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Mediators of adenosine- and ovalbumen-induced bronchoconstriction of sensitized guinea-pig isolated airways

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Abstract

The mediators of bronchoconstriction of isolated lungs and trachea from ovalbumen sensitized guinea-pigs to adenosine and ovalbumen were examined using relevant antagonists. Changes in perfusion pressure and tension of paired lung halves and tracheal spiral strips, respectively, were recorded in response to adenosine (1 mM lung, 300 μM trachea), histamine (10 μM), methacholine (10 μM) and ovalbumen (10 μg). One half was perfused with antagonist while the other received vehicle. Tracheal strips were superfused throughout with the P_1 receptor antagonist 8-phenyltheophylline, to examine 8-phenyltheophylline-resistant responses. The histamine H_1 receptor antagonist, mepyramine (1.5 mM), the cyclooxygenase inhibitors, indomethacin (5 mM) and diclofenac (5 mM), the leukotriene receptor antagonist, zafirlukast (1 mM), and the lipoxygenase inhibitor, zileuton (20 mM), alone failed to inhibit bronchoconstriction by adenosine and ovalbumen of the lung and trachea. When two antagonists were combined, only mepyramine and zafirlukast significantly reduced the lung responses to adenosine and ovalbumen. The tracheal adenosine response was substantially reduced, although not significantly, while ovalbumen was significantly reduced. When mepyramine, indomethacin and zafirlukast were combined, the lung constriction by adenosine and ovalbumen were virtually abolished. Similarly, the combination of mepyramine, diclofenac and zafirlukast significantly attenuated the lung responses to adenosine and ovalbumen. Thus, histamine, cyclooxygenase products and leukotrienes alone are not responsible for the bronchoconstriction of isolated sensitized lung tissues to adenosine or ovalbumen, which appears to be due to the release of all three mediators.

Keywords: Adenosine; Bronchconstriction; Guinea-pig, sensitized; Histamine; Leukotriene; Cyclooxygenase product

1. Introduction

The main mediators involved in the allergic reaction are histamine, leukotrienes and prostaglandins (Pearce, 1990). Histamine is synthesized and stored in granules in guinea-pig and human mast cells which degranulate on immunological challenge and release histamine. Asthmatic patients are more sensitive to inhaled histamine than non-asthmatic patients and therefore display hyperreactivity/hyperresponsiveness (Barnes et al., 1998). Leukotrienes are synthesized mainly by mast cells but they are also produced by macrophages, monocytes and basophils (Lewis et al., 1990). Leukotrienes are formed from arachidonic acid that is released from the cell membrane phospholipid bilayer by phospholipase A_2. The liberated arachidonic acid may then be metabolized by either the cyclooxygenase pathway to generate prostaglandins, thromboxanes and prostacyclin, or the 5-lipoxygenase pathway, to generate leukotrienes C_4, D_4 and E_4 (Samuelson et al., 1987) which are involved in the inflammatory response (O'Hickey et al., 1991; Spector, 1996; Devillier et al., 1999). Leukotrienes are potent contractile agents of human bronchi in vitro, being 1000 times more potent than histamine (Krell et al., 1990). They are able to constrict airways of normal and asthmatic patients (Drazen, 1988), although the airways of asthmatic subjects are 100 to 1000 times more sensitive to inhaled leukotrienes D_4 and E_4 than are airways of normal subjects (Barnes et al., 1984). Leukotriene receptor antagonists, including zafirlukast (Douglas and Hay, 1997), inhibit leukotriene D_4 receptors in human tissue (Chanarin and Johnston, 1994; Drazen, 1998) and in asthmatic patients, caused a 90-fold

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increase in the concentration of leukotriene D_4 required to decrease specific airway conductance by 20% (Smith et al., 1990). The selective reversible 5-lipoxygenase inhibitors, such as zileuton, prevent the generation of leukotriene D_4 (McGill and Busse, 1996). Elevated levels of leukotrienes have been in the plasma and bronchoalveolar fluid of asthmatic patients after an asthma attack or allergen challenge (Wenzel et al., 1995). Studies using leukotriene receptor antagonists and lipoxygenase inhibitors have shown that they protect against the early response to allergen in allergic asthmatics (Taylor et al., 1991) and shift the dose–response curve to the right. This supports the role for mast cell-derived leukotrienes in allergen-induced constrictions. The late phase allergic response in humans can be reduced by the leukotriene receptor antagonist zafirlukast (Taylor et al., 1991).

Prostaglandins are released from mast cells by the breakdown of arachidonic acid by cyclooxygenase (Metcalfe et al., 1997), inhibitors of which, such as indomethacin, block the production of both the bronchoconstricting prostaglandins D_2 and F_2, and the bronchodilating prostaglandins E_2 and I_2. Prostaglandins and thromboxanes cause bronchoconstriction of human airways in vitro (Sheldrick et al., 1995), and inhaled prostaglandins $F_{2\alpha}$ and D_2 cause bronchoconstriction in asthmatic patients (Hardy et al., 1984). Also, the stable thromboxane analogue, U46619, is a potent constrictor in asthmatic patients, mediated in part by acetylcholine release (Saroea et al., 1995). There is considerable evidence obtained with animals to suggest that thromboxane A_2 is involved in airway responsiveness, but this is not supported in asthmatic patients (O'Byrne and Fuller, 1989). After an allergen challenge in humans, there is an increase in prostaglandins D_2 and thromboxane B_2 in macrophage-rich bronchoalveolar lavage fluid (Dworski et al., 1994).

Inhaled adenosine or 5′-AMP cause bronchoconstriction in asthmatic subjects but not in non-asthmatics (Cushley et al., 1983). In sensitized guinea-pigs (Thorne and Broadley, 1992) and rats (Hannon et al., 2001), adenosine has also been shown to induce bronchoconstrictor responses whereas non-sensitized animals show no response. The isolated lungs or tracheae from sensitized guinea-pigs also display a bronchoconstriction to adenosine and it analogues (Thorne and Broadley, 1992; Kehoe and Broadley, 1996; Thorne et al., 1996) which is thought to be due to adenosine A_3 receptor stimulation. The response of non-sensitized tissues is a bronchodilatation, which is regarded as being mediated via adenosine A_2 receptors (Brown and Collis, 1982), which from an agonist profile appears to be of the A_{2b} subtype (Losinski and Alexander, 1995). Isolated bronchi from asthmatic subjects contract in response to adenosine and this is more substantial than in non-asthmatics (Björck et al., 1992). The contraction was inhibited by the combined use of leukotriene receptor antagonists and histamine H_1 and histamine H_2 receptor antagonists. This suggests that the contraction is due to liberation of leukotrienes and histamine, possibly from mast cells.

The bronchoconstriction to inhaled adenosine or 5′-AMP in asthmatic subjects also appears to be through the release of mast cell-derived mediators, including histamine and arachidonic acid breakdown products, since the response has been shown to be inhibited by histamine H_1 receptor antagonists (Rafferty et al., 1987) and by putative mast cell stabilizers such as nedocromil sodium (Crimi et al., 1986, 1987) or sodium cromoglycate (Phillips and Holgate, 1989; Church et al., 1991). However, there is no detailed information on the relative roles of histamine and arachidonic acid products in the responses to adenosine of asthmatics and sensitized animal models of asthma. Therefore, the aim of this study was to investigate by use of relevant antagonists, which mediators are responsible for the constrictor response to adenosine in isolated lungs and trachea from sensitized guinea-pigs. The responses to the sensitizing antigen, ovalbumen, were examined in parallel to determine the relationship between responses to antigen and adenosine challenge.

2. Materials and methods

2.1. Subjects

Male Dunkin–Hartley guinea-pigs weighing 300–400 g at purchase, and 400–500 g at termination, were used throughout. These studies complied with the guidelines for the care and use of laboratory animals according to the Animals (Scientific Procedures) Act 1986 and the procedures have been approved by the Ethical Review Committee of Cardiff University.

2.2. Sensitization procedure

Guinea-pigs were actively sensitized to ovalbumen (100 μg ml^{-1}) plus aluminium hydroxide (100 mg ml^{-1}) in sodium chloride (0.9%). The solution was stirred for 2 h before the guinea-pigs received 2 × 0.5 ml bilateral intraperitoneal (i.p.) injections. Drug solutions were injected directly into the peritoneum, taking care not to damage the internal organs. The guinea-pigs were used 14–21 days after sensitization. This method of sensitization followed the procedure described by Andersson (1980), which in guinea-pigs raises both immunoglobulin (Ig) E and IgG-like antibodies.

2.3. Perfused lung

Guinea-pigs were killed by cervical dislocation and trachea and lungs excised. The trachea was removed 5 mm above the bifurcation. The lungs were separated at the bifurcation and half-lungs perfused via the bronchi and suspended in a heated jacket. Perfusion was with warmed (37 °C) and gassed (5% CO_2 in oxygen) Krebs-bicarbonate solution of composition (mM): NaCl 118, $NaHCO_3$ 24.9, KCl 4.6, $CaCl_2$ 2.5, $MgSO_4$ 1.15, KH_2PO_4 1.15 and glucose 5.5 in twice distilled water. A constant flow rate of 5 ml

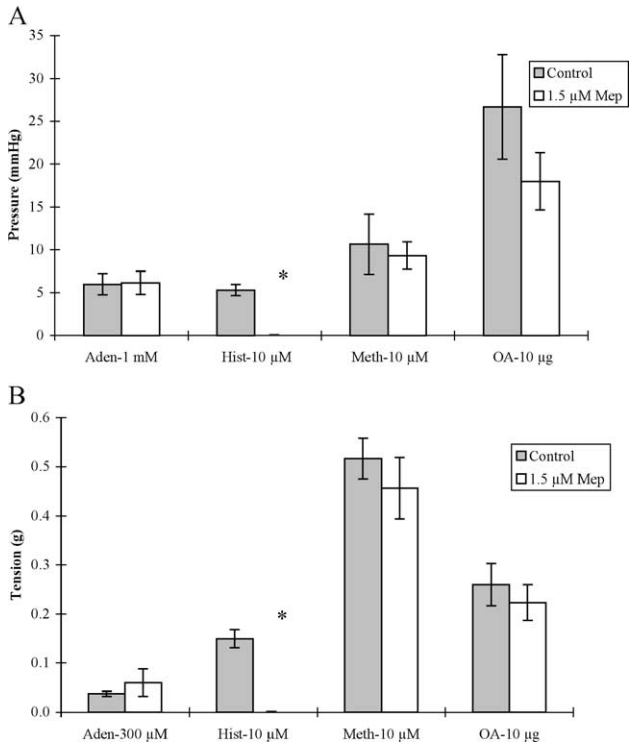

Fig. 1. Effect of mepyramine (Mep) (1.5 μM) on contractile responses of (A) paired lung halves and (B) paired tracheal spirals. Mean (± S.E.M.) increases in perfusion pressure or in tension by adenosine (Aden, lung 1 mM, trachea 300 μM), histamine (Hist, 10 μM), methacholine (Meth, 10 μM) and ovalbumen (OA, 10 μg) in the absence (control) and in the presence of mepyramine ($n=6$) are shown. Tracheas were superfused in the presence of 3 μM 8-phenyltheophylline. * Denotes a significant difference from control ($P<0.05$).

min^{-1} was maintained by a Watson–Marlow peristaltic pump (tube internal diameter 0.5 mm), and changes in back pressure were measured with a physiological pressure transducer (type 4-327-L221, Bell and Howell, Slough, UK), located at the side arm on the perfusion cannula. The Krebs solution leaked out through the wall of the lung halves without scarification. Basal perfusion pressure was 15 ± 4 mm Hg. A Condon mercury manometer was included in the system in series with the pressure transducer to accommodate some degree of volume change during drug responses. Perfusion pressure was recorded on a Devices MX8 polygraph (Ormed, Welwyn Garden, Herts, UK).

2.4. Superfused tracheal spirals

The trachea was removed from the lungs and cut spirally (Constantine, 1965). Lengths of 3–4 cm were suspended in a heated jacket (37 °C) and superfused with Krebs-bicarbonate solution. During periods of equilibration and between agonist exposure, a constant flow rate of 5 ml min^{-1} was maintained by a Watson–Marlow peristaltic pump. However, during agonist superfusion, the flow rate was reduced to 4.75 ml min^{-1}. Changes in isometric tension were measured by attaching the upper end of the spiral to an isometric tension transducer (UF1, 57 g sensitivity range, (Dynamomoter, Ormed)). Intrinsic tone was induced by allowing the spirals to equilibrate under an applied tension of 1 g for 30–45 min and tension was recorded on a Devices MX8 polygraph.

2.5. Agonists

The agonists were slow-infused over the tracheas at a constant rate of 0.25 ml min^{-1} (making a total flow rate of 5 ml min^{-1}) with a 5-ml syringe fitted to a slow infuser (Scientific and Research Instruments, Edenbridge, Kent, UK).

All the agonists were added to the lung halves as 0.1 ml bolus injections made into the perfusion solution before the warming coil.

The lungs and trachea were exposed to single doses or concentrations, respectively, of adenosine (1 mM lung and 300 μM trachea), histamine (10 μM), methacholine (10 μM) and ovalbumen (10 μg) in that order. Subsequent doses were

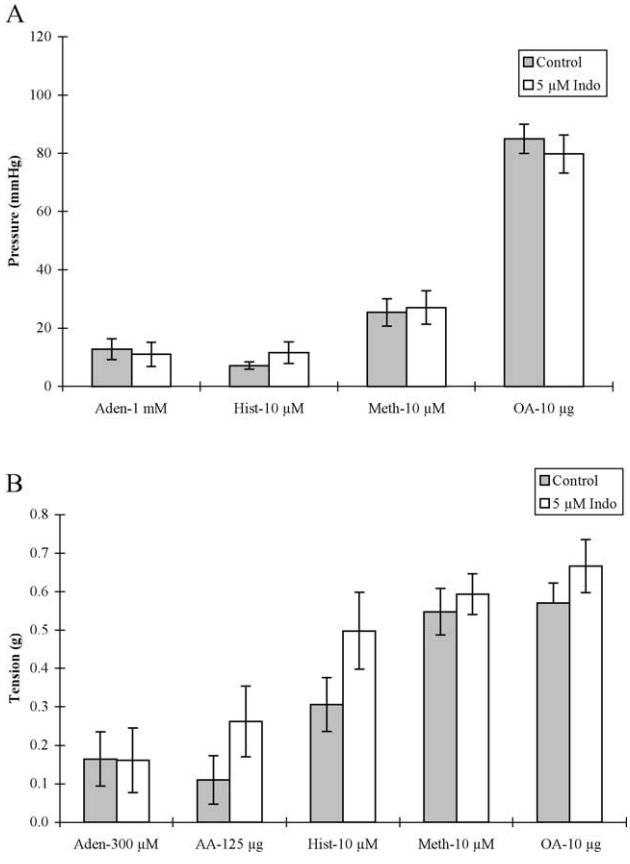

Fig. 2. Effect of indomethacin (Indo) (5 μM) on contractile responses of (A) paired lung halves and (B) paired tracheal spirals from sensitized guinea-pigs. Mean (± S.E.M.) increases in perfusion pressure or tension by adenosine (Aden, lung 1 mM, trachea 300 μM), arachidonic acid (AA, trachea 125 μg), histamine (Hist, 10 μM), methacholine (Meth, 10 μM) and ovalbumen (OA, 10 μg) in the absence (control) and in the presence of indomethacin are shown (lung, $n=11$ and trachea, $n=9$). Tracheas were perfused in the presence of 3 μM 8-phenyltheophylline.

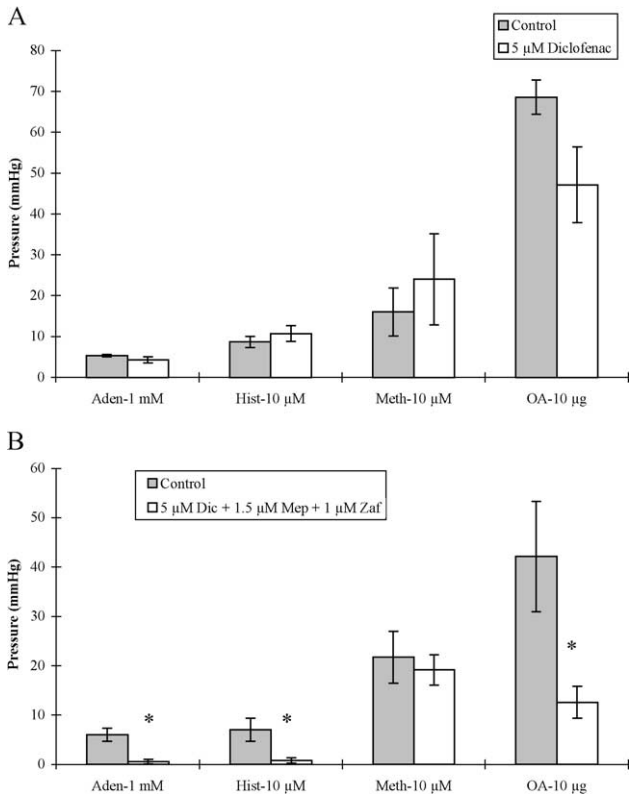

Fig. 3. Effect of (A) diclofenac alone (Dic) (5 μM) and (B) a combination of diclofenac (5 μM), mepyramine (Mep) (1.5 μM) and zafirlukast (Zaf) (1 μM) on the contractile responses of paired lung halves. Mean (± S.E.M.) increases in perfusion pressure by adenosine (Aden, 1 mM), histamine (Hist, 10 μM), methacholine (Meth, 10 μg) and ovalbumen (OA, 10 μg) in the absence (control) and in the presence of diclofenac alone ($n=7$) or the combined inhibitors are shown ($n=6$). * Denotes a significant difference from control ($P<0.05$).

only added after the tissue had returned to its original baseline level. In some experiments, additional agonists were included, such as arachidonic acid or leukotriene D_4, following the initial adenosine dose.

2.6. Antagonists

Antagonists were perfused in the Krebs-bicarbonate solution after the initial equilibration period of 30–45 min. This was commenced 30 min before and then throughout the addition of agonists. One lung half or tracheal half was perfused with antagonist while the other served as a paired control perfused with the relevant antagonist vehicle. In each study group, left and right half-lungs were alternated for antagonist exposure.

We have previously demonstrated that the constrictor response to adenosine of superfused tracheal spirals from sensitized guinea-pigs is resistant to blockade by the P_1-purinoceptor antagonist, 8-phenyltheophylline (Thorne and Broadley, 1992; Thorne et al., 1996). The response to the analogue R-phenylisopropyladenosine, is in fact potentiated by 8-phenyltheophylline (Kehoe and Broadley, 1996). Thus, in the present study, we examine the 8-phenyltheophylline-resistant response in superfused tracheae by inclusion of 8-phenyltheophylline (3 μM) in the Krebs-bicarbonate solution throughout. In the lungs, 8-phenyltheophylline was omitted in order to examine the mediators involved in the net response to adenosine. Concentrations of indomethacin (5 μM) and diclofenac (5 μM) were used in accordance with Devillier et al. (2001) and Przybilla et al. (1987), respectively.

2.7. Data analysis

Responses of the lungs were measured as the peak increase in perfusion pressure (mm Hg) and responses of the tracheae were measured as the peak change in tension (g). Results are expressed as the mean ± S.E.M. Data were analysed for statistical significance by a paired Student's *t*-test. The n values quoted refer to the number of animals used. A P value of less than 0.05 was taken to indicate a significant difference.

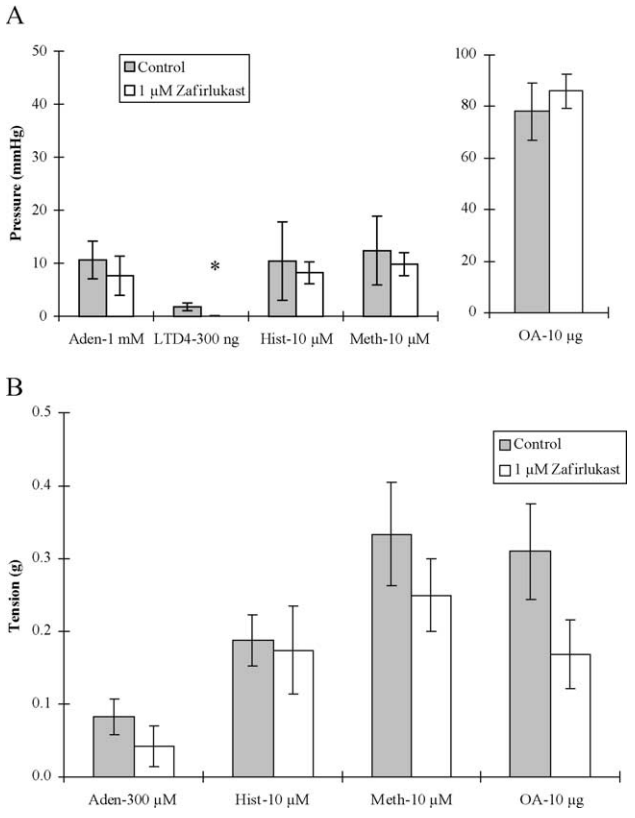

Fig. 4. Effect of zafirlukast (1 μM) on contractile responses of (A) paired lung halves and (B) paired tracheal spirals from sensitized guinea-pigs. Mean (± S.E.M.) increases in perfusion pressure or tension by adenosine (Aden, lung 1 mM, trachea 300 μM), leukotriene D_4 (LTD4 300 ng), histamine (Hist, 10 μM), methacholine (Meth, 10 μM) and ovalbumen (OA, 10 μg) in the absence (control) and in the presence of zafirlukast are shown (lungs, $n=6$ and trachea, $n=5$). Tracheas were perfused in the presence of 8-phenyltheophylline (3 μM). * Denotes a significant difference from control ($P<0.05$).

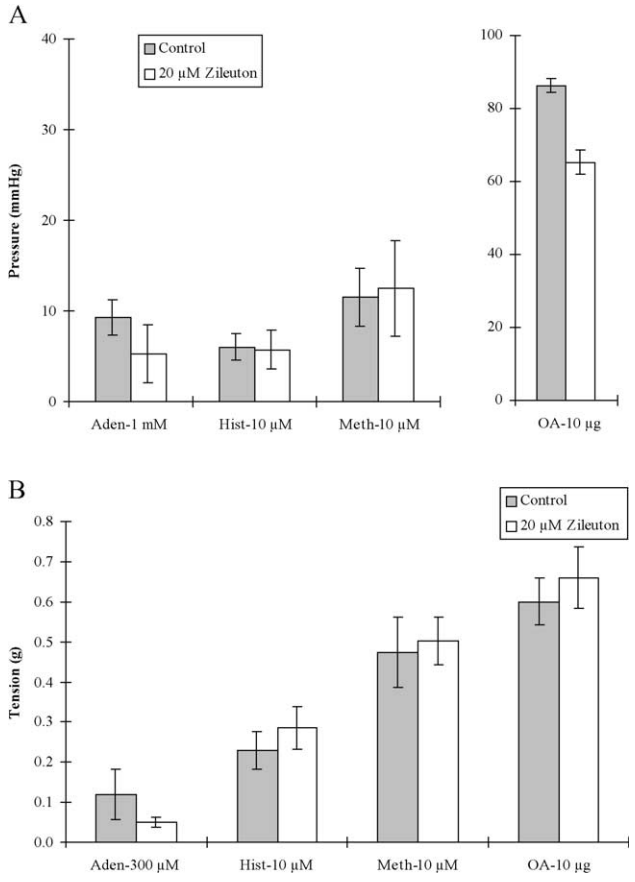

Fig. 5. Effect of zileuton (20 μM) on the contractile responses of (A) paired lung halves and (B) paired tracheal spirals from sensitized guinea-pigs. Mean (± S.E.M.) increases in perfusion pressure or tension by adenosine (Aden, lung 1 mM, trachea 300 μM), histamine (Hist, 10 μM), methacholine (Meth, 10 μM) and ovalbumen (OA, 10 μg) in the absence (control) and in the presence of zileuton ($n=4$) are shown. Tracheas were perfused in the presence of 3 μM 8-phenyltheophylline.

2.8. Drugs

8-Phenyltheophylline, acetyl-methylcholine chloride (methacholine), adenosine, arachidonic acid, diclofenac (sodium salt), dimethylsulphoxide (DMSO), dipyridamole, histamine (diphosphate salt), indomethacin, leukotriene D_4, mepyramine maleate, ovalbumen and polyethylene glycol (grade 200) were obtained from Sigma (Poole, Dorset, UK). Zafirlukast (Abbott Laboratories, UK) and zileuton (Pfizer) were kindly supplied as gifts. Aluminium hydroxide was supplied by BDH (Poole). All agonists stock solutions were prepared freshly each day in distilled water and further serial dilutions made in Krebs-bicarbonate solution. Indomethacin, diclofenac and mepyramine were made up in distilled water. 8-Phenyltheophylline was initially dissolved in 0.1 M NaOH, zafirlukast was dissolved in 30:70 DMSO/0.1 M NaOH, dipyridamole in 0.1 M HCl and zileuton was dissolved in 100% DMSO.

3. Results

3.1. Effect of single antagonists on the bronchoconstrictor response of the sensitized airways to adenosine and ovalbumen

Mepyramine (1.5 μM) failed to significantly ($P>0.05$) reduce the constrictor responses of perfused sensitized guinea-pig half-lungs to adenosine, methacholine or ovalbumen, but the response to histamine was virtually abolished (Fig. 1A) ($n=6$). Mepyramine (1.5 μM) did not significantly ($P>0.05$) reduce the contractile responses of superfused sensitized guinea-pig tracheas to adenosine, methacholine or ovalbumen in the presence of 3 μM 8-phenyltheophylline, but again the response to histamine was abolished (Fig. 1B) ($n=6$).

The cyclooxygenase inhibitor, indomethacin (5 μM), did not significantly ($P>0.05$) reduce the constrictor responses of perfused sensitized guinea-pig half-lungs to adenosine, histamine, methacholine or ovalbumen (Fig. 2A) ($n=11$). Indomethacin (5 μM) also failed to significantly ($P>0.05$) reduce the contractile responses of sensitized guinea-pig tracheas to adenosine, arachidonic acid, histamine, methacholine or ovalbumen (Fig. 2B) ($n=9$).

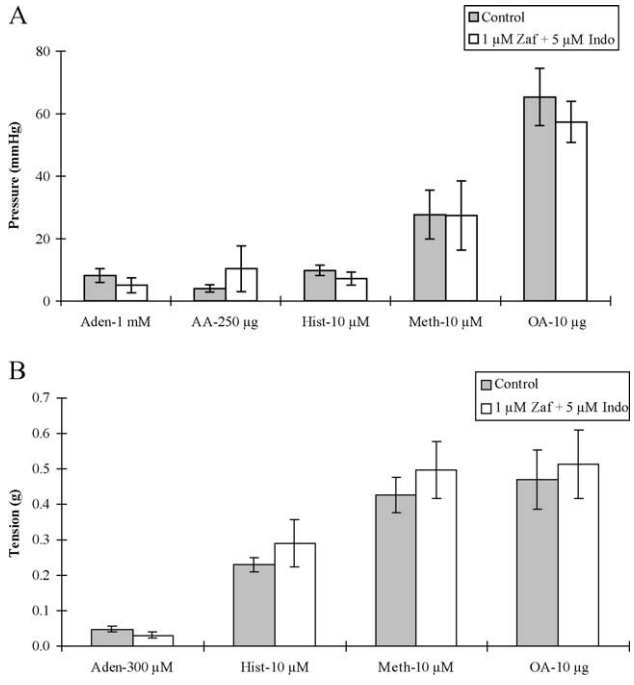

Fig. 6. Effects of a combination of zafirlukast (Zaf) (1 μM) and indomethacin (Indo) (5 μM) on the contractile responses of (A) paired lung halves and (B) paired tracheal spirals from sensitized guinea-pigs. Mean (± S.E.M.) increases in perfusion pressure and tension by adenosine (Aden, lung 1 mM, trachea 300 μM), arachidonic acid (AA, lung 250 μg), histamine (Hist, 10 μM), methacholine (Meth, 10 μM) and ovalbumen (OA, 10 μg) in the absence (control) and in the presence of zafirlukast and indomethacin are shown (lung, $n=5$ and trachea, $n=6$). Tracheas were perfused in the presence of 3 μM 8-phenyltheophylline.

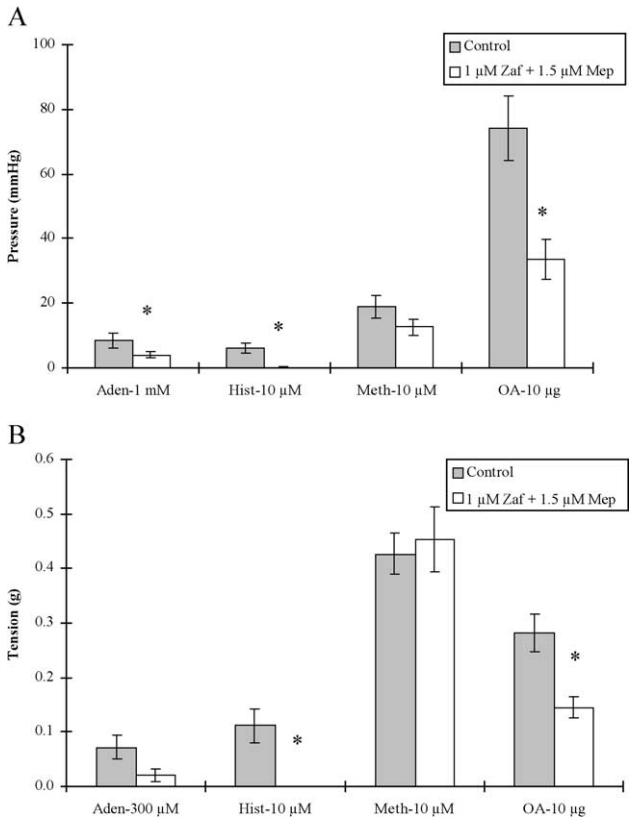

Fig. 7. Effects of a combination of zafirlukast (Zaf) (1 μM) and mepyramine (Mep) (1.5 μM) on the contractile responses of (A) paired lung halves and (B) paired tracheal spirals from sensitized guinea-pigs. Mean (± S.E.M.) increases in perfusion pressure or tension by adenosine (Aden, lung 1 mM, trachea 300 μM), histamine (Hist, 10 μM), methacholine (Meth, 10 μM) and ovalbumen (OA, 10 μg) in the absence (control) and in the presence of zafirlukast and mepyramine are shown (lung, $n=7$ and trachea, $n=8$). Tracheas were perfused in the presence of 3 μM 8-phenyltheophylline. * Denotes a significant difference from control ($P<0.05$).

The cyclooxygenase inhibitor, diclofenac (5 μM), was used as an alternative cyclooxygenase inhibitor in sensitized guinea-pig perfused half-lungs. It did not significantly ($P>0.05$) reduce the constrictor responses to adenosine, histamine, methacholine or ovalbumen (Fig. 3A) ($n=7$).

The leukotriene receptor antagonist, zafirlukast (1 μM), failed to significantly ($P>0.05$) reduce the constrictor responses of sensitized guinea-pig perfused half-lungs to adenosine, histamine, methacholine or ovalbumen. A small constrictor response to leukotriene D_4, however, was abolished (Fig. 4A) ($n=6$). Zafirlukast (1 μM) also failed to significantly ($P>0.05$) reduce the contractile responses of sensitized guinea-pig superfused tracheas to adenosine, histamine, methacholine or ovalbumen (Fig. 4B) ($n=5$).

The lipoxygenase inhibitor, zileuton (20 μM), did not significantly ($P>0.05$) reduce the constrictor responses of sensitized guinea-pig perfused half-lungs to adenosine, histamine, methacholine or ovalbumen (Fig. 5A) ($n=4$). Similarly, in the presence of 3 μM 8-phenyltheophylline, zileuton (20 μM) did not significantly ($P>0.05$) reduce the contractile responses of sensitized guinea-pig superfused tracheas to adenosine, histamine, methacholine or ovalbumen (Fig. 5B) ($n=4$).

3.2. Effects of a combination of two antagonists on the bronchoconstrictor responses of the sensitized airways to adenosine and ovalbumen

A combination of zafirlukast (1 μM) and indomethacin (5 μM) did not significantly ($P>0.05$) reduce the constrictor responses of sensitized guinea-pig perfused half-lungs to adenosine, arachidonic acid, histamine, methacholine or ovalbumen (Fig. 6A) ($n=5$). This combination also failed to significantly ($P>0.05$) reduce the contractile responses of sensitized guinea-pig superfused tracheas to adenosine, histamine, methacholine or ovalbumen (Fig. 6B) ($n=6$).

The combination of zafirlukast (1 μM) and mepyramine (1.5 μM) did not significantly ($P>0.05$) reduce the constrictor responses of sensitized guinea-pig perfused half-lungs to methacholine (Fig. 7A) ($n=7$). There was, how-

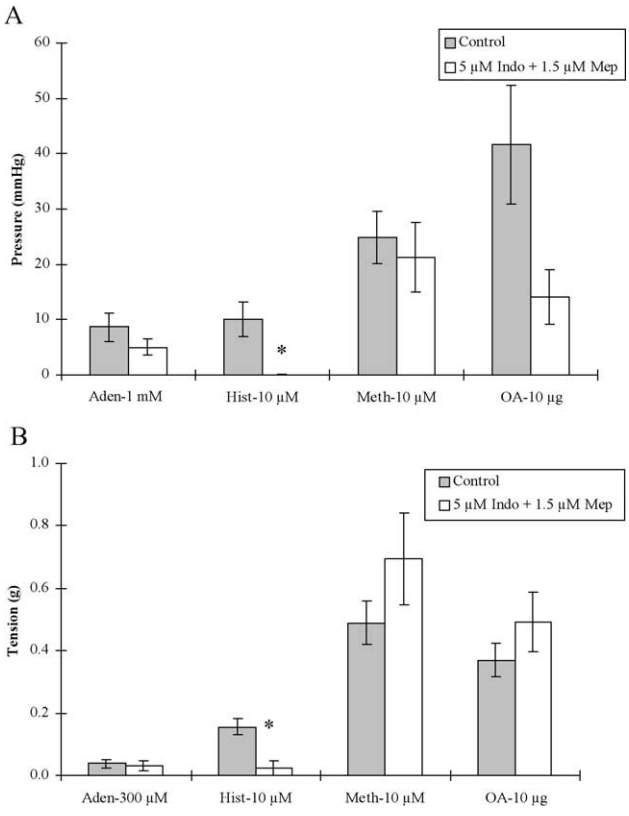

Fig. 8. Effects of a combination of indomethacin (Indo) (5 μM) and mepyramine (Mep) (1.5 μM) on the contractile responses of (A) paired lung halves and (B) paired tracheal spirals from sensitized guinea-pig tracheas. Mean (± S.E.M.) increases in perfusion pressure or tension by adenosine (Aden, lung 1 mM, trachea 300 μM), histamine (Hist, 10 μM), methacholine (Meth, 10 μM) and ovalbumen (OA, 10 μg) in the absence (control) and in the presence of indomethacin and mepyramine are shown ($n=6$). Tracheas were perfused in the presence of 3 μM 8-phenyltheophylline. * Denotes a significant difference from control ($P<0.05$).

ever, a significant ($P<0.05$) reduction in the constrictor responses to adenosine, histamine and ovalbumen. In the trachea, the combination of zafirlukast (1 μM) and mepyramine (1.5 μM) did not significantly ($P=0.09$) affect the contractile response to adenosine, although it was substantially reduced. Methacholine was not affected. However, there was a significant ($P<0.05$) reduction in the response of the trachea to ovalbumen. The response to histamine was abolished (Fig. 7B) ($n=8$).

A combination of indomethacin (5 μM) and mepyramine (1.5 μM) did not significantly ($P>0.05$) reduce the constrictor responses of sensitized guinea-pig perfused half-lungs to adenosine or methacholine. The reduction of ovalbumen approached, but did not achieve, statistical significance ($P=0.06$) (Fig. 8A) ($n=6$). The response of the tissue to histamine was abolished. The combination of indomethacin (5 μM) and mepyramine (1.5 μM) did not significantly ($P>0.05$) reduce the contractile response of the tracheas to adenosine, methacholine or ovalbumen. However, there was a significant ($P<0.05$) reduction in the response of the guinea-pig trachea to histamine (Fig. 8B) ($n=6$).

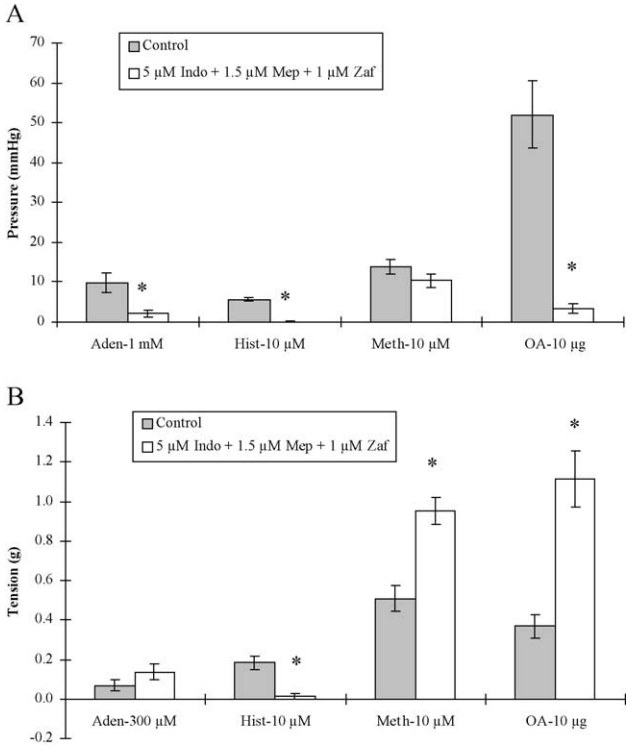

Fig. 9. Effects of a combination of indomethacin (Indo) (5 μM), mepyramine (Mep) (1.5 μM) and zafirlukast (Zaf) (1 μM) on the contractile responses of (A) paired lung halves and (B) paired tracheal spirals from sensitized guinea-pigs. Mean (± S.E.M) increases in perfusion pressure and or tension by adenosine (Aden, lung 1 mM, trachea 300 μM), histamine (Hist, 10 μM), methacholine (Meth, 10 μM) and ovalbumen (OA, 10 μg) in the absence (control) and in the presence of indomethacin, mepyramine and zafirlukast are shown (lung, $n=7$ and trachea, $n=8$). Tracheas were perfused in the presence of 3 μM 8-phenyltheophylline. * Denotes a significant difference from control ($P<0.05$).

3.3. Effects of three antagonists in combination on the bronchoconstrictor responses of the airways to adenosine and ovalbumen

Mepyramine (1.5 μM) in the combination with indomethacin (5 μM) and zafirlukast (1 μM) significantly ($P<0.05$) reduced the constrictor response of perfused half-lungs to adenosine and ovalbumen (Fig. 9A) ($n=7$). The response to histamine was abolished and there was no significant ($P>0.05$) effect on the constriction to methacholine. The combination of indomethacin (5 μM), mepyramine (1.5 μM) and zafirlukast (1 μM) did not significantly ($P>0.05$) reduce the contractile response in sensitized guinea-pig superfused trachea to adenosine. However, the response of the trachea to histamine was virtually abolished. In contrast, the responses to methacholine and ovalbumen were significantly ($P<0.05$) potentiated by this combination (Fig. 9B) ($n=8$).

A combination of diclofenac (5 μM), mepyramine (1.5 μM) and zafirlukast (1 μM) significantly ($P<0.05$) reduced the responses of perfused sensitized half-lungs to adenosine, histamine and ovalbumen (Fig. 3B). The response to methacholine was not inhibited (Fig. 3B) ($n=6$).

4. Discussion

In vivo challenge of sensitized guinea-pigs to inhaled adenosine (Lewis et al., 1994; Thorne and Broadley, 1994) or ovalbumen (Busse et al., 1993; Kageyama et al., 1996) results in a bronchoconstriction. In isolated perfused lungs and superfused tracheal spirals, these responses have been seen as increases in perfusion pressure and tension, respectively (Thorne and Broadley, 1992; Thorne et al., 1996), and this was confirmed in the present study. The possible roles of histamine, leukotriene and prostaglandins in these contractile responses were examined in the present study by use of appropriate antagonists. The histamine H_1 receptor antagonist mepyramine, the leukotriene receptor antagonist zafirlukast, the 5-lipoxygenase inhibitor zileuton, or the cyclooxygenase inhibitors indomethacin and diclofenac were used alone or in combination. None of these antagonists alone had a significant inhibitory action on the bronchoconstrictor responses to adenosine or ovalbumen.

Mepyramine did not inhibit the responses to adenosine or antigen in either the lungs or the trachea. This is in agreement with Takami and Tsukada (1998) and Jonsson and Dahlen (1994), who showed that histamine receptor antagonism with mepyramine had no effect on the antigen response in sensitized guinea-pig isolated lungs. There is mixed clinical evidence as to the relative importance of each mediator involved in the inflammatory response. There is some clinical evidence to support the hypothesis that histamine receptor antagonists alone are not capable of preventing allergen-induced bronchoconstriction. This is in spite of histamine mediating most of effects on airway function

through histamine H_1 receptors. Even when given in large doses, potent histamine H_1 receptor antagonists, such as terfenadine and loratadine, have clinical effects in allergic rhinitis but are far from effective in asthma patients (Van Ganse et al., 1997). Together, this clinical evidence suggests that although histamine is produced from mast cells in asthmatic airways other mediators are also responsible for the bronchoconstrictor response.

The leukotriene receptor antagonist zafirlukast causes a 100-fold shift to the right of the leukotriene D_4 concentration–response curve in humans (Chanarin and Johnston, 1994) and is effective against early and late phase responses to allergens, exercise-induced challenge, cold-induced asthma and chronic asthma (Spector, 1997). However, in the isolated lung and trachea examined here, zafirlukast did not inhibit the responses to either adenosine or antigen nor did it have any non-specific effects on the tracheal responses to histamine or methacholine. This in agreement with Heaslip et al. (1993), who state that the leukotriene D_4 receptor antagonist, LY171883, only inhibited antigen-induced responses in guinea-pig isolated tracheas at high concentrations. The effectiveness as an inhibitor of the leukotriene receptor in our model, however, was shown by inhibition of the small contractile responses to leukotriene D_4.

Zileuton, a novel lipoxygenase inhibitor, alone did not cause a reduction in the response of the lungs or trachea to either adenosine or ovalbumen. This was in agreement with Heaslip et al. (1993), who state that high concentrations of zileuton only produced a partial inhibition of antigen-induced contractions of sensitized guinea-pig tracheas. Abraham et al. (1992), using an in vivo sheep model, tested the effects of zileuton on bronchospasm in the absence of any other inhibitors. They found that zileuton had a significant inhibitory effect against the late phase response, which has a large leukotriene component, but did not inhibit the early phase, which has a significant histamine component. The contraction of the isolated tissues to ovalbumen in the present study may be considered as equivalent to an early phase in vivo response since responses involve substantial bronchoconstriction with an immediate onset. Zileuton did not have any non-specific effects on the spasmogens histamine or methacholine at the concentration used. This is in agreement with Malo et al. (1994), who showed that in guinea-pig tracheal muscle, zileuton had no effect on contractions to acetylcholine, prostaglandin D_2 or the thromboxane A_2 agonist, U-44069.

The cyclooxygenase inhibitor, indomethacin, did not inhibit the responses to adenosine or ovalbumen. This is in agreement with Jonsson and Dahlen (1994), who also state that in isolated lungs, indomethacin failed to inhibit the antigen response. Indomethacin consistently caused an increase in the responsiveness of the trachea not only to adenosine and ovalbumen but also to histamine and methacholine, although this was only significant in Fig. 9B. This observation was not reported by Caparrotta et al. (1984), who state that the adenosine analogue, R-phenylisopropyladenosine, induced a contraction of non-sensitized isolated trachea that was inhibited by indomethacin, or Dunlop and Smith (1976), who report than the in vitro antigen-induced contraction of sensitized human bronchus can be inhibited by indomethacin. One possible explanation for the augmented responses of the isolated trachea to spasmogens in the presence of indomethacin could be that arachidonic acid is diverted to the production of leukotrienes in the absence of the cyclooxygenase pathway and these induce a non-specific hyperresponsiveness on the tissue. Prior treatment of guinea-pig tracheal muscle with leukotriene E_4 has been shown to enhance the responses to histamine and inhaled leukotrienes C_4 and D_4 also cause bronchial hyperresponsiveness to methacholine and histamine (O'Hickey et al.,1991). However, since the potentiation of responses was most marked when indomethacin was combined with the leukotriene receptor antagonist zafirlukast (Fig. 9B), this explanation seems unlikely. In view of this tendency for indomethacin to potentiate the responses, we examined an alternative cyclooxygenase inhibitor, diclofenac (Insel, 1990), in the lungs only. Alone, this agent also failed to block the responses to adenosine and ovalbumen. To test for the effectiveness of indomethacin, an attempt was made to block arachidonic acid-induced contractions (Fig. 2B); however, as with other responses, the trend was for potentiation. That cyclooxygenase was blocked can only be deduced from the fact that the concentrations of indomethacin and diclofenac were as found to be effective by others (Devillier et al., 2001; Przybilla et al., 1987, respectively) and that there was an effect (i.e. potentiation of the responses).

Since none of the inhibitors of the three mediators, histamine, leukotrienes or prostaglandins on their own inhibited the responses of the allergic airways to adenosine or ovalbumen, it might appear that these mediators were not involved in the bronchoconstriction. It could be that although the effect of one mediator was being inhibited, the effects of the remaining mediators were sufficient to produce a sizeable response sufficient to mask any inhibition caused by the single receptor antagonist. In the case of indomethacin and zileuton, any blockade of cyclooxygenase or lipoxygenase would simply result in the conversion of arachidonic acid to prostaglandins and leukotrienes, respectively, and therefore no net reduction in response. Indomethacin inhibits the biosynthesis of prostaglandin E_2, which is a potent inhibitor of mediators released from human (Schulman, 1986) and rat (Metcalfe et al., 1997) mast cells, and thus its decreased production may heighten the release of other mediators, so counteracting the overall effect of the cyclooxygenase inhibitor.

The bronchoconstrictor responses to adenosine and antigen were reduced but not abolished by a combination of zafirlukast and mepyramine (lungs and trachea) but not by a combination of zileuton and mepyramine (lungs and trachea) or zafirlukast and indomethacin (lungs and trachea). These results suggest that leukotrienes must be blocked in addition to histamine if there is to be a net inhibition of both the

adenosine and ovalbumen response. These results were in agreement with the findings of Jonsson and Dahlen (1994), who showed that when 5-lipoxygenase inhibition or leukotriene receptor antagonism were combined with histamine receptor antagonism, there was a major reduction of the antigen response, leaving only a small residual response. Thus, although the antagonists may have little effect on the antigen or adenosine responses in isolation, a combination of antagonists together may have inhibitory effects.

Finally, we have shown that although a combination of two antagonists may reduce the response to adenosine and antigen in the lung and trachea (mepyramine and zafirlukast), to virtually abolish the response in the lung, it was necessary to include a combination of all three types of antagonists. The result with ovalbumen in the lung was in agreement with Malo et al. (1994), whose in vitro studies showed that inhibition of leukotriene production with zileuton caused a reduction in the antigen-induced contraction of sensitized guinea-pig tracheal muscle when in the presence of mepyramine and the cyclooxygenase inhibitor meclofenamic acid. Our result in the trachea, however, differs since the three-inhibitor combination failed to inhibit either adenosine or ovalbumen. This was presumably due to the opposing non-selective potentiation of the constrictor responses by the indomethacin.

Indomethacin also causes a reduction in the adenosine (Crimi et al., 1989) and antigen response (Dunlop and Smith, 1976) in sensitized human bronchus. In addition, Phillips and Holgate (1989) have shown that in human asthmatic subjects, oral administration of flurbiprofen, a potent cyclooxygenase inhibitor, causes a 32% inhibition of 5′-AMP-induced bronchoconstriction. This indicates that cyclooxygenase products, most likely prostaglandin D_2, do have a role to play. Interestingly, when flurbiprofen was given in conjunction with an histamine H_1 receptor antagonist, which in isolation causes a 50% inhibition of the 5′-AMP-mediated bronchoconstriction, the total inhibition was only 60%. This could be because prostaglandin D_2 provides an endogenous signal which, under normal circumstances, serves to potentiate histamine release. This evidence provides a strong case for mast cell-derived histamine as the major component of adenosine-induced bronchoconstriction in asthmatic subjects. However, in this study, for effective inhibition of the in vitro response to adenosine, the leukotriene component must also be inhibited in addition to the histamine component. This suggests that leukotrienes play a major role in the in vitro adenosine response. These results also agree with Sladek et al. (1990), who state that in asthmatics undergoing allergen inhalation, cyclooxygenase products are likely to play only a marginal role in allergic bronchoconstriction as indomethacin failed to affect the reduction in pulmonary function despite inhibiting thromboxane release, while leukotriene E_4 release was unaffected by indomethacin.

Adenosine enhances antigen-induced mast cell degranulation in sensitized/atopic human (Forsythe et al., 1999) and rat (Reeves et al., 1997) tissue, and may therefore contribute the inflammatory changes observed in asthma. Furthermore, the putative mast cell stabilizers, nedocromil sodium (Crimi et al., 1986, 1987) and sodium cromoglycate (Phillips and Holgate, 1989; Church et al., 1991) inhibit the response in human asthmatics. The mast cell would therefore appear to be the likely source of the major mediators, including histamine, leukotriene and prostaglandin, shown in the present study to be involved in the bronchoconstriction by adenosine. The effects of these mast cell stabilizers were not investigated in the present study of guinea-pig isolated tissues as they are ineffective against mast cells from the guinea-pig (Pearce et al., 1989).

Finally, recent advances have suggested a role for adenosine receptor antagonists in the treatment of asthma. The therapeutic effectiveness of enprofylline and other xanthines in human asthma may, in part, be related to the inhibition of adenosine receptors (Pauwels and Joos, 1995), since enprofylline has been found to be a selective adenosine A_{2B} receptor antagonist (Muller et al., 1998). The bronchoconstriction to inhaled 5′-AMP or adenosine in human asthmatics has been shown to be antagonized by theophylline administered by inhalation (Cushley et al., 1983) and orally (Mann et al., 1985; Crimi et al., 1989), suggesting that purine receptors are involved. However, we have failed to antagonize the bronchoconstriction in sensitized guinea-pig airway tissue with the P_1-purinoceptors antagonist 8-phenyltheophylline (Thorne and Broadley, 1992) or in vivo (Spruntulis and Broadley, 2001). Thus, in the present study, we have examined the constriction both in the absence (lungs) and the presence of 8-phenyltheophylline (trachea). Essentially, there was no difference in the profile of antagonism by the various mediator inhibitors whether 8-phenyltheophylline was present or not. Since adenosine A_3 receptors are reputed to be relatively resistant to antagonism by xanthines, such as 8-phenyltheophylline, (Linden, 1994), we conclude that the response is predominantly due to a methylxanthine resistant receptor, possibly adenosine A_3. This response appears to be due to the release of a combination of histamine, leukotrienes and prostaglandins. The remarkable parallel in profile of antagonism between adenosine and ovalbumen suggests that the mediators involved are virtually identical.

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