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The effect of 15-HPETE on airway responsiveness and pulmonary cell recruitment in rabbits

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1 In the present study we have investigated the effect of 15-hydroperoxyeicosatetraenoic acid (15-HPETE) and 15-hydroxyeicosatetraenoic acid (15-HETE) on airway responsiveness to inhaled histamine in rabbits *in vivo*.

2 15-HPETE increased airway responsiveness to histamine 24 h after tracheal instillation and this was associated with a cellular infiltration consisting mainly of neutrophils, as measured by bronchoalveolar lavage. The airway hyperresponsiveness induced by 15-HPETE was still present 72 h after tracheal instillation of 15-HPETE, but had returned to baseline values one week post challenge. The number of neutrophils in bronchoalveolar lavage remained significantly elevated compared to pre-challenge levels. In contrast to 15-HPETE, the major metabolite 15-HETE, failed to alter airway hyperresponsiveness to histamine despite the recruitment of neutrophils into the lung, suggesting that the effect of 15-HPETE was not secondary to the generation of this metabolite nor dependent on the influx of neutrophils.

3 Both capsaicin and atropine but not the peripherally acting μ-opioid receptor agonist, BW443C (H-Tyr-D-Arg-Gly-Phe(4-NO_2)-Pro-NH_4), attenuated 15-HPETE-induced hyperresponsiveness. The increased cellular infiltration induced by 15-HPETE was only attenuated by capsaicin.

4 The results of the present study suggest that the release of 15-HPETE into the airways could contribute to sensitization of afferent nerve endings analogous to the hyperalgesia induced by this mediator in skin.

Keywords: Histamine; airways; 15-HPETE; capsaicin; lipoxygenase

Introduction

Hydroperoxyeicosataetranoic acid (HPETE), mono and dihydroxyeicosatetraenoic acids (HETES) and lipoxins are metabolic products of the mammalian 15-lipoxygenase enzyme which catalyses the insertion of molecular oxygen at carbon 15 of arachidonic acid (Samuelsson *et al*., 1987). Human 15-lipoxygenase activity has been demonstrated within tracheal epithelium (Hunter *et al*., 1985), eosinophils (Turk *et al*., 1982), endothelial cells (Hopkins *et al*., 1984) and monocytes (Conrad *et al*., 1992). This is consistent with the immunohistochemical localization of this enzyme to airway epithelium and eosinophils in man (Nadel *et al*., 1991; Bradding *et al*., 1995).

The products of the 15-lipoxygenase pathway have been implicated as potential mediators of airway inflammation (Samuelsson *et al*., 1987; Sigal & Nadel, 1991). Thus, mono and diHETES are chemotactic for neutrophils and eosinophils (Shak *et al*., 1983; Johnson *et al*., 1985; Kirsch *et al*., 1988; Morita *et al*., 1990; Schwenk *et al*., 1992); 15-HETE stimulates leukotriene C_4 release from mastocytoma cells (Goetzl *et al*., 1983) and mucous secretion from dog trachea (Johnson *et al*., 1985); lipoxins contract airway smooth muscle (Dahlen *et al*., 1987; Meini *et al*., 1992) and activate protein kinase C (Hansson *et al*., 1986). However, there is a paucity of data concerning the role of these mediators in airways hyperresponsiveness. Indeed, 15-HETE has been shown to reduce airway responsiveness to histamine (Lai *et al*., 1990a) but to prolong acute bronchospasm with inhaled antigen (Lai *et al*., 1990b) in asthmatics.

Afferent nerves have been shown to play a role in contributing to airways hyperresponsiveness induced by a number of stimuli including antigen (Ballati *et al*., 1992; Riccio *et al*., 1993; Herd *et al*., 1995), platelet activating-factor (PAF) (Spina *et al*., 1991; Perretti & Manzini, 1993), toluene di-isocyanate (Thompson *et al*., 1987; Marek *et al*., 1996), lipopolysaccharide (Jarreau *et al*., 1994), cigarette smoke (Daffonchio *et al*., 1990; Karlsson *et al*., 1991) and ozone (Tepper *et al*., 1993; Koto *et al*., 1995) in guinea-pigs and rabbits. It is of further interest that 15-lipoxygenase products including 8**R**, 15**S**-diHETE (White *et al*., 1990) and 15-HPETE (Adcock & Garland, 1993) can increase the sensitivity of stimuli that elicit a pain response and increase electrical activity of sensory C-fibres. We have investigated the effect of 15-lipoxygenase products on airways hyperresponsiveness in the rabbit and the involvement of afferent nerves in this phenomenon.

Methods

Animals

Male New Zealand White (NZW) rabbits (2–3 kg) were used throughout the studies and were obtained from Froxfield Farms, Petersfield, U.K. All experimental procedures were carried out in accordance with the U.K. Animals (Scientific procedures) Act, 1986 under a valid Home Office project licence.

Preparation of rabbits for assessment of airways responsiveness

Neuroleptanalgesia was induced in rabbits with a mixture of ketamine hydrochloride (35 mg kg^{-1}) and xylazine (5 mg kg^{-1}) administered intramuscularly (i.m.). Rabbits were laid supine on a small pillow on a moulded animal board and intubated with a 3.0 mm internal diameter (i.d.) endotracheal tube and attached to a Fleish pneumotachograph (size 00). Flow was measured with a differential pressure transducer ($\pm$ 2 cmH_20, Model MP 45-14-871, Validyne Engineering

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Corp. Northridge, CA). Pleural pressure was estimated by placing a latex balloon attached to a polyethylene cannula (i.d. 1.67 mm, outer diameter 2.42 mm) in the lower third of the oesophagus. An index of transpulmonary pressure, the difference between atmospheric and pleural pressure, was recorded by another Validyne differential pressure transducer ($\pm$ 20 cmH_20; Model MP 45-24-871) connected between the outflow of the oesophageal balloon and atmospheric air. The position of the balloon was adjusted in order to maximize the transpulmonary pressure recorded and it remained in this position throughout the experiment. The flow was integrated to give a continuous recording of tidal volume. Total lung resistance (R_L, $cmH_20\ L^{-1}\ s^{-1}$) and dynamic compliance (C_{dyn}; $ml\ cmH_20^{-1}$) were calculated by an online respiratory analyser (Mumed Ltd., London, UK; PMS version 4.0) on a breath by breath basis. Dynamic compliance was obtained by dividing the change in tidal volume by the change in transpulmonary pressure between the points of zero flow. The contribution made to resistance by the endotracheal tube was negligible at flow rates monitored between 0 and 60 ml min^{-1} and therefore not taken into consideration.

Measurement of airways responsiveness to histamine

Aerosols of saline or histamine were generated from a DeVilbiss ultrasonic nebuliser (DeVilbiss Healthcare Ltd, Heston, U.K.) which has previously been demonstrated to generate particles, 80% of which are less than 5 μm in diameter (DeVilbiss). Aerosols were administered directly to the lungs via the endotracheal tube. After measurement of baseline lung function, rabbits were exposed to an aerosol of sterile 0.9% physiological saline for 2 min, followed by a recording of respiratory parameters. Airways hyperresponsiveness was determined by the administration of increasing doubling concentrations of histamine (1.25–80 mg ml^{-1}) for two minutes at each concentration, with measurement of respiratory parameters made immediately after each concentration. The provocative concentrations (PC) of histamine which produced 35% decrease in dynamic compliance (PC_{35}) and a 50% increase in airways resistance (PC_{50}) were determined for each animal, and used as indices of airway responsiveness to histamine.

Bronchoalveolar lavage

A bronchoalveolar lavage (BAL) was performed immediately after the completion of the histamine concentration-response curve. The airways were lavaged through a polyethylene catheter (outer diameter 1.34 mm) inserted into the lungs via the endotracheal tube until it gently wedged against the airway wall. Three millilitres of 0.9% sterile physiological saline was injected into and then immediately aspirated from the lungs into a collection trap. Total cell counts were determined under light microscopy by an improved Neubauer haemocytometer. For differential cell counts, 5–7 drops of BAL fluid were used for centrifugation (Shandon Cytospin 2) and the cells were stained with Lendrum's stain (Lendrum, 1944). A total of 200 cells was counted under light microscopy and classified as either neurophils, eosinophils or mononuclear cells, based on standard morphological criteria.

15-Hydroperoxyeicosatetraenoic acid (15-HPETE) exposure

Twenty four hours following assessment of airways responsiveness to histamine, the same rabbits were re-anaesthetized and intubated with a 3.0 mm endotracheal tube. A polyethylene cannula (outer diameter 1.34 mm) was inserted into the endotracheal tube until it gently wedged and a 0.2 ml aliquot of either 15-hydroperoxyeicosatetraenoic acid (15-HPETE; final concentration 0.1, 0.5, 1.0, 5.0 $\mu g\ kg^{-1}$) or vehicle (10% ethanol and 90% physiological saline) was instilled directly into the lungs via the endotracheal tube. In some experiments baseline recordings of total lung resistance, dynamic compliance, tidal volume, minute volume and respiratory rate were made followed by another recording 15 min post-treatment with 15-HPETE or vehicle. On day 3, airways responsiveness to histamine and a bronchoalveolar lavage were again performed. In another study, airways responsiveness to histamine and bronchoalveolar lavage was determined before and 24 h, 72 h and 1 week following administration of vehicle or 15-HPETE (1 $\mu g\ kg^{-1}$).

Administration of 15-hydroxyeicosatetraenoic acid

In a separate set of experiments, airways responsiveness to histamine was determined before and 24 h following tracheal instillation of 15-hydroxyeicosatetraenoic acid (15-HETE) (0.1, 1 or 3 $\mu g\ kg^{-1}$). Bronchoalveolar lavage was also performed before and 24 h post-challenge as described above.

Drug studies

Administration of atropine and BW443C Atropine (2 mg kg^{-1}), BW443C (1 or 10 mg kg^{-1}) or saline were administered intravenously via the left marginal ear vein 15 min before the administration of 15-HPETE (1 $\mu g\ kg^{-1}$). Each drug was administered in separate animals. Airways responsiveness and cell counts were determined before and 24 h post-challenge with 15-HPETE or vehicle as described above.

Protocol for capsaicin study A total of 80 mg kg^{-1} capsaicin (5 mg kg^{-1} on day 1, 50 mg kg^{-1} on day 2 and 25 mg kg^{-1} on day 3) was administered subcutaneously to the dorsal back of rabbits according to a previously described protocol (Spina *et al*., 1991). Injections were performed every 2 h on days 1 and 2. This protocol has previously been demonstrated to induce a functional desensitization of exogenous capsaicin administration to bronchi *in vitro*. Capsaicin was prepared in 10% ethanol, 10% Tween 80 and 80% saline and control rabbits received the vehicle alone. Four days after the last capsaicin or capsaicin-vehicle injection the rabbits were exposed to 15-HPETE (1 $\mu g\ kg^{-1}$). Airway responsiveness and cell counts were determined before and 24 h post-challenge with 15-HPETE.

Reagents and drugs

Atropine sulphate, bovine serum albumin, capsaicin (8-methyl-N-vanillyl-6-nonemide), histamine diphosphate, ketamine hydrochloride, polyethylene glycol and Tween 80 were obtained from Sigma (U.K.); 15-(**S**)HPETE and 15-(**S**)HETE were purchased from Cascade Biochem (Reading, Berks, U.K.). Sterile saline was from Baxter Healthcare (U.K.) and Xylazine from Veterinary Drug Co. (U.K.) BW443C (H-Tyr-D-Arg-Gly-Phe(4-NO_2)-Pro-NH_4) was a gift from the former Wellcome Research Laboratories (Beckenham, U.K.). In all experiments, the appropriate concentration of 15-HPETE and 15-HETE was prepared under nitrogen gas to prevent the rapid oxidation of these eicosanoids. Vehicle solutions were treated in an identical manner.

Statistical analysis

Results from all studies were expressed as the mean $\pm$ s.e.mean. *In vivo* histamine potency data were derived from measurements of dynamic complicance (PC_{35}) and airway resistance (PC_{50}) and expressed as the geometric means together with the upper and lower values for the s.e.mean. For statistical purposes PC_{35} and PC_{50} values were log transformed. Airway responsiveness data were analysed by use of ANOVA and differences between means evaluated by Student's t test with Bonferroni correction for multiple comparisons where applicable. Nonparametric tests were used in the analysis of cell data. Mean values were considered significant if $P < 0.05$.

Results

The effect of 15-HPETE on lung function parameters

Neither, 15-HPETE (1 μg kg^{-1}, $n=6$) nor vehicle ($n=4$) significantly altered various lung function parameters (Table 1). Respiratory rate was significantly increased 15 min following administration of 15-HPETE but not vehicle ($P<0.05$; Table 1).

Effect of 15-HPETE on airways responsiveness to histamine

Total lung resistance Airways responsiveness to histamine (mg ml^{-1}) was not significantly altered 24 h following administration of vehicle (R_L PC_{50}, 95% confidence limits; 23.4 (12.6–43.6) vs post 20.4 (9.8–42.6), $P>0.05$, $n=7$; Figure 1a). 15-HPETE failed to increase airways responsiveness to histamine significantly at doses of 0.1 and 0.5 μg kg^{-1} ($P>0.05$, Figure 1a). In contrast, 15-HPETE (1 μg kg^{-1}) significantly increased airways responsiveness to histamine (pre PC_{50}/post PC_{50}, 95% confidence limits; 4.7 fold (2.2–9.8), $P<0.001$, $n=7$, Figure 1a). A 1.86 fold (0.95–3.63) increase in airways responsiveness was observed 24 h following 15-HPETE (5 μg kg^{-1}), although this failed to reach statistical significance ($P>0.05$ cf vehicle treatment, Figure 1a).

The increased airways responsiveness to histamine 24 h after administration of 15-HPETE (1 μg kg^{-1}) was not due to an alteration in baseline airway calibre (R_L, cmH_2O L^{-1} s^{-1}; pre = 27.4 ± 5.7 vs post = 22.7 ± 3.7).

Dynamic compliance Airways responsiveness to histamine (mg ml^{-1}) was not significantly altered 24 h following administration of vehicle (C_{dyn} PC_{35}, 95% confidence limits; 16.6 (10–27.5) vs post 17 (8.1–35.5), $P>0.05$, $n=7$; Figure 1b). 15-HPETE failed to increase airways responsiveness to histamine significantly at doses of 0.1 and 0.5 μg kg^{-1} ($P>0.05$, Figure 1b). In contrast, 15-HPETE (1 μg kg^{-1}) significantly increased airways responsiveness to histamine (pre PC_{35}/post PC_{35}, 95% confidence limits; 4.5 fold (1.9–10.5), $P<0.005$, $n=7$, Figure 1b). A 2.5 fold (0.83–7.55) increase in airways responsiveness was observed 24 h following 15-HPETE (5 μg kg^{-1}) although this failed to reach statistical significance ($P>0.05$ cf vehicle treatment, Figure 1b).

The increased airways responsiveness to histamine 24 h after administration of 15-HPETE (1 μg kg^{-1}) was not due to an alteration in baseline airway calibre (C_{dyn}, ml cmH_2O^{-1}, pre = 7.2 ± 0.9 vs post = 7.6 ± 1.2; $P>0.05$).

Effect of 15-HPETE on pulmonary cell recruitment of inflammatory cells

Twenty four hours after administration of 15-HPETE (1 μg kg^{-1}) there was a significant increase in the total number of cells recovered in the BAL fluid compared to vehicle ($P<0.05$, Table 2). Differential counts revealed that the infiltration was associated primarily with neutrophils ($P<0.05$, Table 2).

Time course of 15-HPETE-induced airways hyperresponsiveness to histamine

Total lung resistance Airways responsiveness to histamine was not significantly altered 24 h, 72 h and 1 week following inhalation with vehicle ($P>0.05$, Figure 2a). In contrast, 15-HPETE (1 μg kg^{-1}) significantly increased airways responsiveness over time compared with vehicle ($P=0.01$, ANOVA). This was reflected by a significant increase in airways

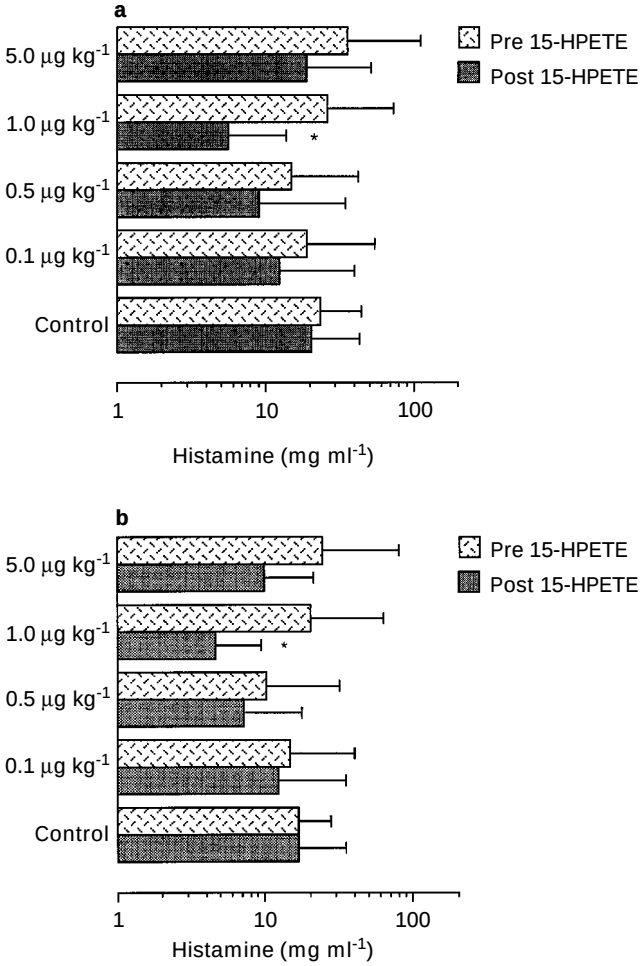

Figure 1 Airways responsiveness to histamine (mg ml^{-1}) from measurements of (a) total airways resistance (R_L PC_{50}); or (b) dynamic compliance (C_{dyn} PC_{35}); before and 24 h following tracheal instillation with vehicle (10% ethanol; control) or 15-HPETE (0.1–5.0 μg kg^{-1}). Horizontal lines represent the upper limit of the 95% confidence interval. Significant increase in airways responsiveness to histamine 24 h following tracheal instillation with 15-HPETE compared with airway responsiveness to histamine before tracheal instillation (*$P<0.05$).

Table 1 The effect of 15-HPETE (1 μg kg^{-1}) or vehicle (10% ethanol, 90% saline) on various lung function parameters in normal rabbits

	Vehicle		*15-HPETE*	
	Pre	*Post*	*Pre*	*Post*
Compliance (ml cmH_20^{-1})	6.5 ± 0.7	5.9 ± 1.2	5.7 ± 0.4	5.4 ± 0.6
Resistance (cmH_20 l^{-1} s^{-1})	24.5 ± 3.4	22.8 ± 2.7	21.0 ± 3.4	14.4 ± 4.5
Tidal volume (ml)	18.2 ± 2.0	17.2 ± 1.8	16.0 ± 0.7	14.0 ± 0.4
Respiratory rate (breath min^{-1})	31.3 ± 3.5	36.8 ± 3.6	31.7 ± 2.4	42.8 ± 4.0*
Minute volume (l min^{-1})	0.56 ± 0.10	0.64 ± 0.06	0.50 ± 0.02	0.59 ± 0.04

Results are expressed as mean ± s.e.mean. Lung function parameters were recorded before and 15 min post application of 15-HPETE ($n=6$) or vehicle ($n=4$). *Significant increase in respiratory rate compared with pre value ($P<0.05$).

responsiveness to histamine 24 h ($P<0.05$), 72 h ($P<0.05$) but not 1 week ($P>0.05$) after administration of 15-HPETE (Figure 2a).

Dynamic compliance Similarly, airways responsiveness to histamine as assessed by measurements of C_{dyn}, was not significantly altered 24 h, 72 h and 1 week following inhalation with vehicle ($P>0.05$, Figure 2b). In contrast, 15-HPETE (1 μg kg^{-1}) significantly increased airways responsiveness over time compared with vehicle ($P=0.01$, ANOVA). This was reflected by a significant increase in airways responsiveness to histamine 24 h ($P<0.01$), 72 h ($P<0.01$) but not 1 week ($P>0.05$) after administration of 15-HPETE (Figure 2b).

Time course of pulmonary cell recruitment of inflammatory cells induced by 15-HPETE

There was a significant increase in neutrophils recovered in bronchoalveolar lavage fluid 24 h ($P<0.05$), 72 h ($P<0.05$) and one week ($P<0.05$) following challenge with 15-HPETE (1 μg kg^{-1}; $n=8$, Table 3). There was no significant increase in total cell, eosinophil and mononuclear cell numbers at any time point following administration of 15-HPETE ($P>0.05$, Table 3).

Tracheal instillation of vehicle resulted in a small but significant increase in neutrophils only, 24 h, 72 h and one week post challenge ($P<0.05$, Table 3).

Effect of 15-HETE on airways responsiveness to histamine and pulmonary cell recruitment

Airways responsiveness to histamine was not significantly increased following intratracheal instillation of 15-HETE (0.1, 1 and 3 μg kg^{-1}) and hence only data for 15-HETE (1 μg kg^{-1}) is presented.

Total lung resistance Airways responsiveness to histamine (mg ml^{-1}) was not significantly increased 24 h after inhalation of 15-HETE compared with vehicle (vehicle: pre R_L PC_{50}, 23.6 (17.5–31.8) vs 24 h post R_L PC_{50}, 20.5 (14.5–28.9) cf 15-HETE: pre R_L PC_{50}, 14.1 (10.6–18.3) cf 24 h post C_{dyn} PC_{35}, 14.7 (11.6–18), $n=8$; $P>0.05$).

Dynamic compliance Airways responsiveness to histamine (mg ml^{-1}) was not significantly increased 24 h after inhalation of 15-HETE compared with vehicle (vehicle: pre C_{dyn} PC_{35}, 16.7 (12.9–21.4) vs 24 h post C_{dyn} PC_{35}, 16.9 (12.0–23.9) vs 15-HETE: pre C_{dyn} PC_{35}; 16.8 (11.4–22.9) vs 24 h post C_{dyn} PC_{35}, 15.2 (12.9–18.8), $n=8$, $P>0.05$).

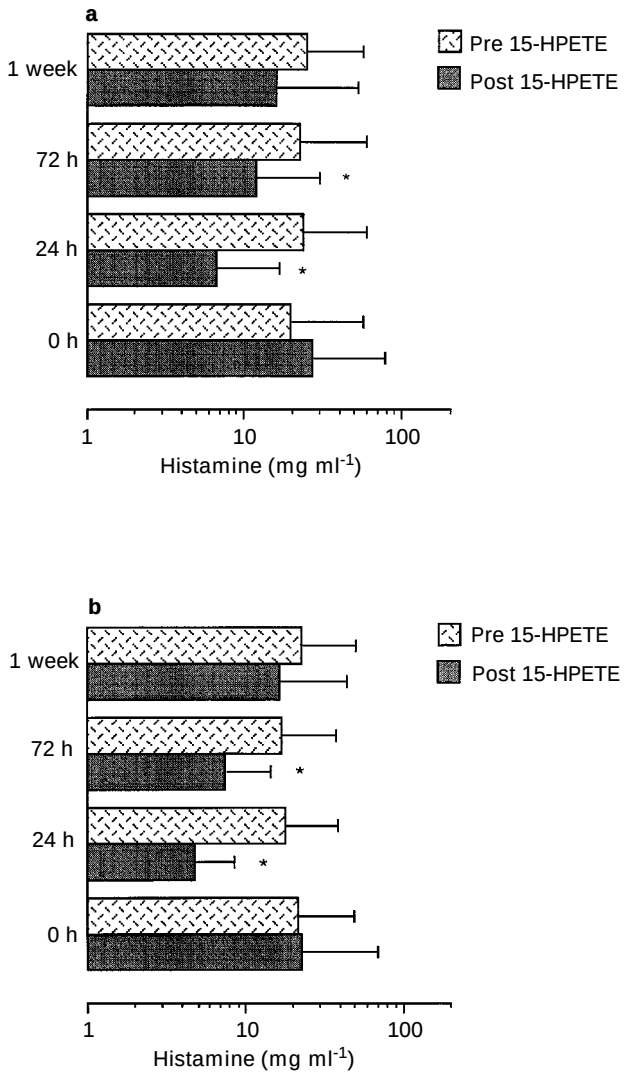

Figure 2 Airways responsiveness to histamine (mg ml^{-1}) from measurements of (a) total airways resistance (R_L PC_{50}); or (b) dynamic compliance (C_{dyn} PC_{35}); before (time 0) and following (24 h, 72 h, 1 week) intratracheal instillation with vehicle or 15-HPETE (1 μg kg^{-1}). Horizontal lines represent the upper limit of the 95% confidence interval. Significant increase in airways responsiveness to histamine following tracheal instillation with 15-HPETE compared

Table 2 The effect of 15-HPETE (1 μg kg^{-1}) or vehicle (10% ethanol, 90% saline) on the number of total cells, eosinophils, neutrophils and monocytes in bronchoalveolar lavage fluid (10^4 ml^{-1}) in normal rabbits

	Total cells	*Eosinophils*	*Neutrophils*	*Monocytes*
Vehicle				
Pre	2.85±0.54	0	0.082±0.055	2.77±0.57
Post	4.14±0.82	0.05±0.03	1.26±0.58	2.83±0.29
15-HPETE (0.1 μg kg^{-1})				
Pre	4.21±1.16	0	0.09±0.03	4.12±1.14
Post	9.07±2.21	0.04±0.03	1.68±0.45	7.35±1.92
15-HPETE (0.5 μg kg^{-1})				
Pre	2.5±0.98	0	0.03±0.026	2.47±0.096
Post	4.43±1.98	0.02±0.008	1.95±0.98	2.46±1.05
15-HPETE (1 μg kg^{-1})				
Pre	2.07±0.34	0	0.012±0.008	2.06±0.33
Post	8.43±1.6*	0.124±0.06	4.07±0.79*	4.26±1.00
15-HPETE (5 μg kg^{-1})				
Pre	2.59±0.4	0	0±0.003	2.58±0.40
Post	7.64±2.07	0.03±0.019	1.01±0.34	6.60±1.77

Results are expressed as mean±s.e.mean from 7 rabbits. Significant increase in cell number compared to pre values compared with vehicle control (*$P<0.05$).

Pulmonary cell recruitment Vehicle treatment failed to increase significantly the total number of cells ($\times 10^4$ ml^{-1}, pre; 2.85 ± 0.54 vs 24 h post, 4.14 ± 0.82, $P > 0.05$), eosinophils (pre; 0 ± 0 vs 24 h post, 0.05 ± 0.03, $P > 0.05$) and monocytes (pre; 2.77 ± 0.57 vs 24 h post, 2.83 ± 0.29, $P > 0.05$) in BAL. However, there was a significant increase in the number of neutrophils recovered in BAL (pre; 0.08 ± 0.06 vs 24 h post, 1.26 ± 0.58, $P < 0.05$).

In contrast, 15-HETE (1 μg kg^{-1}, $n = 8$) significantly increased the total number of cells (pre; 1.75 ± 0.42 vs 24 h post, 5.38 ± 1.39, $P < 0.05$), neutrophils (pre; 0.006 ± 0.005 vs 24 h post, 0.45 ± 0.17, $P < 0.05$) and monocytes (pre; 1.74 ± 0.42 vs 24 h post, 4.83 ± 1.09, $P < 0.05$) but not eosinophils (pre; 0 vs 24 h post, 0.01 ± 0.07, $P > 0.05$).

Effect of BW443C, atropine and vehicle on baseline lung function

On day 2 baseline lung function recordings were made before and 15 min following intravenous administration of vehicle, atropine (2 mg kg^{-1}) or BW443C (1 or 10 mg kg^{-1}). Atropine caused a pronounced bronchodilatation evidenced by an increase in dynamic compliance (pre $= 4.7 \pm 0.8$, post $= 15.9 \pm 3.6$ ml cmH_2O^{-1}; $n = 6$; $P < 0.05$) and a decrease in resistance (pre $= 45.1 \pm 5.8$, post $= 18.8 \pm 3.2$ cmH_2O L^{-1} s^{-1}; $n = 6$; $P < 0.05$).

Effect of atropine and BW443C on 15-HPETE-induced airways hyperresponsiveness to histamine

Airways responsiveness to histamine, as measured by R_L PC_{50} and C_{dyn} PC_{35} was significantly increased 24 h after exposure to 15-HPETE with respect to control experiments (Table 4, $P < 0.05$). Pretreatment with atropine (2 mg kg^{-1}) but not BW443C (1 or 10 mg kg^{-1}) significantly attenuated the 15-HPETE-induced increase in airways responsiveness to histamine (Table 4, $P < 0.05$). Atropine *per se*, did not alter airways responsiveness at 24 h to histamine in untreated animals (pre R_L PC_{50}/post R_L PC_{50}; 1.3 fold (0.4–3.7), $n = 6$ $P > 0.05$; pre C_{dyn} PC_{35}/post C_{dyn} PC_{35}, 1.0 fold (0.5–1.8), $n = 6$, $P > 0.05$).

Effect of capsaicin on 15-HPETE-induced airways hyperresponsiveness to histamine

Chronic treatment with capsaicin failed to alter baseline lung function significantly compared with vehicle (R_L: cmH_2O L^{-1} s^{-1}; vehicle 36.2 ± 6.7 cf capsaicin 35.2 ± 6.6, $P > 0.05$ and C_{dyn}: ml cmH_2O^{-1}; 5.1 ± 1.0 cf 4.5 ± 0.8, $P > 0.05$).

Chronic 3 day treatment of naive rabbits with capsaicin (80 mg kg^{-1}) failed to alter the bronchoconstrictor potency (mg ml^{-1}) to histamine significantly compared with vehicle-treated animals (R_L PC_{50}; vehicle-treated, 35.5 (15.1–83.2), $n = 7$ vs capsaicin-treated, 23.4 (19.7–36.0), $n = 7$, $P > 0.05$; C_{dyn} PC_{35}; vehicle-treated, 23.1 (10.1–55.7), $n = 7$ vs capsaicin-treated, 14.0 (7.8–24.8), $n = 7$, $P > 0.05$; Figure 3a).

Vehicle-treatment failed to alter significantly 15-HPETE-induced increase in airways responsiveness to histamine (pre R_L PC_{50}/post R_L $PC_{50} = 4.1$ fold (1.3–13.4), $n = 7$, $P < 0.05$ and pre C_{dyn} PC_{35}/post C_{dyn} $PC_{35} = 3.5$ fold (1.2–10.2), $n = 7$, $P < 0.05$). In contrast, 15-HPETE failed to induce an increase in airways responsiveness in rabbits chronically treated with capsaicin (80 mg kg^{-1}; $P > 0.05$, Figure 3b).

There was a significant increase in total cell number (pre $2.20 \pm 0.49 \times 10^4$ ml^{-1} vs post $4.7 \pm 0.17 \times 10^4$ ml^{-1}, $P < 0.05$) and neutrophils (pre $0.08 \pm 0.04 \times 10^4$ ml^{-1} vs post $1.15 \pm 0.58 \times 10^4$ ml^{-1}; $P < 0.05$) in the vehicle group 24 h after compared to 24 h before. Capsaicin pretreatment attenuated the 15-HPETE-induced increase in total cell (pre $2.08 \pm 0.51 \times 10^4$ ml^{-1} vs post $2.75 \pm 0.44 \times 10^4$ ml^{-1}; $P < 0.05$ cf vehicle-treated) but not neutrophil numbers (pre $0.002 \pm 0.002 \times 10^4$ ml^{-1} vs post $0.28 \pm 0.06 \times 10^4$ ml^{-1}; $P < 0.05$ cf vehicle-treated).

Discussion

We have demonstrated that tracheal instillation of 15-HPETE but not 15-HETE increases airways responsiveness to histamine in naive rabbits. The increased airways responsiveness to 15-HPETE was concentration- and time-dependent, although interestingly, the effect of 15-HPETE on airway responsiveness

Table 3 Time course for the effect of 15-HPETE (1 μg kg^{-1}) or vehicle (10% ethanol, 90% saline) on the number of total cells, eosinophils, neutrophils and monocytes in bronchoalveolar lavage fluid (10^4 ml^{-1}) in normal rabbits

	Total cells	*Eosinophils*	*Neutrophils*	*Monocytes*
Vehicle ($n = 8$)				
Pre	2.56 ± 0.49	0.014 ± 0.005	0.036 ± 0.009	2.50 ± 0.49
24 h post	3.5 ± 0.63	0.077 ± 0.03	0.61 ± 0.37*	2.83 ± 0.45
72 h post	3.5 ± 0.59	0.009 ± 0.005	0.146 ± 0.05*	3.16 ± 0.55
1 week post	2.88 ± 0.60	0.008 ± 0.004	0.48 ± 0.18*	2.39 ± 0.62
15-HPETE ($n = 8$)				
Pre	2.31 ± 0.33	0.01 ± 0.01	0.027 ± 0.008	2.27 ± 0.32
24 h post	6.315 ± 2.13	0.08 ± 0.05	2.24 ± 1.20*	4.58 ± 0.97
72 h post	3.31 ± 0.65	0.021 ± 0.01	0.60 ± 0.17*	2.48 ± 0.67
1 week post	2.81 ± 0.61	0.024 ± 0.015	0.74 ± 0.15*	2.05 ± 0.56

Results are expressed as mean $\pm$ s.e.mean. Significant increase in cell number compared to pre values (*$P < 0.05$).

Table 4 The effect of atropine and BW443C on 15-HPETE-induced increase in airways responsiveness to histamine as assessed by measurements of R_L PC_{50} and C_{dyn} PC_{35}

	R_L	C_{dyn}	n
Control	2.7 fold (1.2–5.9)	2.4 fold (1.4–4.1)	9
Atropine			
2 mg kg^{-1}	1.1 fold (0.4–2.2)*	0.8 fold (0.3–2.0)*	7
BW443C			
1 mg kg^{-1}	2.2 fold (1.0–4.5)	2.1 fold (1.1–4.2)	8
10 mg kg^{-1}	3.4 fold (1.8–6.6)	3.7 fold (2.4–5.8)	4

Results are expressed as geometric mean together with 95% confidence limits in parentheses. *n* represents the number of rabbits. *Atropine treatment significantly inhibited the ability of 15-HPETE (1 μg kg^{-1}) to increase airways responsiveness to histamine 24 h post exposure ($P < 0.05$).

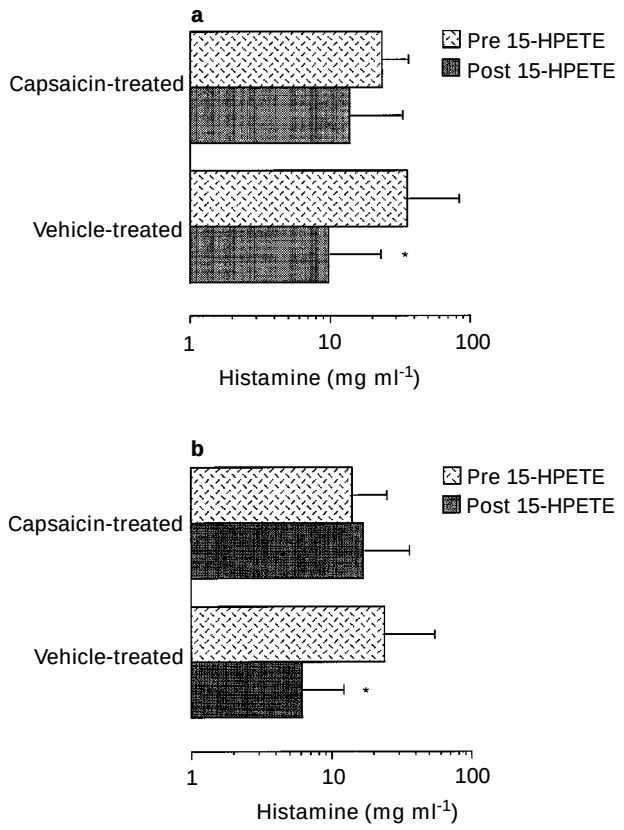

Figure 3 Airways responsiveness to histamine (mg ml^{-1}) from measurements of (a) total airways resistance (R_L PC_{50}); or (b) dynamic compliance (C_{dyn} PC_{35}); in vehicle-treated or capsaicin-treated animals before and 24 h following tracheal instillation with 15-HPETE (1 μg kg^{-1}). Horizontal lines represent the upper limit of the 95% confidence interval. Significant increase in airways responsiveness to histamine 24 h following tracheal instillation with 15-HPETE in vehicle-treated rabbits (*$P<0.05$).

was bell-shaped, a profile that is consistent with the biological response observed previously for 15-HETE and 15-HPETE (Johnson *et al*., 1985). This may be a function of receptor tachyphylaxis. Furthermore, animals chronically treated with capsaicin or naive rabbits pretreated with atropine failed to demonstrate increased airways responsiveness to histamine following 15-HPETE treatment, implicating the involvement of nerves in this response. In contrast, the μ-opioid agonist, BW443C failed to attenuate 15-HPETE-induced hyperresponsiveness in the rabbit.

The lack of effect of 15-HETE on airways responsiveness to histamine suggests that this metabolite is unlikely to mediate the airways hyperresponsiveness induced by 15-HPETE and is consistent with the inability of 15-HETE to increase airways responsiveness to histamine in asthmatics (Lai *et al*., 1990a,b). However, arachidonic acid can be metabolized by both 5- and 15-lipoxygenase to form other products such as the lipoxins (Serhan *et al*., 1984a,b). Lipoxin A_4 has been shown to cause contraction of airway smooth muscle via an action on capsaicin-sensitive nerves (Meini *et al*., 1992), although whether lipoxins induce airways hyperresponsiveness to spasmogens remains to be established.

Studies in the skin have shown that 15-HPETE can decrease the threshold of stimuli to elicit a pain response and increase electrical activity following stimulation of sensory C-fibres in the saphenous nerves (Follenfant *et al*., 1990). Similarly, a number of inflammatory mediators, including bradykinin, prostaglandins, leukotrienes, platelet activating factor (PAF; Adcock & Garland, 1993), substance P (Nakamura-Craig & Smith, 1989; Nakamura-Craig & Gill, 1991), neurokinin A (Nakamura-Craig & Gill, 1991), cytokines, including interleukin-1β (IL-1β; Ferreira *et al*., 1988; Safieh-Garabedian *et al*., 1995) and neurotrophins, including nerve growth factor (Woolf *et al*., 1994; Safieh-Garabedian *et al*., 1995), are known to increase the sensitivity of tissues to painful stimuli, termed hyperalgesia. It has been proposed that airways hyperresponsiveness may be analogous to hyperalgesia in the skin (Adcock & Garland, 1993), a hypothesis consistent with the ability of capsaicin to abrogate airway hyperesponsiveness induced by non-allergic stimuli including PAF (Spina *et al*., 1991; Perretti & Manzini, 1993), toluene di-isocyanate (Thompson *et al*., 1987; Marek *et al*., 1996), lipopolysaccharide (Jarreau *et al*., 1994), cigarette smoke (Daffonchio *et al*., 1990; Karlsson *et al*., 1991), poly-L-lysine (Coyle *et al*., 1994), and ozone (Tepper *et al*., 1993; Koto *et al*., 1995). Capsaicin also abrogates airway hyperresponsiveness induced by allergen in adult guinea-pigs (Ballati *et al*., 1992), rabbits (Herd *et al*., 1995) and neonatal rabbits (Riccio *et al*., 1993). Furthermore, a role for sensory nerves in airways hyperresponsiveness is also supported by the finding that neurokinin-2 receptor antagonists can attenuate airways hyperresponsiveness to allergen (Boichot *et al*., 1995; 1996; Mizuguchi *et al*., 1996), PAF (Perretti *et al*., 1995), poly-L-lysine (Coyle *et al*., 1994) and toluene di-isocyanate (Marek *et al*., 1996). The ability of capsaicin to reduce airways hyperresponsiveness induced by 15-HPETE in the present study, provides further evidence that airway sensory nerves may act as a common pathway by which a variety of stimuli induce airways hyperresponsiveness to spasmogens. It is well established that histamine-induced bronchoconstriction is, in part, a consequence of the reflex activation of parasympathetic nerves in the rabbit (Tanaka *et al*., 1991). However, in the present study atropine was without effect on histamine induced bronchoconstriction in control experiments, although it reduced airways hyperresponsiveness to inhaled histamine induced by 15-HPETE. These observations support the concept that 15-HPETE leads to a heightened parasympathetic reflex response which is susceptible to inhibition by atropine. Thus, both sensory and cholinergic nerves play a role in the 15-HPETE-induced airway hyperresponsiveness. This is consistent with previous studies demonstrating the ability of sensory neuropeptides to facilitate cholinergic neurotransmission (Tanaka & Grunstein, 1986; John *et al*., 1993; Belvisi *et al*., 1994), and of atropine to attenuate allergen-induced hyperresponsiveness to histamine in the rabbit (Tanaka *et al*., 1991). In contrast, the μ-opioid receptor agonist, BW443C did not attenuate airways hyperresponsiveness induced by 15-HPETE; this may be due to the charged nature of BW443C and its metabolism by neutral endopeptidase, which would thus, impede access of this drug to airway sensory nerves (Choudry *et al*., 1991), or a lack of effect on the sensitization process of airway afferent nerves.

Both 15-HPETE and 15-HETE induced an increase in total cell number 24 h following exposure that was composed primarily of an influx of neutrophils into the lung, as assessed by bronchoalveolar lavage. This is consistent with the ability of 15-lipoxygenase products including 15-HETE (Johnson *et al*., 1985) and (8s) (15s)-diHETE (Shak *et al*., 1983; Kirsch *et al*., 1988) to act as chemoattractants for neutrophils. However, the accumulation of neutrophils into the lung did not correlate with the increased airways responsiveness to histamine, as shown by the lack of effect of 15-HETE on airway responsiveness despite the presence of neutrophils in bronchoalveolar lavage fluid. In addition, airways hyperresponsiveness induced by 15-HPETE disappeared one week after challenge, despite the fact that the number of neutrophils in bronchoalveolar lavage fluid was still significantly above baseline. These results suggest that the infiltrating neutrophils do not contribute to airway hyperresponsiveness, although they were partly inhibited by capsaicin pretreatment, consistent with the ability of capsaicin to inhibit PAF-induced neutrophil accumulation (Spina *et al*., 1991). In conclusion, 15-HPETE induces airways hyperresponsiveness via a capsaicin- and atropine-sensitive pathway which appears to be unrelated to either neutrophil influx or the generation of 15-HETE.

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