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Evidence for reduction of bradykinin-induced bronchoconstriction in guinea-pigs by release of nitric oxide

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1 In this study the influence of nitric oxide (NO) on the bronchoconstriction induced by bradykinin in anaesthetized and artifically ventilated guinea-pigs pretreated with atropine was investigated.

2 Aerosol administration of bradykinin (0.1–1 mM, 40 breaths) caused a dose-dependent increase in lung resistance (R_L): maximum increase in R_L was 2.5 fold the baseline value. Pretreatment with aerosolized N^G-nitro-L-arginine methyl ester (L-NAME) or N^G-monomethyl-L-arginine (L-NMMA) (1 mM, 10 breaths every 5 min for 30 min), NO synthase inhibitors, markedly increased the bronchoconstrictor response to bradykinin. L-Arginine, but not D-arginine, (3 mM, 10 breaths every 5 min for 30 min) reversed the hyperresponsiveness to aerosolized bradykinin caused by L-NAME and L-NMMA.

3 L-NAME (1 mM, 10 breaths every 5 min for 30 min) increased the bronchoconstriction induced by intravenous bradykinin (1–10 nmol kg^{-1}). L-Arginine, but not D-arginine, (10 breaths every 5 min for 30 min) reversed the hyperresponsiveness to intravenous bradykinin caused by L-NAME.

4 The increase in R_L induced by capsaicin, either aerosol (10 μM, 10 breaths) or i.v. (20 nmol kg^{-1}) was not affected by L-NAME (1 mM, 10 breaths every 5 min for 30 min). Acute resection of the vagi did not affect the bronchoconstriction evoked by bradykinin in guinea-pigs, either in the absence or presence of L-NAME (1 mM, 10 breaths every 5 min for 30 min).

4 These results suggest that, irrespective of the route of administration, bradykinin releases NO or a related molecule which exerts a bronchodilator action that opposes the bronchoconstrictor mechanisms activated by bradykinin itself.

Keywords: Bradykinin; nitric oxide; capsaicin; guinea-pig airways; bronchoconstriction; epithelium; N^G-nitro-L-arginine methyl ester (L-NAME); N^G-monomethyl-L-arginine (L-NMMA); nitric oxide synthase inhibitors

Introduction

The nonapeptide, bradykinin and the decapeptide, kallidin are kinins released from plasma and tissue precursors during inflammation (Regoli & Barabe, 1980). Kinins exert a variety of inflammatory responses which in the airways include increases in plasma protein extravasation and bronchoconstriction. Bradykinin given by aerosol to anaesthetized guinea-pigs increases plasma extravasation mainly by releasing the tachykinins substance P (SP) and neurokinin A (NKA) from sensory nerve endings and also it appears, albeit in a smaller contribution, by a direct action on endothelial cells of post-capillary venules (Lundberg & Saria, 1983; Saria *et al.*, 1983; Bertrand *et al.*, 1993a). The atropine-insensitive component of the bronchoconstriction induced by local application of bradykinin into the guinea-pig airways is mediated by similar mechanisms: most of the response is due to tachykinin release and a minor component is mediated by a direct action on tracheobronchial smooth muscle cells (Ichinose *et al.*, 1990). Capsaicin, the hot component of the plants of the genus *Capsicum*, stimulates selectively a subpopulation of sensory nerves and causes the release of neuropeptides, including tachykinins (Holzer, 1991). In the airways of anaesthetized guinea-pigs, capsaicin increases plasma extravasation an causes an atropine-insensitive bronchoconstriction, effects that are entirely due to tachykinins released from sensory nerve endings (Ballati *et al.*, 1992; Bertrand *et al.*, 1993b; Saria *et al.*, 1983).

In the first part of this study we observed that aerosolized capsaicin is more potent than aerosolized bradykinin in causing bronchoconstriction in anaesthetized guinea-pigs pretreated with atropine and phosphoramidon. In contrast, we found that aerosolized bradykinin is almost equipotent to aerosolized capsaicin in causing plasma extravasation in the trachea of these animals. Furthermore, the dose of bradykinin that caused half maximal plasma extravasation was not sufficient to cause any bronchoconstriction. The mechanism by which bradykinin and capsaicin induce bronchoconstriction and plasma extravasation in the experimental conditions mentioned above is similar and mostly due to tachykinin release from sensory nerves (Ichinose *et al.*, 1990; Bertrand *et al.*, 1993b). Nevertheless, bradykinin, in contrast to capsaicin, is a less potent agent in causing bronchoconstriction than in increasing plasma extravasation. We hypothesizd that one possible explanation for the apparent discrepancy was that bradykinin, like capsaicin, induces the release of tachykinins which cause bronchoconstriction and increase plasma protein extravasation, but that bradykinin, in contrast to capsaicin, also releases a bronchodilator agent.

Bradykinin releases a variety of prostaglandins (Leikauf *et al.*, 1985). However, the observation that bronchoconstriction following bradykinin applied locally to the guinea-pig airways is not affected by indomethacin (Ichinose *et al.*, 1990) rules out the possibility that a bronchodilator prostaglandin released by bradykinin opposes markedly bronchoconstriction induced of bradykinin. Activation of bronchodilator β-adrenoceptors, probably due to catecholamines released from the adrenal medulla, occurs following intravenous administration by bradykinin (Piper *et al.*, 1967). However, this bronchodilator mechanism has not been reported after aerosolized bradykinin. More recently, it has been proposed that the L-arginine-nitric oxide (NO) pathway plays a role in the regulation of bronchomotor tone (Belvisi *et al.*, 1991; Li

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& Rand, 1991; Lei et al., 1993; Sekizawa et al., 1993). Therefore, we tested the hypothesis that bradykinin causes the release of NO or a related molecule from the airways of guinea-pigs and by this mechanism reduces its bronchoconstrictor action. The present data indicate that NO or a related molecule released by bradykinin markedly inhibits the bronchoconstrictor response to bradykinin. An attempt to determine the neural or non-neural source of NO released by bradykinin was also performed. The present data and recent studies (Nijkamp et al., 1993; Schempler & Calixto, 1994) suggest that bradykinin does not release NO from neural elements, but more probably from the epithelium.

Methods

Animals

Male Hartley guinea-pigs (Simonsen Laboratories, Inc., Gilroy, CA, U.S.A.) weighing 350–400 g at the time of housing, were used in this study. They were kept in a temperature-controlled environment with standard laboratory food and water freely available.

Measurement of total pulmonary resistance (R_L)

Animals were anaesthetized with sodium pentobarbitone (45 mg kg^{-1}, i.p.; Anthony Product Corp., Arcadia, CA, U.S.A.) and then ventilated artificially through a tracheal cannula, using a constant-volume ventilator (model 683; Harvard Apparatus Co., Inc) at a frequency of 80 breaths min^{-1}. The tidal volume was adjusted to maintain normal arterial blood gases as described previously (Dusser et al., 1988). Airflow was monitored coninuously with a pneumotachograph A. Fleisch, Medical Inc.) connected to a differential pressure transducer (model DP45; Validyne Engineering Corp.). A fluid-filled polyethylene catheter was introduced into the oesophagus to measure the oesophageal pressure as an approximation of pleural pressure. Intratracheal pressure was measured with a polyethylene catheter inserted into a short tube connecting the tracheal cannula to the pneumotachograph. The transpulmonary pressure (defined as the pressure difference between the intratracheal and the oesophageal pressures) was measured with a differential pressure transducer (model DP7; Valdyne Engineering Corp.). Output signals representing transpulmonary pressure and airflow were amplified with an amplifier (model CD19; Validyne Engineering Corp.) and recorded on a polygraph recorder (model 1508 B Visicorder; Honeywell, Inc.). R_L was calculated as previously described (Dusser et al., 1988). The right jugular vein and the left carotid artery were cannulated to permit administration of drugs and to withdraw a sample of blood for arterial blood gas measurement, respectively.

Measurement of plasma extravasation

Evans blue dye (3% solution in 0.9% saline; Polysciences, Inc., Warrington, PA, U.S.A.) was injected immediately before the use of the aerosol. Five minutes after injection of the tracer, the chest was opened, a cannula was inserted into the ascending aorta through the left ventricle, and the circulation was perfused for 2 min with phosphate buffer (pH 5; Sigma Chemical Co., St Louis MO, U.S.A.) at a pressure of 120 mmHg. The trachea was dissected and opened along the ventral midline, blotted, weighed, and then incubated in 3 ml of formamide (Fisher Scientific, Santa Clara, CA) at 50°C for 18 h to extract the extravasated Evans blue dye. The extravasation of Evans blue-labelled macromolecules from the microcirculation in different tissues was quantified by measuring the optical density of the formamide extracts at a wavelength of 620 nm with a spectrophotometer (Model UV160U, Shimatzu Scientific Instruments, Inc., Columbia, MD, U.S.A.). The amount of Evans blue dye extravasated in the tissues, expressed in nanograms per milligram of wet weight, was interpolated from a standard curve of Evans blue concentrations (0.1 to 5 μg ml^{-1}).

Measurement of arterial blood pressure

A polyethylene catheter (i.d. 0.8 mm, length 2.5 cm; Angiocath, Deseret Medical) was inserted into the left carotid artery and then connected to a pressure transducer (model 1270A, Hewlett-Packard) for measurement of arterial pressure. The amplified signal from the transducer (module M2102B, Electronics for Medicine) was displayed continuously on a video monitor (model OM, Electronics for Medicine) and recorded with an oscillographic recorder (model DASH-8, Astro-Med). Heart rate was derived from the pressure pulse signal by a cardiotachometer coupler.

Experimental design

Baseline R_L remained stable for at least 2 h, and no significant changes were produced by aerosol administration (5 breaths) or i.v. injection (1 ml kg^{-1}) of saline (0.9% NaCl) after a stabilization period of 30 min. Aerosols of saline or drugs were generated from an ultrasonic nebulizer (Pulmo-Sonic model 25, DeVilbiss Co., Somerset, PA, U.S.A.) and were delivered into the airways by the respirator via the tracheal cannula (aerosol delivery rate, 0.2 ml min^{-1}). To deliver NO synthase inhibitors, we used the protocol used previously (Nijkamp et al., 1993): guinea-pigs inhaled 10 breaths of an aerosol containing 0.9% saline (controls), N^G-nitro-L-arginine methyl ester (L-NAME, 1 mM) or N^G-monomethyl-L-arginine (L-NMMA, 1 mM). This procedure was repeated every 5 min for 30 min (total 60 breaths). Five minutes after the last inhalation, bradykinin or capsaicin were given. A single dose of bradykinin or capsaicin was given to each animal. Ten breaths of an aerosol containing L-arginine (L-Arg, 3 mM), D-arginine (D-Arg, 3 mM) or 0.9% saline were administered every 5 min for 30 min to L-NAME pretreated animals. All animals were pretreated with atropine (1.4 μmol kg^{-1}, i.v., 15 min before the stimulus). To increase the tachykinin-mediated bronchoconstriction, 5 min before the bradykinin aerosol, guinea-pigs received phosphoramidon (4.5 μmol kg^{-1}, i.v.). To block the effect of bronchodilator catecholamines released by i.v. bradykinin, guinea-pigs were also treated with propranolol (3.4 μmol kg^{-1}, i.v., 15 min before bradykinin i.v.). To maintain identical experimental conditions, propranolol was also given to guinea-pigs treated with capsaicin i.v. and phosphoramidon was also given to guinea-pigs treated with capsaicin aerosol. Vagotomy was performed by a standard procedure by exposing both cervical vagus nerves and sectioning them below the nodose ganglion 10 min before the delivery of the stimulus. Drugs given by i.v. route were diluted in saline (1 ml kg^{-1}).

Drugs

L-Arg, D-Arg, L-NAME, L-NMMA, capsaicin, propranolol, atropine and histamine were obtained from Sigma Chemical (St. Louis, MO, U.S.A.). Phosphoramidon and bradykinin were purchased from Peninsula Laboratories, Inc. (Belmont, CA, U.S.A.). All drugs were dissolved in 0.9% saline. Capsaicin was dissolved in a solution containing 10% ethanol, 5% Tween 80 and 85% 0.9% NaCl. Further dilutions were made in saline (0.9% NaCl).

Statistical analysis

Values in the text and figures are the mean ± standard error of the mean (s.e.mean). Statistical comparisons were performed by one way analysis of variance and Dunnett's test or Student's bilateral unpaired t tests, when appropriate. In all cases, a P value of less than 0.05 was considered significant.

Results

Plasma extravasation

In guinea-pigs pretreated with 0.9% saline (10 breaths every 5 min for 30 min), atropine (1.4 μmol kg^{-1}, i.v.) and phosphoramidon (4.5 μmol kg^{-1}, i.v.) baseline plasma extravasation in the trachea was 19.6 ± 2.3 ng mg^{-1} of tissue ($n = 4$). Bradykinin aerosol (40 breaths) and capsaicin aerosol (10 breaths) caused a dose-dependent increase in the extravasation of the Evans blue dye in the guinea-pig trachea (Figure 1b). The two drugs were equipotent in increasing plasma extravasation (Figure 1b).

Bradykinin aerosol

In guinea-pigs pretreated with 0.9% saline (10 breaths every 5 min for 30 min), atropine (1.4 μmol kg^{-1}, i.v.) and phosphoramidon (4.5 μmol kg^{-1}, i.v.), baseline R_L was 0.18 ± 0.012 mmH$_2$O ml^{-1} min^{-1} ($n = 6$). Aerosolization of 0.9% NaCl, L-NAME (1 mM) or L-NMMA (1 mM) (all, 10 breaths every 5 min for 30 min) did not change the baseline value of R_L (data not shown). Aerosolized bradykinin (0.1–1 mM, 40 breaths) induced a dose-dependent increase in R_L (Figures 1 and 2). The maximum increase obtained with 1 mM bradykinin was approximately 2.5 fold higher than the baseline value. After administration of L-NAME (1 mM, 10 breaths every 5 min for 30 min) the bronchoconstrictor response to each dose of bradykinin was markedly increased (Figure 2). The administration of L-Arg (3 mM, 10 breaths every 5 min for 30 min), but not the administration of D-Arg (3 mM, 10 breaths every 5 min for 30 min), after the aerosolization of L-NAME, reversed the potentiating effect of L-NAME on bradykinin-induced bronchospasm (Figure 2). Pretreatment with L-NMMA (1 mM, 10 breaths every 5 min for 30 min) increased the bronchoconstriction induced by aerosolized bradykinin (1 mM, 40 breaths) (Figure 3). Pretreatment with L-Arg (3 mM, 10 breaths every 5 min for 30 min), but not the administration of D-Arg (3 mM, 10 breaths every 5 min for 30 min), abolished the L-NMMA-induced increase in the bronchoconstriction evoked by bradykinin (Figure 3).

Intravenous bradykinin

In guinea-pigs pretreated with atropine (1.4 μmol kg^{-1}, i.v.) and propranolol (34 μmol kg^{-1}, i.v.) baseline R_L was 0.20 ± 0.024 mmH$_2$O ml^{-1} min^{-1} ($n = 6$). Bradykinin (1–10 nmol kg^{-1},

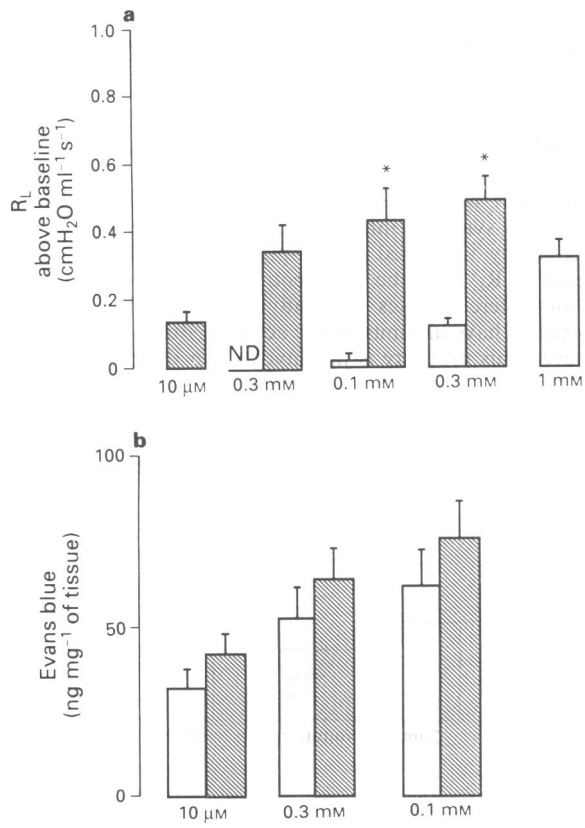

Figure 1 Increase in total pulmonary resistance (R_L) (a) and Evans blue dye extravasation (b) in the trachea induced by increasing doses of aerosolized bradykinin (open columns) or capsaicin (hatched columns) in anaesthetized and artifically ventilated guinea-pigs in the presence of atropine (1.4 μmol kg^{-1}, i.v.) and phosphoramidon (4.5 μmol kg^{-1}, i.v.). Each column is the mean ± s.e.mean of at least four experiments. ND, not detectable. *$P < 0.01$.

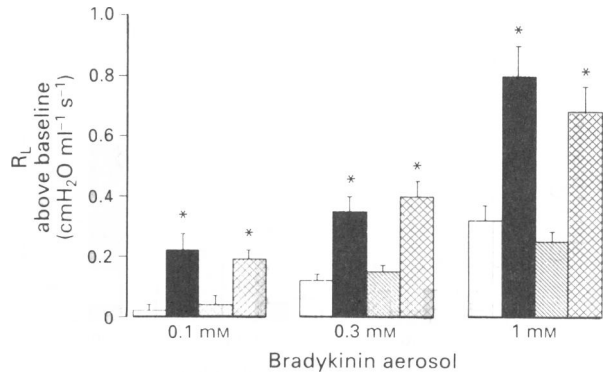

Figure 2 Effect of aerosolized 0.9% saline (open columns), N^G-nitro-L-arginine methyl ester (L-NAME, 1 mM) (solid columns), L-NAME (1 mM) plus L-arginine (L-Arg, 3 mM) (hatched columns), L-NAME (1 mM) plus D-arginine (D-Arg, 3 mM) (cross-hatched columns) on the increase in total pulmonary resistance (R_L), induced by different doses of aerosolized bradykinin in anaesthetized and artificially ventilated guinea-pigs in presence of atropine (1.4 μmol kg^{-1}, i.v.) and phosphoramidon (4.5 μmol kg^{-1}, i.v.). Saline and L-NAME were administered by giving 10 breaths every 5 min for 30 min. L-Arg and D-Arg (3 mM, 10 breaths every 5 min for 30 min) were given 5 min after the last administration of L-NAME. Each column is the mean ± s.e.mean of five experiments. *$P < 0.05$ versus saline group.

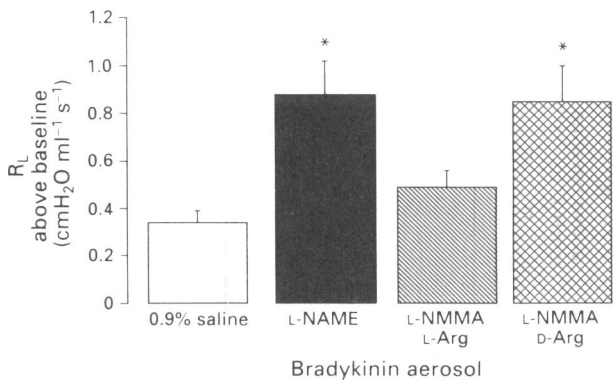

Figure 3 Effect of aerosolized 0.9% saline (open column), N^G-monomethyl-L-arginine (L-NMMA, 1 mM, solid column), L-NMMA (1 mM) plus L-arginine (L-Arg, 3 mM, hatched column), L-NMMA (1 mM) plus D-arginine (D-Arg, 3 mM, cross-hatched column) on the increase in total pulmonary resistance (R_L), induced by aerosolized bradykinin (1 mM, 40 breaths) in anaesthetized and artificially ventilated guinea-pigs in presence of atropine (1.4 μmol kg^{-1}, i.v.) and phosphoramidon (4.5 μmol kg^{-1}, i.v.). Saline and L-NAME were administered by giving 10 breaths every 5 min for 30 min. L-Arg and D-Arg (3 mM, 10 breaths every 5 min for 30 min) were given 5 min after the last administration of L-NAME. Each column is the mean ± s.e.mean of five experiments. *$P < 0.05$ versus saline group.

i.v.) induced a dose-dependent increase in R_L (Figure 4). The maximum increase obtained with 10 nmol kg^{-1} was approximately 2.5 fold higher than the baseline value. In guinea-pigs pretreated with L-NAME (1 mM, 10 breaths every 5 min for 30 min) bronchoconstrictor response to each dose of bradykinin were significantly increased. L-Arg (3 mM, 10 breaths every 5 min for 30 min), but not D-Arg (3 mM, 10 breaths every 5 min for 30 min), abolished the potentiation induced by L-NAME of the bronchoconstrictor response to i.v. bradykinin (Figure 4).

Capsaicin and vagotomy

In guinea-pigs pretreated with atropine (1.4 μmol kg^{-1}, i.v.) and phosphoramidon (4.5 μmol kg^{-1}, i.v.) baseline R_L was 0.19 ± 0.016 mmH_2O ml^{-1} min^{-1} ($n = 5$). Aerosolization of capsaicin (10 breaths) increased R_L in a dose-dependent manner (Figure 1a). Capsaicin caused a much higher bronchoconstrictionr than bradykinin at any concentration tested (Figure 1a). Pretreatment with L-NAME (1 mM, 10 breaths every 5 min for 30 min) did not affect the bronchoconstrictor response to capsaicin (10 μM, 10 breaths). In guinea-pigs pretreated with atropine (1.4 μmol kg^{-1}, i.v.) and propranolol (3.4 μmol kg^{-1}, i.v.) the bronchoconstriction induced by intravenous injection of 20 nmol of capsaicin was not affected by pretreatment with L-NAME (1 mM, 10 breaths every 5 min for 30 min) (Figure 5).

In guinea-pigs pretreated with atropine (1.4 μmol kg^{-1}, i.v.) and phosphoramidon (4.5 μmol kg^{-1}, i.v.) acute resection of both vagus nerves slightly, but not significantly, reduced the bronchoconstrictor response to bradykinin (1 mM, 40 breaths). In guinea-pigs pretreated with L-NAME (1 mM, 10 breaths every 5 min for 30 min) vagotomy had no effect on the potentiating effect of L-NAME on bradykinin-induced bronchoconstriction (Figure 6).

Cardiovascular measurements

Baseline heart beat frequency and arterial blood pressure were not changed after aerosolization of 0.9% saline (10 breaths every 5 min for 30 min). Aerosolized L-NAME or L-NMMA (1 mM, 10 breaths every 5 min for 30 min) did not affect baseline heart beat frequency and arterial blood pressure (Table 1). L-Arg and D-Arg (3 mM, 10 breaths every 5 min for 30 min) did not alter basal arterial pressure (Table 1). Bradykinin aerosol (1 mM) did not change blood pressure and heart rate (Table 1). Intravenous administration of bradykinin dose-dependently decreased blood pressure and heart beat frequency. The effect of bradykinin was short-lived and blood pressure and heart rate returned to baseline values within 60–120 s. Maximum changes induced by bradykinin 10 nmol kg^{-1} are reported in Table 1. Capsaicin aerosol did not affect cardiovascular parameters. Capsaicin i.v. caused a transient (20–40 s) decreases in blood pressure and a transient (20–40 s) increase in heart rate (Table 1).

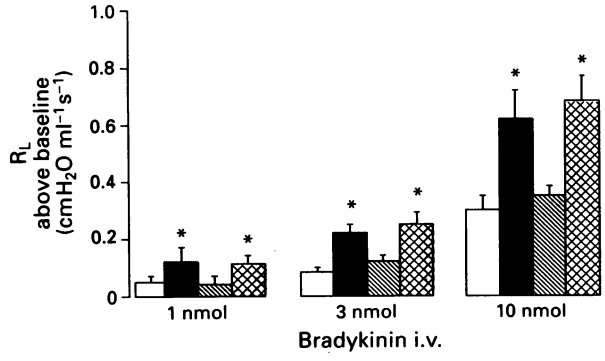

Figure 4 Effect of aerosolized 0.9% saline (open columns), N^G-nitro-L-arginine methyl ester (L-NAME, 1 mM, solid columns), L-NAME (1 mM) plus L-arginine (L-Arg, 3 mM, hatched columns) and L-NAME (1 mM) plus D-arginine (D-Arg, 3 mM, cross-hatched columns) on the increase in total pulmonary resistance (R_L), induced by intravenous administration of bradykinin at different doses in anaesthetized and artifically ventilated guinea-pigs in presence of atropine (1.4 μmol kg^{-1}, i.v.) and propranolol (3.4 mmol kg^{-1}, i.v.). Saline and L-NAME were administered by giving 10 breaths every 5 min for 30 min. L-Arg and D-Arg (3 mM, 10 breaths every 5 min for 30 min) were given 5 min after the last administration of L-NAME. Each column is the mean ± s.e.mean of five experiments. $*P < 0.05$ versus saline group.

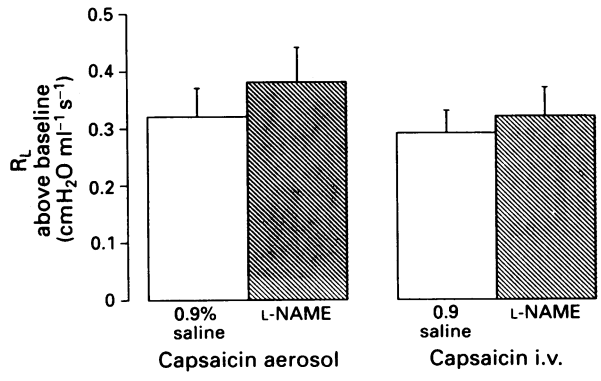

Figure 5 Effect of aerosolized 0.9% saline and N^G-nitro-L-arginine methyl ester (L-NAME, 1 mM, hatched column) on the increase in total pulmonary resistance (R_L), induced by aerosol (10 μM, 10 breaths) and intravenous (20 nmol) administration of capsaicin in anaesthetized and artifically ventilated guinea-pigs. Capsaicin aerosol was given to guinea-pigs pretreated with atropine (1.4 μmol kg^{-1}, i.v.) and phosphoramidon (4.5 mmol kg^{-1}, i.v.). Capsaicin i.v. was given to guinea-pigs pretreated with atropine (1.4 μmol kg^{-1}, i.v.) and propranolol (3.4 mml kg^{-1}, i.v.). Saline and L-NAME were administered by giving 10 breaths every 5 min for 30 min. Each column is the mean ± s.e.mean of five experiments. $*P < 0.05$ versus saline group.

Discussion

In this study we have demonstrated that aerosol administration of NO-synthase inhibitor, L-NAME (Rees *et al.*, 1990) to artificially ventilated giunea-pigs resulted in a significant increase in R_L after the intravenous and aerosol administration of different doses of bradykinin. L-NAME has been reported to have antimuscarinic activity (Buxton *et al.*, 1993). However, this property is not involved in the potentiation of

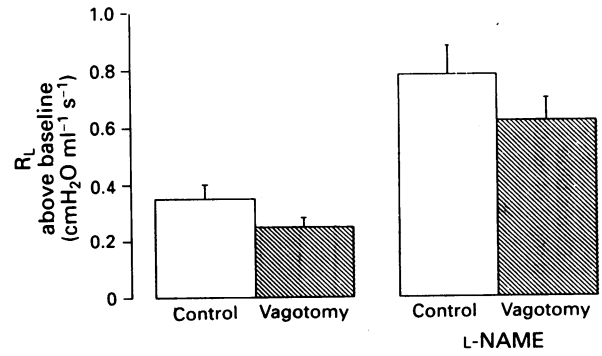

Figure 6 Effect of aerosolized 0.9% saline and N^G-nitro-L-arginine methyl ester (L-NAME, 1 mM) and vagotomy (hatched column) on the increase in total pulmonary resistance (R_L), induced by aerosolized bradykinin (1 mM, 40 breaths) in anaesthetized and artifically ventilated guinea-pigs pretreated with atropine (1.4 μmol kg^{-1}, i.v.) and phosphoramidon (4.5 μmol kg^{-1}, i.v.). Saline and L-NAME were administered by giving 10 breaths every 5 min for 30 min. Each column is the mean ± s.e.mean of five experiments. $*P < 0.05$ versus saline group.

Table 1 Mean arterial blood pressure and heart beat frequency before and after administration of the indicated drugs in one pentobarbitone anaesthetized guinea pigs

Treatment	*Blood pressure* (mmHg)	*Heart beat* (beats min^{-1})
Baseline	82 ± 5	283 ± 7
Bradykinin 1 mM (40 breaths, aerosol)	72 ± 6	288 ± 7
Capsaicin 10 μM (10 breaths, aerosol)	78 ± 4	286 ± 8
Baseline	85 ± 4	282 ± 8
Bradykinin 10 nmol, i.v.	40 ± 3*	210 ± 11*
Capsaicin 20 nmol, i.v.	54 ± 3*	320 ± 11*
Baseline	89 ± 5	280 ± 3
L-NAME	83 ± 5	273 ± 8
L-NMMA	85 ± 3	285 ± 6
L-NAME + L-Arg	85 ± 3	279 ± 4
L-NAME + D-Arg	83 ± 4	279 ± 5
L-NMMA + L-Arg	84 ± 5	283 ± 6
L-NMMA + D-Arg	85 ± 3	279 ± 5

Values are mean ± s.e.mean of at least five experiments. Guinea-pigs receiving i.v. drugs were pretreated with propranolol (3.4 μmol kg^{-1}) and atropine (1.4 μmol kg^{-1}); guinea-pigs receiving aerosol drugs were pretreated with phosphoramidon (4.5 μmol kg^{-1}) and atropine (1.4 μmol kg^{-1}). N^G-nitro-L-arginine methyl ester (L-NAME), N^G-monomethyl-L-arginine (L-NNMA), L-arginine (L-Arg) and D-Arg were administered by aerosol according to the protocol and doses reported in the text.
*$P < 0.05$ vs baseline.

the bradykinin-induced increase in R_L as the experiments were performed in the presence of atropine. Furthermore, L-NMMA, a NO-synthase inhibitor which does not have significant affinity for muscarinic receptors (Buxton *et al.*, 1993), also potentiated the bronchoconstrictor response to bradykinin. Finally, administration of the NO precursor, L-Arg, but not the inactive enantiomer D-Arg (Moncada & Higgs, 1993), given after the administration of L-NAME or L-NMMA reversed the increased airway responsiveness to bradykinin induced by the NO-synthase inhibitors. Therefore, these findings provide evidence that bradykinin administration releases NO or a related molecule which counteracts the bronchoconstrictor response to bradykinin.

Administration of bradykinin causes bronchoconstriction in guinea-pigs by multiple mechanisms which depend on the route of administration of the peptide (Ichinose *et al.*, 1990). Most of the response to aerosolized bradykinin is due to activation of neural pathways, e.g. cholinergic reflexes and axon reflexes and the subsequent release of tachykinins (Ichinose *et al.*, 1990). A minor component is apparently due to the direct action of bradykinin on smooth muscle cells (Ichinose *et al.*, 1990). After intravenous administration, bradykinin causes bronchoconstriction by activating cholinergic reflexes and by releasing prostanoids. Furthermore, bronchodilator catecholamines are released from the adrenal medulla following intravenous bradykinin (Piper *et al.*, 1967). The present results indicate that the bronchodilator agent, NO, is released by bradykinin, irrespective of the method of administration of the peptide. Thus, L-NAME increased the response to both aerosol and i.v. bradykinin.

NO released by bradykinin may originate from the airways or from a tissue outside the airways. Bradykinin aerosol, even at the highest dose, did not affect baseline blood pressure and heart rate. This observation suggests that bradykinin does not reach the systemic circulation and its action is confined to the airways. Hence, we postulate that the source of the NO released by bradykinin aerosol is located within the airway tissue. Decreases in arterial blood pressure and in heart rate were caused by i.v. bradykinin administration, thus indicating that systemic effects were produced by bradykinin given by this route of administration. However, it is possible that the source of NO released by i.v. and aerosol bradykinin is the same.

NO-synthase is present in different anatomical structures of the airways, which include non-cholinergic non-adrenergic nerves (Li & Rand, 1991; Lei *et al.*, 1993), macrophages and other inflammatory cells (Jorens *et al.*, 1993), and endothelial and epithelial cells (Fischer *et al.*, 1993; Kobzik *et al.*, 1993). To determine whether NO released by bradykinin derives from neural or non-neural elements, we studied the effect of L-NAME on the bronchoconstrictor response to capsaicin and to bradykinin after resection of the vagus nerves. Bronchoconstriction evoked by aerosolized capsaicin is due entirely to activation of cholinergic reflexes and release of tachykinins from sensory nerves (Ballati *et al.*, 1992; Bertrand *et al.*, 1993b). Bradykinin-induced bronchoconstriction is largely due to these two mechanisms (Ichinose *et al.*, 1990; Geppetti, 1993). We found that in guinea-pigs pretreated with atropine and phosphoramidon, capsaicin is more potent than bradykinin in causing bronchoconstriction, whereas the two drugs were equipotent in causing plasma extravasation. In these experimental conditions bradykinin and capsaicin cause bronchoconstriction and plasma extravasation by a common mechanism, e.g. tachykinin release from sensory nerves (Ichinose *et al.*, 1990; Bertrand *et al.*, 1993b). These observations support the hypothesis that bradykinin, but not capsaicin, releases bronchodilator agents. In addition, the present and previous (Lei *et al.*, 1993) observations that capsaicin-induced bronchoconstriction was not increased by L-NAME indicate that sensory nerves are not the source of NO released by bradykinin. Bradykinin and capsaicin seem to stimulate the same type of afferent fibres of the guinea-pig vagus nerve, e.g. C-fibres, but not Aδ-fibres (Fox *et al.*, 1993). This observation and the fact that acute vagotomy did not affect the bronchoconstrictor response to bradykinin and that L-NAME did not affect the response to capsaicin suggest that activation of neural cholinergic reflexes is not involved in the release of NO by bradykinin and that NO released by bradykinin does not originate from postganglionic cholinergic nerves. However, the present experimental data cannot exclude the possibility that bradykinin releases NO from neural elements by a direct action on NANC nerves containing NO-synthase and releasing NO.

An alternative hypothesis is that bradykinin releases NO from non-neural elements. The airway epithelium is a possible candidate as the site of NO release based on the following rationale: bradykinin B_2 receptors have been identified in guinea-pig airway epithelial cells (Proud *et al.*, 1993); bradykinin increases the mobilization and intracellular Ca^{2+} in bronchial epithelial cells (Geppetti *et al.*, 1994); increased intracellular Ca^{2+} concentration is required to activate constitutive NO-synthase (Moncada *et al.*, 1991); and the epithelium of various species (Fischer *et al.*, 1993; Kobzik *et al.*, 1993), including guinea-pig, is positively stained for NO-synthase. Recently, Nijkamp *et al.* (1993) reported that in anaesthetized spontaneously breathing guinea-pigs, histamine i.v. causes a bronchoconstrictor response which was potentiated by NO-synthase inhibitors. Potentiation by L-NAME and L-NMMA also was observed *in vitro* when histamine was administered to the guinea-pig trachea through the luminal surface and was abolished in tracheae in which the epithelium was removed. These data led the authors to suggest that histamine releases the bronchodilator agent, NO, from the epithelium. We propose that bradykinin, delivered either from the intravascular space or from the airway lumen, releases NO from the airway epithelium and by this mechanism opposes its own bronchoconstrictor action. This hypothesis is supported by the recent observation by Schlemper & Calixto (1994), who reported that the epithelium-dependent relaxant effect of bradykinin in strips of guinea-pig trachea is largely mediated by release of NO. However, further study is needed to test definitively the hypothesis that bradykinin releases NO from the airway epithelium of guinea-pigs *in vivo*.

Bradykinin aerosol does not substantially affect the bronchomotor tone in healthy volunteers. However, bradykinin causes bronchoconstriction in asthmatic patients (Herxheimer & E., 1961; Simonsson *et al.*, 1973; Fuller *et al.*, 1987). Histamine does not produce more than 20% reduction in FEV_1 in healthy subjects, whereas asthmatic patients exhibit hyperresponsiveness to histamine (Woolcock *et al.*, 1984). The ability of bradykinin and histamine to release the bronchodilator agent, NO, leads to the speculation that NO-synthase activity may be diminished in the airway epithelium of asthmatic patients and this reduction may contribute to the hyperresponsiveness to these mediators. The possibility that airway epithelial cells contain NO-synthase that is activated by bradykinin and other mediators adds further support to the view that these cells are a major pathophysiological component of asthma (Bertrand & Tschirhart, 1993).

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