pi), to assess airway narrowing, and the content of Evans blue dye in airway tissue, to assess plasma exudation.

4 Bradykinin instillation into the trachea caused an increase in Pi and extravasation of Evans blue dye. The increase in Pi was significantly attenuated by atropine or the TxA₂ receptor antagonist, but not by hexamethonium, vagotomy or the LT receptor antagonist.

5 The bradykinin-induced exudation of Evans blue dye was significantly attenuated in the intrapulmonary airways by the TxA₂ receptor antagonist, but not by atropine, hexamethonium, cervical vagotomy or the LT receptor antagonist.

6 A thromboxane-mimetic, U-46619 (20 nmol kg⁻¹, i.v. or 10 nmol intratracheally), caused both an increase in Pi and extravasation of Evans blue dye at all airway levels. Atropine pretreatment slightly attenuated the peak Pi after the intratracheal administration of U-46619, but not after i.v. administration.

7 We conclude that peripheral cholinergic nerves are involved in bradykinin-induced airflow obstruction but not plasma exudation, and that TxA₂ is involved in both airflow obstruction and airway plasma exudation induced by bradykinin given via the airway route. TxA₂-induced airflow obstruction is mediated only to a minor degree, via the release of acetylcholine in the airways.

Keywords: Asthma; bronchoconstriction; airway oedema; inflammation; plasma exudation; cholinergic nerves; bradykinin; thromboxane; leukotrienes

Introduction

Bradykinin, a plasma-derived inflammatory peptide, has been implicated in asthma (Regoli & Barabe, 1980; Barnes *et al.*, 1988). Bradykinin produces bronchoconstriction in asthmatics, but to a lesser extent in normal subjects (Simonsson *et al.*, 1973; Fuller *et al.*, 1987). It has also been shown that asthmatics have elevated concentrations of bradykinin in plasma during active disease and of the bradykinin precursor kininogen in broncho-alveolar lavage fluid after allergen challenge (Abe *et al.*, 1967; Christiansen *et al.*, 1987).

In experimental animals, bradykinin causes airway narrowing *in vivo* (Collier *et al.*, 1960; Ichinose *et al.*, 1990; Lötval *et al.*, 1991), exudation of plasma from the airway microcirculation (Saria *et al.*, 1983; Erjefält & Persson, 1989; Lötval *et al.*, 1991) and airway smooth muscle contraction *in vitro* (Bhoola *et al.*, 1962; Dusser *et al.*, 1988).

The mechanisms of bradykinin-induced effects in the airways have been the subject of many studies. It has been suggested that cholinergic nerves (Ichinose *et al.*, 1990), sensory neuropeptides (Kaufman *et al.*, 1980; Saria *et al.*, 1988; Ichinose *et al.*, 1990), metabolites of arachidonic acid (Collier *et al.*, 1960; Ichinose *et al.*, 1990) and platelet activating factor (Rogers *et al.*, 1990) are involved in these responses.

Moreover, peptide-degrading enzymes, such as neutral endopeptidase 24.11 and angiotensin-converting enzyme, modulate the responses to bradykinin (Dusser *et al.*, 1988; Lötval *et al.*, 1991). The relationships between the different mediators which are involved in bradykinin responses and the exact metabolites of arachidonic acid that are involved, have not yet been elucidated. Furthermore, the results of different studies are sometimes contradictory. For example, Collier *et al.* (1960) found that neither anticholinergic drugs nor section of the cervical vagi had any effect on the bronchoconstrictor response to bradykinin, whereas Ichinose *et al.* (1990) found that atropine had a significant inhibitory effect.

In this study we wanted to investigate the role of cholinergic nerves and of metabolites of arachidonic acid, as well as their relationship in the mechanism of bradykinin-induced effects in the airways of guinea-pigs given bradykinin directly into the tracheal lumen. To evaluate the role of cholinergic nerves, we used atropine or hexamethonium pretreatment, or cervical vagotomy before bradykinin instillation. To elucidate the importance of arachidonic acid metabolites in bradykinin-induced airway responses, a thromboxane A₂ receptor antagonist (ICI 192,605) or a leukotriene C₄/D₄/E₄ receptor antagonist (ICI 198,615) was given before bradykinin instillation. Finally, we studied the relationship between cholinergic nerves and thromboxane A₂ by administering U-46619, a stable thromboxane A₂-mimetic, to animals pretreated with

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atropine. In all these experiments, we monitored airway insufflation pressure as a measurement of airway narrowing and quantified the amount of Evans blue dye in the airways as a measurement of plasma exudation.

Methods

Preparation

This study was approved by the Animal Ethics Committee in Göteborg. We used male Dunkin-Hartley guinea-pigs weighing 350–550 g for the study involving bradykinin and 450–700 g for the study involving U-46619 (total $n = 118$). The animals were anaesthetized with ketamine (100 mg kg⁻¹, i.p.) and xylazine (10–12 mg kg⁻¹, i.m.). Additional doses were given as required to maintain adequate anaesthesia. The animals were placed on a heated blanket (Harvard model 507061, Harvard Apparatus Ltd., Edenbridge, Kent), which maintained body temperature at approximately 37°C. The left carotid artery was cannulated and the catheter was connected to a pressure transducer (Spectramed, Viggo, Helsingborg, Sweden). Another polyethylene catheter was inserted into the right external jugular vein for the i.v. administration of drugs and fluids. A tracheal cannula (10 mm length and 2.7 mm internal diameter) was inserted into the upper cervical trachea through a tracheostomy, secured with a suture and connected to a constant volume mechanical ventilator (Harvard model 50-1718, Harvard Apparatus Ltd., Edenbridge, Kent). Animals were placed in a supine position at an angle of 20°, with the head at a higher level to allow the instilled material to reach the lower airway levels. A tidal volume of 10 ml kg⁻¹ and a frequency of 60 breaths per min was used. The ventilatory circuit had a total volume of 18 ml. Insufflation pressure (Pi) was measured with a differential pressure transducer (Model FCO40; $\pm 1,000$ mmH₂O, Furness Controls Ltd., Bexhill, Sussex) which was attached to a catheter connected to a side port of the intratracheal cannula. The signals from the blood pressure transducer were amplified with an analogue preamplifier (Kungsbacka mät & registerteknik AB, Sweden). All the signals were digitised using a 12-bit analog-digital board (National Instruments, Austin, TX, U.S.A.) connected to a Macintosh II computer (Apple Computer Inc., Cupertino, CA, U.S.A.) and monitored with software (LabView, National Instruments, Austin, TX, U.S.A.).

Protocol

After preparation, all the animals were treated with suxamethonium (5 mg, i.v.) to avoid artefacts of spontaneous breathing and with propranolol (1 mg kg⁻¹, i.v.) to inhibit the sympathomimetic effects of bradykinin administration (Piper *et al.*, 1967). The animals were divided into twelve groups, to study the effect of atropine, hexamethonium, cervical vagotomy, ICI 192,605 or ICI 198,615 on bradykinin-induced effects in the airways. Each group had a separate control group, which was given the vehicle for each drug or which was not vagotomized before the airway instillation of bradykinin (150 nmol). The eleventh group was a control which was not treated and which was given a tracheal instillation of the vehicle for bradykinin (0.9% saline). The twelfth group was a control pretreated with the vehicle for ICI 198,615 before the instillation of the vehicle for bradykinin (0.9% saline) into the tracheal lumen. Ten minutes after the administration of suxamethonium and propranolol, the animals were pretreated with the relevant drugs or vehicles: atropine (1 mg kg⁻¹, i.v.); hexamethonium (2 mg kg⁻¹, i.v.); ICI 192,605 (10⁻⁶ mol kg⁻¹, i.v.); ICI 198,615 (10⁻⁶ mol kg⁻¹, i.v.), or the vehicle for each drug; 0.9% saline, NaHCO₃ in 0.9% saline, 0.9% saline and [1 M NaOH, PEG 400 and 10 nM NaH₂PO₄] in 0.9% saline respectively. In a separate group, both cervical vagi were sectioned by surgical proce-

dures. The control group for vagotomy was dissected around the vagi, but the nerves were not cut. Eight minutes later, Evans blue dye (20 mg kg⁻¹) was administered i.v. for 1 min. Two minutes later, the lungs were hyperinflated with twice the tidal volume, by manually blocking the outflow of the ventilator, and bradykinin or saline was instilled into the airways. Instillation into the tracheal lumen was performed by flushing 50 µl of 3 mM bradykinin with 1 ml of air through a needle, directly into the tracheal lumen via the tracheal cannula, with the point of the needle inside the lower part of the tracheal cannula. The distribution of instilled liquid into the airway tree has been studied by use of pen ink and Evans blue dye. We found that most of the ink/dye was macroscopically located at the level of the trachea and main bronchi immediately after intratracheal instillation, but we also found staining around the intrapulmonary airways, suggesting that the dye had also reached this airway level (unpublished observation).

Airway insufflation pressure (Pi) and mean systemic blood pressure were monitored every 15 s over 1 min and subsequently every 30 s for another 5 min after bradykinin instillation. The animals were disconnected from the ventilator and the thoracic cavity was opened. A catheter was inserted into the aorta through the left ventricle and the systemic circulation was perfused with 50 ml of 0.9% NaCl to remove the Evans blue dye from the bronchial circulation. Another catheter was inserted via the right ventricle into the pulmonary artery and the pulmonary circulation was perfused with 20 ml of 0.9% NaCl.

Another four groups of animals were used to evaluate the role of cholinergic nerves in airway responses induced by U-46619, a thromboxane mimetic. These groups were anaesthetized, tracheostomized and given propranolol, suxamethonium and Evans blue dye in the same way as all the other animals. U-46619 was administered intravenously (20 nmol kg⁻¹) or intratracheally (10 nmol in 50 µl of 0.9% NaCl) after the animals were pretreated with atropine (1 mg kg⁻¹, i.v.) or saline (0.9%). Airway insufflation pressure (Pi) and mean systemic blood pressure were monitored and the amount of Evans blue dye in the airway tissue was quantified.

Measurement of plasma exudation

After perfusion, the trachea and lungs were dissected out *en bloc*, the parenchyma was carefully scraped off and the extraneous tissue was removed. The trachea, main bronchi and intrapulmonary airways were then separated from one another and the intrapulmonary airways were divided into two portions of approximately equal length, arbitrarily called proximal and distal. We did not attempt to remove any plasma from the intraluminal portion of the airways. All the tissues were dried in a freeze-drier for 24 h (MicroModulyo, Edwards High Vacuum International, West Sussex), weighed when dry and Evans blue dye was extracted by keeping the tissues in 2 ml of formamide in a 40°C water bath for another 16 h. Absorption at 620 nm was measured in a spectrophotometer (Beckman DB, Ingeniörsfirman Hugo Tillquist, Stockholm, Sweden). Extravasated Evans blue dye was quantified by interpolation on a standard curve of dye concentrations in the range of 0–15 µg ml⁻¹ and expressed as ng dye mg⁻¹ dry tissue. The Evans blue dye measurement has previously been shown to correlate with the extravasation of radiolabelled albumin into guinea-pig airways (Rogers *et al.*, 1989).

Drugs

The following drugs were used: ketamine (Parke-Davis, S.A. Barcelona, Spain), xylazine (Bayer Leverkusen, Bayer Sverige AB, Mölndal, Sweden), Evans blue dye (Aldrich Chemical Co., Inc., Milwaukee, U.S.A.), propranolol (ICI Pharmaceuticals, Macclesfield, Cheshire), suxamethonium (Kabi-

Vitrum AB, Stockholm, Sweden), atropine, hexamethonium and bradykinin (Sigma Chemical Co. Ltd., Poole, Dorset).

U-46619 (9 α , 11 α -dideoxymethanoeperoxy-prostaglandin F_{2a}) (Cayman Chemical Co Inc., Ann Arbor, MI, U.S.A.), ICI 192,605 (4(z)-6-(2-o-chlorophenyl-4-o-hydroxyphenyl-1,3-dioxan-*cis*-5-yl)hexenoic acid), a thromboxane A₂ receptor antagonist (Jessup *et al.*, 1988), and ICI 198,615 ([1-[[2-methoxy-4[[[phenylsulphonyl]amin]carbonyl]phenyl]methyl]-1H-indazol-6-yl]carbamic acid cyclopentylester) a leukotriene C₄/D₄/E₄ receptor antagonist (Shirley & Cheng, 1991), were kindly donated by ICI Pharmaceuticals.

Data analysis

Data are reported as the mean $\pm$ s.e.mean. The peak Pi, immediate area under the curve (i-AUC = from 0 min to 1.5 min), more sustained AUC (s-AUC = from 1.5 min to 6 min), total AUC (t-AUC = from 0 min to 6 min) were calculated to evaluate the differences in airway narrowing. The amounts of Evans blue dye at the four airway levels were compared to determine any differences in plasma exudation. The mean baseline blood pressure and the lowest mean blood pressure after bradykinin instillation were calculated to estimate the effect of bradykinin on the systemic circulation. Non-parametric analysis (Mann-Whitney U-test) was used to determine significance of differences between groups. A value of $P < 0.05$ was considered significant. Data were analysed with a Macintosh computer using StatView II.

Results

Role of cholinergic nerves in bradykinin-induced airway effects

Airway insufflation pressure There was no significant difference in baseline pulmonary insufflation pressure (Pi) between atropinized or untreated animals (Figure 1a). The instillation of 50 μ l of 0.9% saline into the tracheal lumen induced a small increase in Pi (from 8.9 ± 0.6 cmH₂O to 16.5 ± 1.2 cmH₂O). Bradykinin instilled into the trachea (50 μ l of 3 mM; 150 nmol) produced a biphasic response in Pi. The immediate sharp peak reached a maximum between 30–45 s after instillation (Figure 1a; peak Pi 35 ± 4 cmH₂O). A more slowly progressing sustained response was observed between 3.5 and 4 min (Figure 1a; Pi 28 ± 3 cmH₂O). Atropine pretreatment significantly inhibited both the immediate and the more sustained response to bradykinin ($P < 0.05$; Figure 1a and Table 1). Peak Pi and the area under the curves were not significantly different in the group pretreated with atropine before bradykinin instillation compared with sham stimulation (50 μ l of 0.9% saline; Table 1).

In animals pretreated with hexamethonium, the baseline Pi was not significantly affected (10.4 ± 0.2 mmHg) compared with untreated animals (11.2 ± 0.3 mmHg). The bradykinin-induced increase in Pi (peak Pi: 37 ± 2.7 mmHg) was not significantly affected by hexamethonium (peak Pi: 47 ± 5.8 mmHg, $P = 0.2$; Figure 2a and Table 1).

There was no significant difference in baseline Pi between vagotomized animals compared with control animals (8 ± 0.3 and 8 ± 0.4 cmH₂O respectively). Cervical vagotomy did not significantly affect the changes in Pi induced by bradykinin instillation (Figure 3a and Table 1; Peak Pi 41 ± 5 and 35 ± 4 cmH₂O in vagotomized and control animals respectively).

Evans blue dye Bradykinin (150 nmol) instilled into the lumen of the trachea produced a significant extravasation of Evans blue dye into the trachea, main bronchi and intrapulmonary airways, as compared with instilled 0.9% saline ($P < 0.005$). Atropine (Figure 1b) or hexamethonium (Figure 2b) did not influence this effect. In vagotomized animals, however, we found a slight but significant enhancement of

Figure 1 (a) Time course of the increase in airway insufflation pressure (Pi) induced by a tracheal instillation of 0.9% saline (sham, $\square$, $n = 8$), bradykinin in untreated animals ($\circ$, $n = 13$) and bradykinin after pretreatment with atropine ($\bullet$, $n = 11$). There was a significant difference in the response to bradykinin between the untreated and atropinized group ($P < 0.05$). (b) The amount of Evans blue dye at different airway levels after an intratracheal instillation of 0.9% saline (sham, $n = 8$), bradykinin (BK) in untreated animals ($n = 13$), and bradykinin after pretreatment with atropine ($n = 11$). IPA = intrapulmonary airways. Data are shown as mean $\pm$ s.e.mean, * $P < 0.05$; NS $P > 0.05$.

the extravasation of Evans blue dye in the proximal intrapulmonary airways (Figure 3b; $P < 0.05$), but no significant effect was found at any other airway level.

Mean blood pressure There was no significant difference in baseline mean systemic blood pressure between the group pretreated with atropine and its control group (37 ± 3 and 37 ± 2 mmHg respectively) or with hexamethonium (36 ± 2 mmHg in the group pretreated with hexamethonium and 38 ± 2 mmHg in controls), whereas the vagotomized group had a higher mean blood pressure compared with its control group (52 ± 3 mmHg in the vagotomized group and 39 ± 2 mmHg in controls, $P < 0.05$). Instilled bradykinin produced a significant fall in systemic blood pressure, reaching the lowest values approximately 2 min after bradykinin (lowest mean blood pressure 20 ± 2 mmHg after bradykinin instillation compared with 29 ± 3 mmHg after the instillation of

Table 1 The role of cholinergic nerves in bradykinin (BK)-induced airway effects

	Sham	BK	Atropine + BK	BK	Hexamethonium + BK	BK	Vagotomy + BK
i-AUC	21 ± 2	37 ± 3*	26 ± 3†	46 ± 2*	53 ± 5*	35 ± 4*	53 ± 5*
s-AUC	60 ± 5	112 ± 12*	75 ± 8†	128 ± 12*	175 ± 27*	111 ± 17*	119 ± 10*
t-AUC	82 ± 6	148 ± 15*	100 ± 10†	174 ± 14*	228 ± 31*	147 ± 21*	160 ± 13*

The effect of atropine, vagotomy and hexamethonium on i-AUC (immediate area under the curve = 0–1.5 min), s-AUC (sustained area under the curve = 1.5–6 min) and t-AUC (total area under the curve = 0–6 min) in guinea-pigs given bradykinin into the tracheal lumen.

* $P < 0.05$ compared with sham exposed animals.

† $P < 0.05$ compared with untreated animals exposed to bradykinin.

Values are shown as mean ± s.e.mean.

Figure 2 (a) Time course of increase in airway insufflation pressure (Pi) induced by a tracheal instillation of 0.9% saline (sham, □, $n = 8$), bradykinin in untreated animals (○, $n = 6$) and bradykinin after pretreatment with hexamethonium (●, $n = 6$). (b) The amount of Evans blue dye at different airway levels after an intratracheal instillation of 0.9% saline (sham, $n = 8$), or after an intratracheal instillation of bradykinin (BK) in untreated animals ($n = 6$) and in animals pretreated with hexamethonium (C6, $n = 6$). IPA = intrapulmonary airways. Data are shown as mean ± s.e.mean, * $P < 0.05$; NS $P > 0.05$.

Figure 3 (a) Time course of the increase in airway insufflation pressure (Pi) induced by a tracheal instillation of 0.9% saline (sham; □, $n = 8$), bradykinin in untreated animals (○, $n = 11$) and bradykinin after cervical vagotomy (●, $n = 9$). (b) The amount of Evans blue dye at different airway levels after an intratracheal instillation of 0.9% saline (sham, $n = 8$), bradykinin (BK) in untreated animals ($n = 11$) and bradykinin after cervical vagotomy ($n = 9$). IPA = intrapulmonary airways. Data are shown as mean ± s.e.mean, * $P < 0.05$; NS $P > 0.05$.

0.9% saline; $P < 0.05$). Atropine or hexamethonium pretreatment, or vagotomy, had no significant effect on the lowest mean blood pressure measured after bradykinin instillation (20 ± 2 mmHg in the group pretreated with atropine compared with 20 ± 2 mmHg in its control group, 22 ± 2 mmHg in the group pretreated with hexamethonium compared with 19 ± 2 mmHg in its control group and 21 ± 1 mmHg in the vagotomized group compared with 20 ± 2 mmHg in its control group).

Role of arachidonic acid metabolites in bradykinin-induced airway effects

Thromboxane A_2 The thromboxane A_2 receptor antagonist, ICI 192,605 (10^{-6} mol kg $^{-1}$, i.v.), did not influence baseline Pi compared with the vehicle (Figure 4a). Pretreatment with

ICI 192,605 significantly suppressed both the immediate and the more sustained response in Pi to bradykinin (Figure 4a; $P < 0.005$). Peak Pi, i-AUC, s-AUC and t-AUC were significantly higher in the group pretreated with ICI 192,605 before bradykinin instillation compared with sham stimulation ($P < 0.05$; Table 2).

The bradykinin-induced extravasation of Evans blue dye was significantly attenuated in both the proximal and distal intrapulmonary airways after pretreatment with ICI 192,605, but it was not significantly affected in the trachea and main bronchi (Figure 4b; $P < 0.05$).

Animals pretreated with ICI 192,605 had a significantly lower mean baseline blood pressure (27 ± 2 mmHg) than the group pretreated with the vehicle and given bradykinin (46 ± 4 mmHg, $P < 0.05$). However, the lowest mean blood pressure measured after bradykinin instillation was not significantly different in animals pretreated with ICI 192,605 compared with their controls (21 ± 2 and 21 ± 1 mmHg respectively).

Leukotrienes The leukotriene $C_4/D_4/E_4$ receptor antagonist, ICI 198,615 (10^{-6} mol kg $^{-1}$, i.v.), did not affect baseline Pi compared with the vehicle (Figure 5a). Neither the bradykinin-induced increase in Pi (Table 2) nor the effect on Evans blue dye extravasation were significantly affected by ICI 198,615 (Figure 5b). ICI 198,615 had no significant effect on baseline mean blood pressure (32 ± 3 and 39 ± 5 mmHg in animals pretreated with ICI 198,615 and vehicle respectively) or lowest mean blood pressure after bradykinin instillation (15 ± 1 and 19 ± 2 mmHg respectively).

Thromboxane-mimetic, U-46619

Intravenous U-46619 There was no difference in baseline Pi between atropinized and untreated animals (10 ± 1 and 10 ± 1 cmH $_2$ O respectively). The administration of U-46619 (20 nmol kg $^{-1}$, i.v.) caused an increase in airway insufflation pressure, which was not significantly influenced by atropine pretreatment (Table 3). Furthermore, the time course of the Pi response to U-46619, measured as the areas under the curves, was not significantly different in the group pretreated with atropine before U-46619 administration compared with the animals pretreated with the vehicle (Table 3). The intravenous administration of U-46619 produced an extravasation of Evans blue dye in the trachea, main bronchi and both the proximal and distal intrapulmonary airways. Atropine appeared to have a small potentiating effect on the U-46619-induced plasma exudation in the intrapulmonary airways (Table 4).

There was no difference in baseline mean systemic blood pressure between animals pretreated with atropine and the control group (42 ± 4 and 44 ± 5 mmHg, respectively). Intravenous U-46619 caused an increase in mean blood pressure, which reached its maximum level after approximately 60 s, but atropine did not influence this effect.

U-46619 administered into the trachea U-46619 (10 nmol in 50μ l of 0.9% NaCl) administered directly into the tracheal lumen produced an immediate increase in Pi, which reached a peak within 1 min (Table 3). When the thromboxane-mimetic was administered via this route, there was a slight but significant attenuation of the peak Pi, but the immediate, sustained or total areas under the curve were not significantly affected (Table 3). The tracheal administration of U-46619 produced an extravasation of Evans blue dye in the trachea, main bronchi and both the proximal and distal intrapulmonary airways (Table 4). Atropine appeared to have a slight attenuating effect on the U-46619-induced plasma exudation in the distal intrapulmonary airways.

The administration of U-46619 into the trachea caused an increase in mean systemic blood pressure, which reached its maximum level at approximately 60 s. Atropine did not influence this effect of U-46619 (data not shown).

Figure 4 (a) Time course of the increase in airway insufflation pressure (Pi) induced by a tracheal instillation of 0.9% saline (sham, □, $n = 8$), bradykinin in untreated animals (150 nmol, ○, $n = 6$) and bradykinin after pretreatment with ICI 192,605 (10^{-6} mol kg $^{-1}$, i.v., ●, $n = 7$), ($P < 0.005$). (b) The amount of Evans blue dye at different airway levels after an intratracheal instillation of 0.9% saline (sham, $n = 8$), bradykinin (BK) and bradykinin after pretreatment with ICI 192,605. IPA = intrapulmonary airways. Data are shown as mean $\pm$ s.e.mean, * $P < 0.05$; NS $P > 0.05$.

Table 2 The role of eicosanoids in bradykinin (BK)-induced airway effects

	Sham	BK	TxA ₂ antagonist + BK	Vehicle + sham	BK	LT antagonist + BK
i-AUC	21 ± 2	53 ± 4*	28 ± 1††	20 ± 2	42 ± 2*	44 ± 5*
s-AUC	60 ± 5	187 ± 19*	74 ± 4††	46 ± 4	143 ± 7*	145 ± 20*
t-AUC	82 ± 6	241 ± 22*	103 ± 4††	66 ± 7	185 ± 8*	189 ± 24*

The effects of ICI 192,605, a TxA₂ receptor antagonist and ICI 198,615, a LT receptor antagonist on i-AUC (immediate area under the curve = 0–1.5 min), s-AUC (sustained area under the curve = 1.5–6 min) and t-AUC (total area under the curve = 0–6 min) in guinea-pigs given bradykinin into the tracheal lumen.

**P* < 0.05 compared with sham exposed animals.

††*P* < 0.05 compared with untreated animals exposed to bradykinin.

Values are shown as mean ± s.e.mean.

Figure 5 (a) Time course of the increase in airway insufflation pressure (Pi) induced by a tracheal instillation of 0.9% saline in animals pretreated with the vehicle for ICI 198,615 (sham, □; *n* = 5), bradykinin instillation in untreated animals (○, *n* = 6) and bradykinin instillation after pretreatment with ICI 198,615 (●, *n* = 6). (b) The amount of Evans blue dye at different airway levels after an intratracheal instillation of 0.9% saline in animals pretreated with the vehicle for ICI 198,615 (sham, *n* = 5), bradykinin (BK) in untreated animals and bradykinin after pretreatment with ICI 198,615. IPA = intrapulmonary airways. Data are shown as mean ± s.e.mean, *P* < 0.05; NS *P* > 0.05.

Discussion

In the present study, we have investigated the role of cholinergic nerves and arachidonic acid metabolites in bradykinin-induced airflow obstruction and airway plasma exudation in the guinea-pig. We found that atropine, but not hexamethonium or cervical vagotomy, attenuated the airflow obstruction induced by bradykinin, but not the induced plasma exudation. By contrast, in animals pretreated with the thromboxane A₂ receptor antagonist, both bradykinin-induced airflow obstruction and plasma exudation in the intrapulmonary airways were attenuated, suggesting that thromboxane A₂ plays an important role in bradykinin-induced airway responses. The release of leukotrienes did not appear to be involved, because a leukotriene C₄/D₄/E₄ receptor antagonist produced no effect.

Cholinergic nerves

Our finding that atropine, but not cervical vagotomy, attenuated bradykinin-induced airflow obstruction suggests that bradykinin activates peripheral cholinergic fibres, which cause the release of acetylcholine, without any activation of central reflexes. It is likely that anaesthetized guinea-pigs have an intact central nervous cholinergic innervation to the airways, because Hay *et al.* (1990) showed that cholinergic airflow obstruction can be induced in this species by electrical stimulation of specific areas in the dorsal medulla. Ichinose *et al.* (1990) used different anaesthetics from the present study (urethane versus ketamine and xylazine) and still demonstrated that atropine had an inhibitory effect on bradykinin-induced airflow obstruction. Furthermore, hexamethonium did not influence the responses, implying that activation of cholinergic nerves via cholinergic ganglia was unimportant in the responses to bradykinin. Our data thus suggest that, in this model, the bradykinin-induced activation of cholinergic nerves is located at the level of postganglionic fibres within the airway wall, and excludes central cholinergic reflexes.

The amount of Evans blue dye exudation in airway tissue after bradykinin was not inhibited by atropine, hexamethonium or cervical vagotomy, thus implying that plasma exudation is independent of the release of acetylcholine. This is not surprising, since the cholinergic agent, methacholine, does not produce airway microvascular leakage in guinea-pigs (Lötvall *et al.*, 1990). By contrast, there was a trend towards a potentiation of bradykinin-induced Evans blue dye extravasation in the peripheral airways after atropine, cervical vagotomy and hexamethonium. These findings are not completely explained by the present study. Possibly a relative increase in the tone of sympathetic nerves after inhibition of cholinergic nerves may influence the bronchial blood flow, and subsequently the plasma exudation induced by bradykinin.

Arachidonic acid metabolites

To evaluate the role of metabolites of arachidonic acid, we pretreated animals with either a TxA₂ receptor antagonist

Table 3 The effect of atropine on U46619-induced airflow obstruction

	Intravenous		Intratracheal	
	U-46619 (n = 6)	Atropine + U-46619 (n = 6)	U-46619 (n = 6)	Atropine + U-46619 (n = 6)
Peak Pi	58 ± 2	60 ± 1	66 ± 3	55 ± 2*
i-AUC	68 ± 3	70 ± 2	66 ± 5	59 ± 2
s-AUC	179 ± 35	193 ± 9	241 ± 21	217 ± 9
t-AUC	280 ± 30	263 ± 10	307 ± 26	276 ± 11

The effect of atropine on peak Pi, i-AUC (immediate area under the curve = 0–1.5 min), s-AUC (sustained area under the curve = 1.5–6 min) and t-AUC (total area under the curve = 0–6 min) in guinea-pigs given U-46619, intravenously or into the tracheal lumen.

Values are shown as mean ± s.e.mean, **P* < 0.05.

Table 4 The effect of atropine on U46619-induced airway plasma exudation

	Intravenous		Intratracheal	
	U-46619 (n = 6)	Atropine + U-46619 (n = 6)	U-46619 (n = 6)	Atropine + U-46619 (n = 6)
Trachea	244 ± 41	255 ± 37	158 ± 34	129 ± 15
Main bronchi	350 ± 38	352 ± 47	279 ± 60	223 ± 60
Proximal IPA	391 ± 40	501 ± 66*	452 ± 50	389 ± 53
Distal IPA	390 ± 21	529 ± 61*	452 ± 50	309 ± 30*

The effect of atropine on exudation of Evans blue dye into airway tissue (ng mg⁻¹ dry tissue) in guinea-pigs given U-46619 intravenously or into the tracheal lumen.

IPA = intrapulmonary airways.

Values are shown as mean ± s.e.mean, **P* < 0.05.

(ICI 192,605) or a LT C₄/D₄/E₄ receptor antagonist (ICI 198,615) at doses that are known strongly to inhibit responses to the thromboxane-mimetic (Jessup *et al.*, 1988; Lötvall *et al.*, 1992) or leukotrienes (Shirley & Cheng, 1991). We found that the TxA₂ receptor antagonist incompletely suppressed the increase in airway insufflation pressure caused by bradykinin instilled into the tracheal lumen. The TxA₂ receptor antagonist significantly decreased, but did not abolish, the extravasation of Evans blue dye in the intrapulmonary airways, thereby suggesting that TxA₂ plays a partial role in bradykinin-induced airway microvascular leakage at this airway level. These two findings imply that TxA₂ is one of the important mediators of bradykinin-induced airway responses in the guinea-pig, but that other mechanisms are also involved. The involvement of prostanoids in bradykinin-induced plasma exudation has recently been implied by Rogers *et al.* (1990), who found that indomethacin had an inhibitory effect in intrapulmonary airways after i.v. bradykinin. This finding is further supported by another study in our laboratory, where we found that the thromboxane synthase inhibitor, OKY-046, significantly suppressed airway narrowing and plasma exudation in the intrapulmonary airways after bradykinin (Arakawa *et al.*, 1992). In addition, a TxA₂-mimetic (U-46619) alone produces airway microvascular leakage in guinea-pigs when administered by the i.v. route (Lötvall *et al.*, 1992) or via the airway route as shown in the present study.

Ichinose and co-workers (1990) found no effect of indomethacin on airflow obstruction induced by bradykinin administered via tracheal instillation. This discrepancy could be explained by small differences in the experimental protocol. Both we and Rogers *et al.* (1990) gave each animal a single dose of bradykinin, whereas Ichinose *et al.* (1990) gave increasing doses of bradykinin.

The specific leukotriene receptor antagonist, ICI 198,615, did not significantly affect the observed bradykinin-induced airflow obstruction or airway microvascular leakage, in con-

trast to the obvious effects of the thromboxane A₂ receptor antagonist. This finding is in agreement with Rogers *et al.* (1990) who also found that the same leukotriene receptor antagonist failed to influence the extravasation of Evans blue dye in airway tissue caused by i.v. bradykinin. In our laboratory, we have previously found that ICI 198,615, used at the same dose as in the present study, attenuates the allergen-induced exudation of plasma in the airways of guinea-pigs, further urging that the drug is active (Lötvall *et al.*, 1993). In another study, we have observed a lack of effect by BW B70C, a 5-lipoxygenase inhibitor, (3 mg kg⁻¹, i.v.) on bradykinin-induced airflow obstruction and airway plasma exudation (Kawikova & Lötvall, unpublished observations), which further argues against any involvement of leukotrienes in bradykinin-induced airway responses.

Thromboxane versus cholinergic nerves

We have found that both cholinergic nerves and thromboxane A₂ are involved in the mechanism of bradykinin-induced airway responses. Chung *et al.* (1985) showed that a low dose of U-46619, a TxA₂-mimetic, enhanced the airway response to electrical field stimulation, but not to methacholine, thereby suggesting an increase in the prejunctional release of acetylcholine. We administered U-46619 to animals both i.v. and via the tracheal route and expected to observe an attenuation of airflow obstruction in animals pretreated with atropine. However, atropine did not have any significant effect on this response when U-46619 was given i.v., but we observed a small attenuating effect when it was administered via the trachea. The bradykinin-induced activation of peripheral cholinergic nerves may therefore, in part, be mediated via thromboxane A₂, although additional mechanisms are also likely to work in parallel. It is likely that the bradykinin-induced activation of cholinergic fibres and the release of thromboxane is due primarily to the activation of different receptors for bradykinin (Farmer *et al.*, 1989; 1991). How-

ever, further studies are required to support this hypothesis.

In animals given i.v. U-46619, atropine appeared to have a small augmenting effect on Evans blue dye extravasation in the intrapulmonary airways, but not when the thromboxane-mimetic was given via the trachea. It is possible that some variation in parasympathetic activity may influence the bronchial vasculature and thus the bronchial blood flow in response to the thromboxane-mimetic. This response may also be different when U-46619 is administered by the different routes. To our knowledge, no studies have been conducted to evaluate the effect of local or systemic thromboxane on bronchial blood flow in guinea-pigs. It is unlikely that the local release of acetylcholine affects airway plasma exudation, because the inhalation of methacholine does not produce plasma exudation in the guinea-pig (Lötvall *et al.*, 1990).

We conclude that, in the guinea-pig, both cholinergic nerves and thromboxane A₂ are involved in bradykinin-induced airflow obstruction and that thromboxane A₂ is

involved in bradykinin-induced plasma exudation in the intrapulmonary bronchi. In man, the mechanisms of bradykinin-induced airflow obstruction are also controlled in part via the activation of cholinergic mechanisms (Simonsson *et al.*, 1973; Fuller *et al.*, 1987). In one small study, the inhibition of cyclo-oxygenase in asthmatics had no significant effect on bradykinin-induced airway responses, but a small but significant inhibitory effect was found in a larger study (Fuller *et al.*, 1987; Polosa *et al.*, 1990). However, the exact role of thromboxane A₂ in bradykinin-induced airway plasma exudation in man remains to be investigated.

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