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Contractile responses to adenosine, R-PIA and ovalbumen in passively sensitized guinea-pig isolated airways

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1 Responses to adenosine, R-PIA and ovalbumen were examined in guinea-pig isolated superfused tracheal spirals to determine the effects of passive sensitization by overnight incubation in serum from ovalbumen (OA)-sensitized or non-sensitized guinea-pigs.

2 Tissues incubated with serum from non-sensitized and OA-sensitized guinea-pigs contracted (0.07 ± 0.02 and 0.04 ± 0.01 g, respectively) to adenosine (300 μM) whereas non-incubated or Krebs-incubated tissues produced no contractions to adenosine or ovalbumen (10 μg). Ovalbumen caused substantial contractions (0.40 ± 0.09 g) after OA-sensitized serum incubation and significantly ($P < 0.05$) smaller contractions (0.08 ± 0.03 g) after non-sensitized serum incubation. Tracheae from guinea-pigs actively sensitized to ovalbumen 14–21 days beforehand also contracted to adenosine, R-PIA (3 μM) and ovalbumen.

3 The A_1/A_2 adenosine receptor antagonist, 8-phenyltheophylline (8-PT, 3 μM), failed to antagonize these contractions, suggesting that A_1/A_2 adenosine receptors were not involved.

4 Unlike adenosine, R-PIA (3 μM) produced contractions in non-incubated (0.23 ± 0.04 g) or Krebs-incubated (0.15 ± 0.04 g) tracheae, as well as after passive and active sensitization. None of these responses were blocked by 8-PT.

5 The A_3 receptor agonist, IB-MECA, in the presence of 8-PT produced small contractions in passively sensitized tracheae (10 μM, 0.02 ± 0.003 g) and, in larger doses (100 μM and 1 mM), contracted actively sensitized tracheae.

6 In actively sensitized trachea, the A_3 receptor antagonist, MRS-1220 (100 nM), significantly ($P < 0.05$) attenuated adenosine contractions in the presence of 8-PT from 0.23 ± 0.07 g to 0.07 ± 0.03 g.

7 These results show that passive, like active sensitization, reveals bronchoconstrictions to adenosine of isolated tracheae. The insensitivity to 8-PT blockade, the antagonism by MRS-1220, and the fact that the A_3 receptor agonist, IB-MECA, mimics this response, suggest involvement of A_3 receptors. R-PIA, however, has a different profile of adenosine receptor activity.
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Keywords: Adenosine; guinea-pig tracheal spirals; ovalbumen; passive sensitization; R-PIA; bronchoconstriction

Abbreviations: 5′-AMP, 5′-adenosine monophosphate; DMSO, dimethylsulphoxide; IB-MECA, N^6-3-iodobenzyl-5′-N-methyl-carbamoyladenosine; MRS-1220, N-[9-chloro-2-(2-furanyl)[1,2,4,]-triazolo[1,5-c]quinazolin-5-beneneacetamide; 8-PT, 8-phenylethophylline; R-PIA, R-phenylisopropyladenosine; OA, ovalbumen; PEG, polyethyleneglycol

Introduction

Inhaled adenosine (or 5′-AMP) has little effect in normal subjects but causes bronchoconstriction in asthmatic and atopic non-asthmatics (Cushley *et al*., 1983). Similarly, inhalation of adenosine by sensitized guinea-pigs causes bronchoconstriction but non-sensitized animals show no response (Thorne & Broadley, 1994; Spruntulis & Broadley, 2001). The *in vitro* response of the airways to adenosine has been studied in isolated tissues from both sensitized and non-sensitized guinea-pigs. Adenosine usually causes a relaxation of non-sensitized guinea-pig tracheas (Brown & Collis, 1982; Darmani & Broadley, 1986). However, Advenier *et al*. (1982), Karlsson *et al*. (1982) and Farmer *et al*. (1988) have reported that adenosine can cause a small contraction of non-sensitized guinea-pig tracheas. In contrast, Pauwels & Van der Straeten (1987), Thorne & Broadley (1992), Lewis *et al*. (1994) and Thorne *et al*. (1996) have shown that adenosine causes a more substantial contraction of the ovalbumen sensitized guinea-pig isolated airways. The mechanism responsible for this phenomenon is not understood. The bronchoconstriction in asthmatics can be inhibited by theophylline, an adenosine receptor antagonist (Cushley *et al*., 1983). Bjorck *et al*. (1992) have shown enhanced bronchoconstrictor responses to adenosine of isolated bronchi from asthmatics, which are mediated via adenosine A_1 receptors. It has therefore been suggested by Ali *et al*. (1994) that there may be increased expression of A_1 receptors in the asthmatic airways. However, Walker *et al*. (1997) reported that A_3 receptor expression was increased in asthmatic lung eosinophils. There are conflicting reports on which adenosine receptor mediates the adenosine-induced bronchoconstriction. El-Hashim *et al*. (1996) and Ghai *et al*. (1987) claim an A_1 receptor involvement, whereas Thorne & Broadley (1992) and Kehoe & Broadley (1996) suggest that

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A_3 receptors mediate the response in sensitized guinea-pigs. Recently, Hannon *et al.* (2002) have suggested that a novel atypical receptor, distinct from the four known categorized adenosine receptors, mediates the bronchoconstriction in sensitized rats.

Sensitization of the animal to ovalbumen also results in a bronchoconstriction of guinea-pig airways to ovalbumen *in vivo* (Howell *et al.*, 1993; Thorne & Broadley, 1994; Spruntulis & Broadley, 2001) and *in vitro* (Thorne & Broadley, 1992; Lewis *et al.*, 1994; Broadley, 1995; Chabot-Fletcher *et al.*, 1995). Active sensitization of guinea-pigs has long been used as a technique for the study of airway responsiveness and inflammation *in vivo* after challenge of the animal with specific allergens (Busse *et al.*, 1993; Pretolani *et al.*, 1994; Danahay & Broadley, 1997). In active sensitization, animals are pretreated with a sensitizing medium to raise antibodies to the particular antigen, in our experiments ovalbumen, together with the adjuvant, aluminium hydroxide. In the procedure described for guinea-pigs by Andersson (1980), the immunoglobulins that were raised were of the E and G types. Exposure of sensitized airways to the specific antigen *in vitro* results in an anaphylactic response, the release of mast cell mediators and a contractile response (Metcalfe *et al.*, 1997).

Passive sensitization by incubation of isolated human lung tissue with non-sensitized or sensitized serum is an alternative sensitization process (Tunon de Lara *et al.*, 1995), which has been used in a number of studies to investigate the mediators and mechanisms involved in allergic reactions (Rabe, 1998; Schmidt *et al.*, 2000). In passive sensitization, the isolated tissue from a non-sensitized subject is incubated with serum containing elevated levels of antibodies to a specific allergen. This is sufficient to cause the passive transfer of specific immunoglobulins from the sensitizing serum, which bind with high affinity to solubilized Fc receptors on the surface of mast cells in non-sensitized tissues. Mast cell activation by interaction of a multivalent antigen with its specific antibody attached to the cell membrane *via* its high affinity FcRI receptor brings about mediator release (Buisseret, 1982; Metcalfe *et al.*, 1997). Human allergic, immediate hypersensitivity reactions are mainly mediated through the IgE subtype, whereas the serum collected from guinea-pigs sensitized using the technique described by Andersson (1980), will have raised levels of IgE and IgG.

In this study, the technique of passive sensitization was therefore developed in guinea-pig isolated trachea to establish whether, like active sensitization, it revealed a contractile response to adenosine and the adenosine analogue R-PIA. Guinea-pigs were selected because the active sensitization protocols adopted have been well-characterized in this species and shown to generate both IgE and IgG antibodies (Andersson, 1980), a situation that closely approximates the human allergic condition. The responses of the tracheas to ovalbumen were used to establish effective passive sensitization. Since passive sensitization may also lead to a change in smooth muscle unit type that could lead to an increased responsiveness of the tissues to a variety of stimuli (Kroeger & Stephens, 1975; Kannan *et al.*, 1983), the responses to the spasmogens histamine and methacholine were also examined.

Methods

Subjects

Male Dunkin-Hartley guinea-pigs weighing 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.

Active sensitization procedure

Guinea-pigs were actively sensitized to ovalbumen (10 μg ml^{-1}) plus aluminium hydroxide (100 mg ml^{-1}) in sodium chloride (0.9%). The suspension 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 IgE- and IgG-antibodies.

Passive sensitization procedure

Blood was collected from non-sensitized or actively sensitized guinea-pigs into polypropylene centrifuge tubes immediately after cervical dislocation. The blood was left to clot before being centrifuged (Mistral 3000, Fisons, Loughborough, U.K.) at $200 \times g$ for 10 mins at 21°C. After spinning, the serum was removed and a 1 : 10 dilution was made with Krebs-bicarbonate solution. The serum was diluted to avoid the excessive formation of bubbles during subsequent incubation and aeration.

The tracheae were removed from non-sensitized guinea-pigs, cut spirally (Constantine, 1965), and divided in two pieces of length 3–4 cm. One half of the non-sensitized trachea was then incubated in the serum solution for 24 h at room temperature while gassing with 5% CO_2 in 95% O_2. Experiments were also performed after incubating overnight in Krebs solution or in non-incubated tracheal spirals, set up immediately after killing. Tracheae used in non-incubated experiments were paired with tissues from the Krebs-incubated experiments and tracheae incubated in serum from non-sensitized animals were paired with tracheae incubated in sensitized serum.

The levels of antibodies in the serum were not measured directly. However, the tracheae from the sensitized guinea-pigs from which the serum was taken were routinely tested for ovalbumen sensitivity. If ovalbumen caused an *in vitro* bronchoconstriction this was a strong indicator that the animal had been successfully actively sensitized and that the antibody titre of the serum was raised. Serum was not used for incubation if there was a lack of an *in vitro* constriction to ovalbumen in the donor trachea.

Tissue set-up

After incubation, or in the case of the experiments investigating the responses of actively sensitized or non-sensitized guinea-pig tracheae which required no incubation, tracheae spirals were suspended in a heated jacket. They were superfused 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. 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). Changes in tension were recorded on a Devices MX8 polygraph (Ormed, Welwyn Garden, Herts, U.K.). Intrinsic tone was induced by allowing the spirals to equilibrate under an applied tension of 1 g for 30–45 mins.

Drug administration

After equilibration, agonists were added to the trachea by slow infusion. Single doses of adenosine (300 μM) or the adenosine analogues R-PIA (3 μM) or IB-MECA (100 μM) were superfused over the tracheal preparations, followed by single superfused doses of histamine (10 μM) and methacholine (10 μM). Dose-response curves to R-PIA (0.1–100 μM) and IB-MECA (100 μM–1 mM) were also constructed in actively sensitized guinea-pig isolated tracheae. Finally, all tissues were exposed to a single superfused dose of ovalbumen (10 μg) to check for passive or active sensitization. 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 Ltd., Edenbridge, Kent, U.K.). Subsequent doses were only added after the tissue had returned to its original baseline level. In experiments performed in the presence of the adenosine receptor antagonists (8-phenyltheophylline (8-PT) and MRS-1220), they were included in the Krebs-bicarbonate solution after the initial equilibration period of 30–45 mins, commencing 30 mins before and then throughout the addition of agonists.

Data analysis

Responses of the trachea were measured as the peak change in tension (g) and expressed as the mean ± s.e.mean. Data were analysed for statistical significance by a paired or unpaired Student's *t*-test as appropriate. A *P*-value of less than 0.05 was taken to indicate a significant difference.

Drugs

8-phenyltheophylline, acetyl-methylcholine chloride (methacholine), adenosine, histamine (diphosphate salt), ovalbumen and R-phenylisopropyladenosine (R-PIA) were obtained from Sigma (Poole, Dorset, U.K.). N-[9-chloro-2-(2-furanyl)[1,2,4]-triazolo[1,5-c]quinazolin-5-benzeneacetamide (MRS-1220) and N^6-3-iodobenzyl-5′-N-methylcarbamoyladenosine (IB-MECA) were supplied by Tocris, U.K. Aluminium hydroxide was supplied by BDH (Poole, Dorset, U.K.). All drug stock solutions were made up in distilled water, with the exception of 8-PT which was dissolved in 0.1 M NaOH and MRS-1220 and IB-MECA which were dissolved in dimethylsulphoxide (DMSO) and 50/50 polyethyleneglycol (PEG)/distilled water respectively. DMSO and PEG were supplied by Sigma, U.K. All drugs were then serially diluted in Krebs-bicarbonate solution.

Results

Effect of 24 h sham incubation with Krebs solution on responses to adenosine

In tissues that received no incubation or a 24 h Krebs incubation there was no significant ($P>0.05$) response to adenosine or ovalbumen. Histamine and methacholine caused contractions in both non-incubated trachea and 24 h Krebs-incubated trachea (histamine, 0.25 ± 0.02 and 0.24 ± 0.05 g, respectively; metacholine, 0.44 ± 0.03 and 0.45 ± 0.03 g). There was no significant ($P>0.05$) difference in spasmogen responsiveness between the two groups (Figure 1). In the presence of 3 μM 8-PT, the responses of the trachea were similar to those described above (data not shown). There were no responses to adenosine or ovalbumen and histamine and methacholine contractions did not differ between non-incubated and 24 h Krebs-incubated tracheae.

Effect of 24 h incubation with non-sensitized and sensitized serum on the responses to adenosine

Tissues incubated with serum from non-sensitized or ovalbumen sensitized guinea-pigs displayed contractile responses to adenosine (non-sensitized serum-incubated 0.07 ± 0.02 g; sensitized serum-incubated 0.04 ± 0.01 g) and ovalbumen

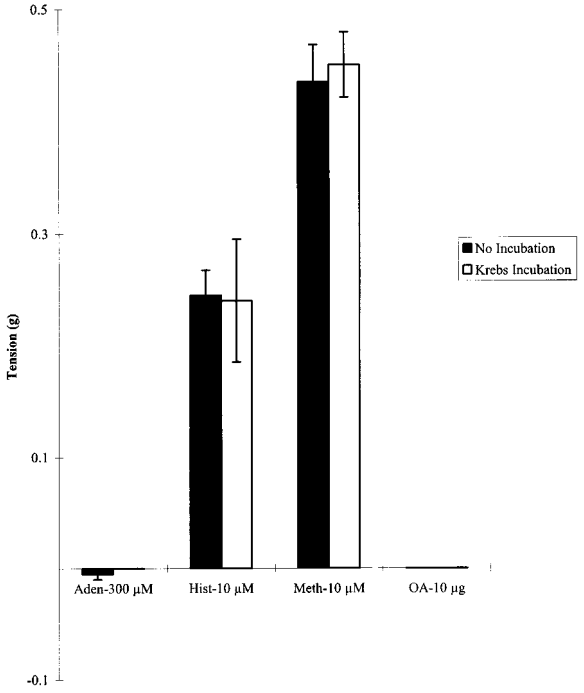

Figure 1 Effects of adenosine (Aden, 300 μM), histamine (Hist, 10 μM), methacholine (Meth, 10 μg) and ovalbumen (OA, 10 μg) on guinea-pig isolated tracheae incubated in Krebs solution for 24 h (open histograms) compared with non-incubated tissues (solid histograms). Responses are the mean changes ($n=5$) in tension (g) with error bars representing the s.e.m. Tissues are in the absence of 8-PT.

(0.08±0.03 and 0.40±0.09 g) in the absence of 8-PT. These contractions were significantly different ($P<0.05$, Student's unpaired *t*-test) from those in non-incubated or Krebs-incubated tracheae shown in Figure 1. Histamine and methacholine also caused contractions in both non-sensitized and sensitized serum-incubated tracheae (histamine, 0.29±0.05 and 0.31±0.03 g; methacholine, 0.50±0.08 and 0.45±0.02 g). There was no significant ($P>0.05$) difference in adenosine, histamine and methacholine responsiveness between the non-sensitized and sensitized serum incubated tracheae. The response to ovalbumen in the tracheae incubated with sensitized serum was, however, significantly ($P<0.05$) greater than in the non-sensitized serum-incubated tracheae (Figure 2A). The responses to ovalbumen and adenosine in the non-sensitized serum incubated tracheae did not differ significantly ($P>0.05$). The ovalbumen response of the tracheae from the sensitized donors was 0.41±0.07 g.

In the presence of 3 μM 8-PT to block A_1/A_2 purinoceptors, tissues that were incubated with serum from non-sensitized or ovalbumen-sensitized guinea-pigs contracted in response to adenosine (non-sensitized serum-incubated 0.04±0.01 g; sensitized serum-incubated 0.03±0.01 g) and ovalbumen (0.04±0.01 and 0.33±0.07 g). Histamine and methacholine also caused a contraction in both non-sensitized and sensitized serum-incubated tracheae (histamine, 0.23±0.03 and 0.25±0.03 g; methacholine, 0.49±0.10 and 0.44±0.05 g). There was no significant ($P>0.05$) difference in adenosine, histamine and metacholine responses between the non-sensitized and sensitized serum incubated tracheae. The contractions to ovalbumen in tracheae incubated with sensitized serum was, however, significantly ($P<0.05$) greater than in the non-sensitized serum incubated tracheas (Figure 2B). The ovalbumen response of the trachea from the sensitized serum donors was 0.58±0.16 g. There were no significant ($P>0.05$, Student's unpaired *t*-test) differences for any responses obtained in the absence (Figure 2A) or presence (Figure 2B) of 3 μM 8-PT.

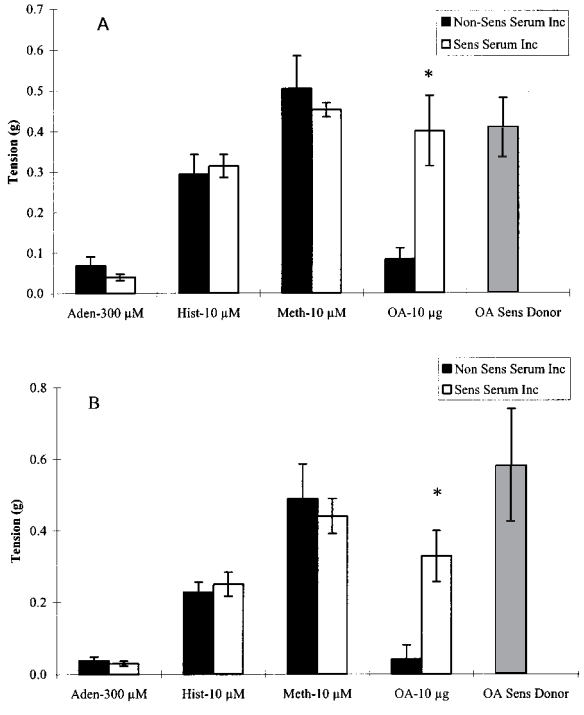

Figure 2 Effects of passive sensitization by incubation with serum from non-sensitized (solid histograms) and ovalbumen sensitized guinea-pigs (open histograms) on the responses of guinea-pig tracheae to adenosine (Aden, 300 μM), histamine (Hist, 10 μM), methacholine (Meth, 10 μg) and ovalbumen (OA, 10 μg). Also shown is the ovalbumen response of the tracheae from sensitized animals used to provide the serum for passive sensitization (hatched histogram, Donor). Responses are the mean increases ($n=5$) in tension (g) with error bars representing the s.e.m. *Denotes a significant ($P<0.05$) difference between sensitized and non-sensitized serum, determined by Student's paired *t*-test. Tissues were in the absence (A) or the presence (B) or 8-phenyltheophylline (8-PT, 3 μM).

Effect of 24 h sham incubations with Krebs solution on responses to R-PIA

In tissues that received no incubation or Krebs 24 h incubation, there was a sizeable response to R-PIA (non incubated 0.23±0.04 g; 24 h Krebs incubated 0.15±0.04 g) in the absence of 8-PT. There were, however, no responses to ovalbumen. Histamine and methacholine caused contractions in both non-incubated and 24 h Krebs-incubated tracheae (histamine, 0.41±0.08 and 0.23±0.07 g; methacoline, 0.80±0.07 and 0.43±0.11 g). The histamine response was significantly ($P<0.05$) greater in the non-incubated than in the Krebs-incubated tissues, but there was no significant ($P>0.05$) difference in the responsiveness to R-PIA or methacholine (Figure 3A).

In the presence of 8-PT, tissues that received no incubation or 24 h Krebs-incubation revealed sizeable contractile responses to R-PIA (non-incubated 0.13±0.01 g; 24 h Krebs-incubated 0.08±0.03 g). There were, however, no responses to ovalbumen. Histamine and methacholine caused contractions in both non-incubated and Krebs-incubated tracheae (histamine, 0.34±0.04 and 0.15±0.02 g; methacoline, 0.68±0.06 and 0.36±0.06 g). The responses to histamine and methacholine were significantly ($P<0.05$) less after 24 h Krebs-incubation, but there was no significant ($P>0.05$) differences in the contraction to R-PIA (Figure 3B).

Effect of 24 h incubation with non-sensitized and sensitized serum on responses to R-PIA

In tissues incubated with serum from non-sensitized or sensitized guinea-pigs, there were sizeable contractile response to R-PIA (non-sensitized serum-incubated, 0.10±0.03 g; sensitized serum incubated, 0.08±0.01 g) in the absence of 8-PT. Histamine and methacholine also caused contractions in both non-sensitized and sensitized serum-incubated tracheae (histamine, 0.17±0.05 and 0.17±0.05 g; methacholine, 0.44±0.11 and 0.35±0.06 g). There was no significant ($P>0.05$) difference in R-PIA, histamine or methacholine responses between the non-sensitized and sensitized serum-incubated tracheae. There was no contraction to ovalbumen in the tracheae incubated with serum from non-sensitized guinea-pigs but a significant ($P<0.05$) contraction in tissues incubated in serum from ovalbumen sensitized guinea-pigs (0.28±0.07 g) (Figure 4A). The ovalbumen response of the tracheae from the sensitized serum donors was 0.42±0.10 g.

In the presence of 8-PT, tissues that were incubated with serum from non-sensitized or sensitized guinea-pigs revealed sizeable contractile responses to R-PIA (non-sensitized serum

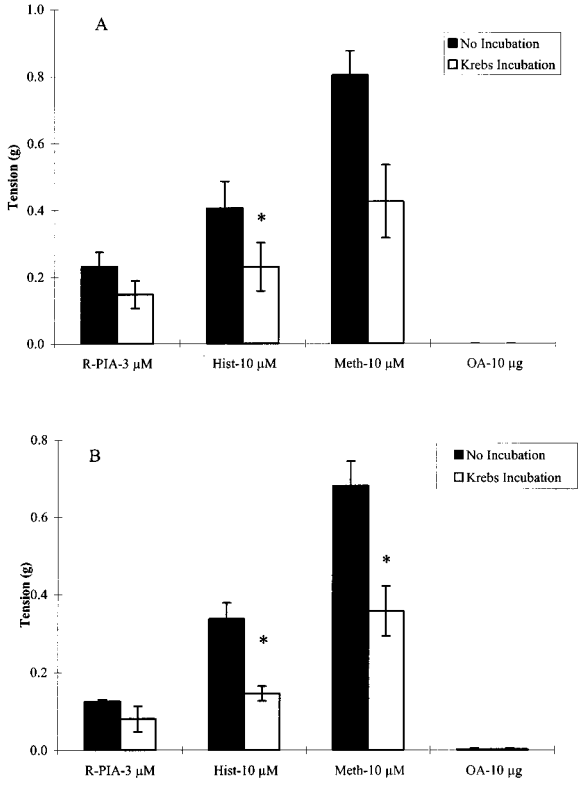

Figure 3 Effects of R-PIA (3 μM), histamine (Hist, 10 μM), methacholine (Meth, 10 μg) and ovalbumen (OA, 10 μg) on guinea-pig isolated tracheae incubated in Krebs-solution for 24 h (open histograms) compared with non-incubated tissues (solid histograms). Responses are the mean changes ($n=5$) in tension (g) with error bars representing the s.e.m. *Denotes a significant ($P<0.05$) difference between non-incubated and 24 h Krebs-incubated tissues, determined by Student's paired t-test. Tissues were in the absence (A) or presence (B) of 8-phenylehtophylline (8-PT, 3 μM).

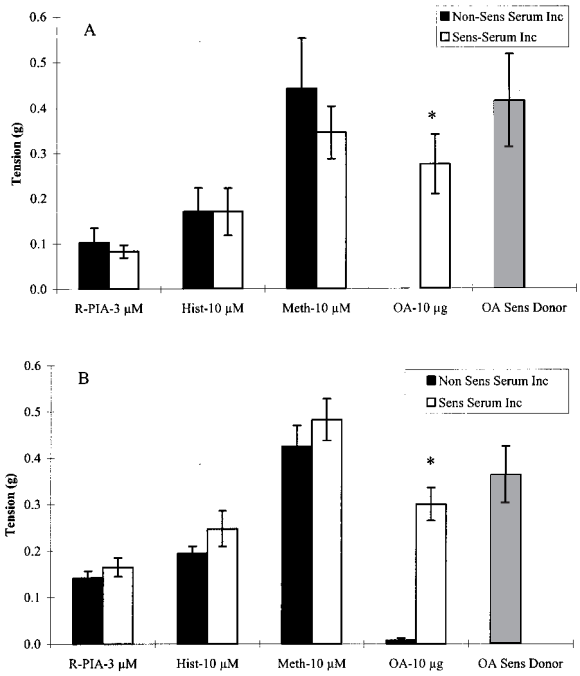

Figure 4 Effects of passive sensitization by incubation with serum from non-sensitized (solid histogram) and ovalbumen-sensitized guinea-pigs (open histogram) on the responses of guinea-pig paired tracheae to R-PIA (3 μM), histamine (Hist, 10 μM), methacholine (Meth, 10 μM) and ovalbumen (OA, 10 μg). Also shown is the ovalbumen response of the tracheae from actively sensitized animals used to provide the serum for passive sensitization (hatched histogram, Donor). Responses are the mean increase ($n=5$) in tension with error bars representing s.e.m. *Denotes a significant ($P<0.05$) difference from non-sensitized serum incubation, determined by Student's paired t-test. Tissues were in the absence (A) or in the presence (B) of 8-phenyltheophylline (8-PT, 3 μM).

incubated 0.14 ± 0.01 g; sensitized serum incubated 0.17 ± 0.02 g). Histamine and methacholine also caused contractions in both non-sensitized and sensitized-serum incubated tracheae (histamine, 0.20 ± 0.02 and 0.25 ± 0.04 g; methacholine, 0.43 ± 0.05 and 0.48 ± 0.05 g). There was no significant ($P>0.05$) difference in R-PIA, histamine or methacholine responses between the non-sensitized and sensitized serum-incubated tracheae. There was a substantial response to ovalbumen in the tracheae incubated in serum from sensitized animals (0.34 ± 0.04 g) which was significantly ($P<0.05$) larger than the minor response in the non-sensitized serum-incubated tissues (0.01 ± 0.01 g) (Figure 4B). The ovalbumen response of the tracheae from sensitized serum donors was 0.36 ± 0.06 g.

Effect of 24 h incubation with non-sensitized and sensitized serum on the responses to the adenosine A_3 receptor agonist IB-MECA

In tissues that received non-sensitized or sensitized serum incubation, small contractile responses were obtained to IB-MECA (10 μM) (non-sensitized serum incubated, 0.01 ± 0.01 g; sensitized serum incubated 0.02 ± 0.003 g) in the presence of 8-PT, which were not significantly different ($P>0.05$). Histamine and methacholine caused contractions in both non-sensitized and sensitized serum-incubated tracheae (histamine, 0.21 ± 0.04 and 0.24 ± 0.06 g; methacholine, 0.48 ± 0.03 and 0.52 ± 0.10 g) which were not significantly ($P>0.05$) different in each case (Figure 5). Ovalbumen produced a substantial contraction in tracheae incubated in serum from sensitized guinea-pig (0.41 ± 0.10 g) which was significantly ($P<0.05$) greater than the small response seen in tracheae incubated with non-sensitized serum (0.02 ± 0.01 g). The ovalbumen response of the tracheae from sensitized serum donors was 0.35 ± 0.10 g.

Actively sensitized guinea-pig isolated trachea

In actively sensitized guinea-pig tracheae there was a contractile response to adenosine. This response was significantly ($P<0.05$) greater in the presence of 8-PT (0.06 ± 0.01 g) than in its absence (0.03 ± 0.01 g). The contractions to histamine, methacholine and ovalbumen were not significantly ($P>0.05$) different in the absence (0.2 ± 0.03, 0.39 ± 0.07 and 0.29 ± 0.02 g) and in the presence of 8-PT (0.17 ± 0.04, 0.42 ± 0.05 and 0.27 ± 0.05 g) (Figure 6A).

R-PIA caused concentration-related contractions of actively sensitized guinea-pig trachea reaching a maximum

(0.13±0.01 g) at 10 μM. 8-PT significantly ($P<0.05$) increased the response to R-PIA. The maximum response (0.42±0.03 g) occurred at 30 μM and was of the same magnitude as the response to ovalbumen. 8-PT did not significantly ($P>0.05$) increase the response to either methacholine (0.42±0.05, 0.52±0.08 g) or ovalbumen (0.38±0.09, 0.33±0.09 g) (Figure 6B). IB-MECA, the adenosine A_3 receptor agonist, caused dose-related contractions of actively sensitized guinea-pig trachea. These contractions were not affected by 8-PT which also had no effect on the responses to methacholine (0.46±0.12, 0.49±0.15 g or ovalbumen (0.52±0.10, 0.41±0.11 g) (Figure 7).

In actively sensitized guinea-pig tracheae in the presence of 8-PT, the adenosine A_3 receptor antagonist, MRS-1220, significantly ($P<0.05$) reduced the contractile response to adenosine (control 0.23±0.07 g; MRS-1220, 0.07±0.03 g. MRS-1220 had no significant ($P>0.05$) effect on the responses to histamine (21±0.03 and 0.27±0.04 g), methacholine (0.50±0.05 and 0.54±0.08 g) or ovalbumen (0.37±0.03 and 0.41±0.06 g) (Figure 8).

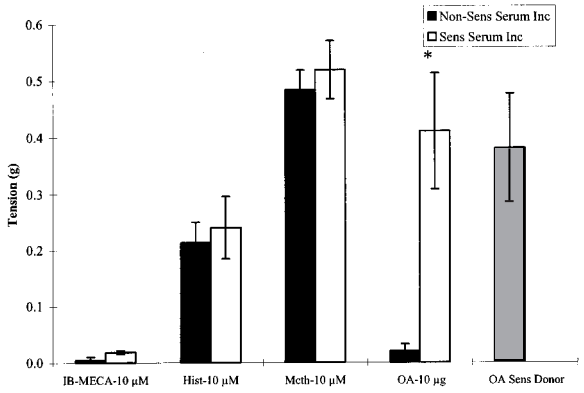

Figure 5 Effects of passive sensitization by incubation with serum from non-sensitized (solid histogram) and ovalbumen sensitized guinea-pigs (open histogram) on the response of guinea-pig paired tracheae to IB-MECA (10 μM), histamine (Hist, 10 μM), methacholine (Meth, 10 μg) and ovalbumen (10 μg) in the presence of 3 μM 8PT. Also shown is the ovalbumen response of the tracheae from actively sensitized animals used to provide the serum for passive sensitization (hatched histogram, Donor). Responses are the mean increases ($n=5$) in tension with error bars representing the s.e.m. *Denotes a significant ($P<0.05$) difference from non-sensitized serum incubation, determined by Student's paired t-test.

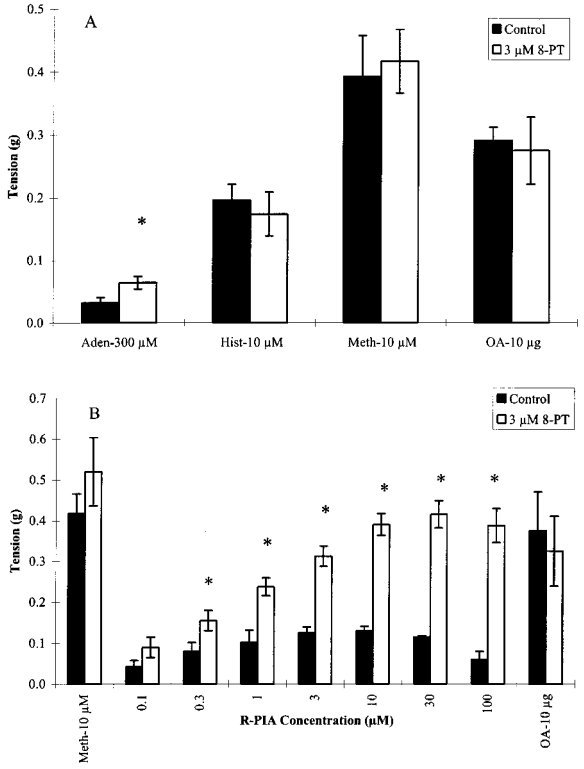

Figure 6 Effects of adenosine (300 μM) (A) and R-PIA (0.1–100 μM) (B) on paired tracheae from actively sensitized guinea-pigs in the absence (Control, solid histogram), and in the presence of 8-phenyltheophilline (8-PT, 3 μM, open histogram). Responses are compared with histamine (Hist, 10 μM), methacholine (Meth, 10 μM) and ovalbumen (OA, 10 μg). Responses are the mean increases ($n=5$) in tension with error bars representing the s.e.m. *Denotes a significant ($P<0.05$) difference from controls in the absence of 8-PT, determined by Student's paired t-test.

Discussion

This study confirms previous findings from these laboratories (Darmani & Broadley, 1986; Thorne & Broadley, 1992) and by others (Brown & Collis, 1982) which show that non-sensitized guinea-pig tracheae do not display a contractile

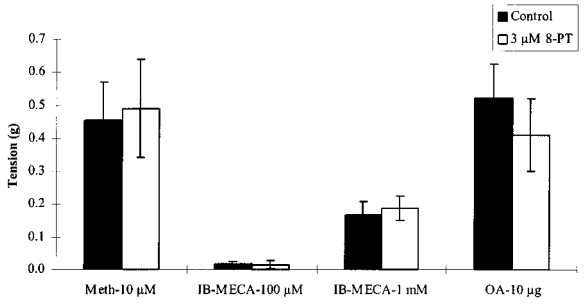

Figure 7 Effects of IB-MECA (100 μM and 1 mM), methacholine (Meth, 10 μM) and ovalbumen (OA, 10 μg) on paired tracheae from actively sensitized guinea-pigs in the absence (Control, solid histogram) and in the presence of (8-phenyltheophylline, 8PT, 3 μM, open histogram). Responses are the mean increase ($n=5$) in tension with error bars representing the mean s.e.m.

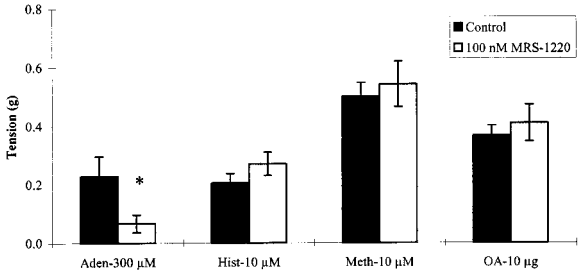

Figure 8 Effect of adenosine (Aden, 300 μM), histamine (Hist, 10 μM), methacholine (Meth, 10 μM) and ovalbumen (OA, 10 μM) on paired tracheae from actively sensitized guinea-pig in the absence (Control, solid histogram) and in the presence of 100 nM MRS-1220 (open histogram). Responses are the mean increase ($n=5$) in tension with error bars representing the s.e.m. Both tracheal halves were superfused with 8-phenyltheophylline, (8-PT, 3 μM) throughout. *Denotes a significant difference ($P<0.05$) from controls in the absence of MRS-1220, determined by Student's paired t-test.

response to adenosine. Other studies, however, have reported a small contractile response to adenosine in non-sensitized guinea-pig tissue before a dominant relaxatory response (Advenier *et al*., 1982; Karlsson *et al*., 1982; Farmer *et al*., 1988). This ability of tissues to exhibit a contractile or relaxatory response may depend on the initial state of tone. The degree of relaxation is lower in guinea-pig tissues with intrinsic tone compared with precontracted tissues (Darmani & Broadley, 1986).

Passive sensitization of the isolated tracheae by incubation with serum from sensitized guinea-pigs, however, revealed contractile responses to both adenosine and the sensitizing antigen, ovalbumen. These responses were similar to those observed in actively sensitized guinea-pig isolated trachea in this study and previously (Thorne & Broadley, 1992; Thorne *et al*., 1996). This was in agreement with Watson *et al*. (1997) and Schmidt *et al*. (2000), who showed that incubation of human trachea, with the allergen from *Dermatophagoides farinae*, induces a contractile response to the specific sensitizing allergen compared with non-sensitized tissues. They proposed that the degree of specificity was related to the concentration of allergen-specific IgE in the sensitizing serum. Black *et al*. (1989), Marthan *et al*. (1992), Ben-Jebria *et al*. (1993), Mitchell *et al*. (1994) and Watson *et al*. (1997) describe a non-specific increase in responsiveness of human tissue to agonists such as histamine, KCl and tachykinins which were not seen in the present study. Watson *et al*. (1997) suggested that hyperresponsiveness to histamine was unrelated to the concentration of specific IgE in the sensitizing serum but related to the level of total IgE in the serum. The total IgE level in the present study may therefore have been lower than used by Watson *et al*. (1997) possibly due to the 1 in 10 dilution before incubation. In retrospect, this was an advantage since the increase in sensitivity to adenosine could be distinguished from a general non-specific hyperresponsiveness. By using the active sensitizing technique described by Andersson (1980), levels of both IgE and IgG should be raised in the guinea-pig. Heating the serum from actively sensitized guinea-pigs to inactivate IgE and not IgG, does not remove its capacity to passively sensitize guinea-pig lung parenchymal strips *in vitro* (Souhrada & Souhrada, 1994). Thus, both IgE and IgG appear to be responsible for the passive sensitization. Further studies are required to evaluate whether heat treatment of serum from sensitized animals results in a reduced responsiveness to adenosine or ovalbumen. Although immunoglobulin levels were not measured directly in the present study, the tracheae of guinea-pigs from which sensitized serum was taken were routinely tested *in vitro* for ovalbumen sensitivity and consistently showed substantial contractile responses. The serum was not used for incubation if the trachea of the donor did not respond to ovalbumen with a contraction.

The contractions to adenosine and OA were not due to incubation alone as adenosine and ovalbumen had no effect in sham 24 h Krebs-incubated tissues. This lack of responsiveness in Krebs incubated tissues, was not due to non-specific loss of viability, as responses to histamine and methacholine were observed after by sham incubation. In some experiments, however, they were reduced compared with non-incubated tissues. This suggests that the tissue was still viable after a 24 h incubation and any small reduction in responsiveness during incubation was due to a small time-dependent deterioration of the tissue.

Passive sensitization involves the passive transfer of antibodies from sensitized guinea-pig serum to non-sensitized guinea-pig trachea (Berger *et al*., 1998; Rabe, 1998). Incubation of tissues with serum from animals sensitized to ovalbumen caused induction of a contractile response of the tissue to the specific antigen, ovalbumen. This was consistent with the concept that incubation of tissues with allergen-specific serum results in the loading of high affinity immunoglobulin receptors ($Fc_{\varepsilon}RI$) on mast cells (Watson *et al*., 1997). In humans, IgE alone does not mediate the allergic reaction, but it primes the mast cells. The surface of the human lung mast cell is studded with between 100,000 and 500,000 receptors for IgE molecules. When specific IgE antibodies are synthesized in the millions by the binding of an allergen they move through the blood stream to mast cells in connective tissue and become firmly fixed to the receptors (Buisseret, 1982). However, in passively sensitized human tissues, incubation alone is sufficient to induce sensitization *via* the transfer of specific immunoglobulins from the sensitized serum to receptors on the mast cell. Then, exposure of these airways to the specific allergen results in the antigen binding to the immunoglobulins fixed on mast cells (Watson *et al*., 1997). This results in degranulation of the mast cells, brought about by two adjacent IgE molecules on the surface of the mast cell being bridged by two epitopes on an antigen molecule. This results in the increased permeability of the membrane to calcium ions which enter the cell, activate phospholipase A_2, which then metabolizes phosphatidyl choline to lysophosphatidyl choline and arachidonic acid. The granules then move towards the surface of the cell because the calcium ions also activate enzymes that release energy and promote the assembly in the cell of microtubules. Contraction of these microtubules then cause the granules to move towards the cell membrane, fuse with it and discharge histamine (Buisseret, 1982). In addition to the preformed mediators, there are potent bronchoconstricting mediators released by antigen stimulation, including prostaglandins and leukotrienes (Metcalfe *et al*., 1997).

The IgE-dependence of this response has been demonstrated in human tissue by Tunon de Lara *et al*. (1995), who showed that IgG was unable to elicit this response. The importance of the IgE-mediated response has been shown in actively sensitized guinea-pigs (Andersson, 1980). Although a variety of cell types express the high affinity receptor for IgE ($Fc_{\varepsilon}RI$), such as basophils, monocytes and Langerhans cells, mast cells are the predominant cell type involved in passive IgE sensitization of human tissue (Berger *et al*., 1998). Berger *et al*. (1998) have also shown that passive sensitization reduces the number of cells bearing unoccupied IgE receptors and so there are increased cell numbers available for antigen stimulation. It has also been shown that incubation of isolated bronchi with serum containing a high concentration of IgE alters the mechanical response to a number of non-specific agonists such as histamine, KCl and tachykinins in human tissue (Black *et al*., 1989; Marthan *et al*., 1992; Ben-Jebria *et al*., 1993; Mitchell *et al*., 1994). Black *et al*. (1989) showed that passive sensitization of human bronchial tissue may be associated with increased potassium mobilization in airway smooth muscle. Alternatively, the attachment of the Fc fragment of IgE to mast cells or smooth muscle cells may

alter calcium flux through voltage-dependent channels, with a concomitant change in membrane potential (Souhrada & Souhrada, 1984).

These results show that guinea-pig tracheae incubated in serum from a sensitized guinea-pig also contract when exposed to adenosine. This was similar to the response seen in tissues from actively sensitized guinea-pigs in this study and previously (Thorne & Broadley, 1992; Thorne *et al*., 1996). More surprising, however, was the finding that tissues incubated overnight in serum from non-sensitized guinea-pigs were also capable of producing contractile responses to both adenosine and ovalbumen. Although the ovalbumen response was significantly smaller than that for tissues incubated in ovalbumen sensitized serum, it was still a sizeable response. The explanation for this response was not clear. It could be that although the serum has come from non-sensitized guinea-pigs, it contains a low, non-specific background level of immunoglobulins. Although the levels of antibodies are not great enough to produce a response to adenosine or ovalbumen *in vivo* or *in vitro* in the donor animal the low background concentration levels of antibodies could occupy sufficient immunoglobulin receptors on mast cells for subsequent stimulation by antigen. Maximal histamine release from rat basophil leukaemia cells is obtained when 10% of the receptors are bound, but a detectable response is achieved when only 0.03% receptors are bound (Menon *et al*., 1984). Also, the specificity of antibodies are not due to each antibody reacting exclusively with the inducing antigen; antibodies are therefore potentially multivalent in their reaction with antigen (Owen & Steward, 1996). Therefore, in the non-sensitized serum there would be antibodies present that may not be specific for ovalbumen, but have a recognizable site to which the ovalbumen antigen can bind. Further studies are required to examine whether an immune or non-immune principle is present to account for this potentiation of adenosine and ovalbumen responses, for example, by use of inactivation by heat or denaturation.

It was interesting that, although the ovalbumen response in tracheae incubated in non-sensitized serum was significantly smaller than in tracheae from either actively sensitized guinea-pigs (e.g. donors) or those incubated with sensitized serum, the contractile response to adenosine was virtually identical in the tracheae incubated in non-sensitized and sensitized serum. The adenosine response was also of the same magnitude as the ovalbumen response obtained after incubation in non-sensitized serum. This suggests that a common non-specific sensitization mechanism is required for adenosine and ovalbumen to induce contraction. The larger response obtained with ovalbumen in tissues incubated with ovalbumen-sensitized serum indicated a specific mechanism allowing a greater release of mediators. In non-sensitized serum incubated tissues, there was no non-specific increase in responsiveness to other spasmogens. This agrees with Watson *et al*. (1997) who have also reported a lack of non-specific hyperresponsiveness in human tissues that have been incubated with low levels of IgE-containing serum.

The constrictor response to adenosine of superfused tracheal spirals from actively sensitized guinea-pig was resistant to the blockade by 8-phenyltheophylline (8-PT), an antagonist of adenosine A_1 and A_2 receptors. This confirms previous observations from these laboratories (Thorne & Broadley, 1992; Thorne *et al*., 1996). Similarly, when the tracheal spirals incubated with sensitized guinea-pig serum were examined in the presence of 8-PT (3 μM), they still displayed a contraction to adenosine. Incubation of tracheas in non-sensitized serum also resulted in adenosine producing a contraction in the presence of 8-PT. Since the A_3 receptors of certain species are regarded as resistant to block by xanthines such as 8-PT (Linden, 1994), the receptors mediating the bronchoconstriction by adenosine could be of the A_3 subtype. To examine this further, the effect of a selective human A_3 receptor agonist, IB-MECA (Ezeamuzie & Philips, 1999) was examined in both actively and passively sensitized tracheae in the presence of 8-PT. IB-MECA at a dose of 10 μM caused small contractions in both non-sensitized and sensitized serum-incubated tissues. The concentration of IB-MECA was considered to be more than enough for A_3 receptor agonism as previous studies have shown significant responses at this concentration, including enhanced antigen-induced release of 5-hydroxytryptamine from rat mast cells (Reeves *et al*., 1997), neutrophil degranulation (Bouma *et al*., 1997) and inhibition of eosinophil peroxidase release from human eosinophils (Ezeamuzie & Philips, 1999). In actively sensitized guinea-pig trachea, larger concentrations produced concentration-related contractions that were not antagonized by 8-PT. To further determine whether A_3 receptors were involved in the bronchoconstrictor response to adenosine, the effect of the human A_3 receptor selective antagonist, MRS-1220 (Fredholm *et al*., 2001) was examined in actively sensitized guinea-pig tracheae. In the presence of 8-PT to block A_1/A_2 receptors, the contractile response was significantly inhibited by MRS-1220. The responses to histamine, methacholine and ovalbumen were unaffected. This, suggests that the responses to adenosine is probably mediated *via* A_3 receptors.

Unlike adenosine, the adenosine analogue, R-PIA, produced sizeable contractile responses in both non-incubated and 24 h Krebs-incubated tracheae. This was not expected as R-PIA is recognized as an adenosine analogue with affinity for P_1-purinoceptors (Dalziel & Westfall, 1994) and would therefore be expected to act in a similar way to adenosine, i.e. no contractile effect on naïve tissues but a contraction after sensitization (Thorne & Broadley, 1992). A contractile response to R-PIA in non-sensitized guinea-pig trachea has been reported by Caparrotta *et al*. (1984), who stated that the contraction was through A_1 receptors. R-PIA also caused a contractile response in actively sensitized guinea-pig tissue, and this response was potentiated in the presence of 8-PT, presumably through the blockade of the underlying relaxatory adenosine A_2 receptors. This confirms our earlier studies (Kehoe & Broadley, 1996; Thorne *et al*., 1996) where, since non-sensitized tissues were not examined, it was not recognized that they could produce contractile responses to R-PIA. The present study has therefore demonstrated that R-PIA exerts a contractile response in both unsensitized and passively and actively sensitized tracheae. This suggests that the contraction by R-PIA was not through the same mechanisms as adenosine or antigen. Whether R-PIA has an additional effect in sensitized tissue in a similar manner to adenosine through mast cell degranulation is not certain. Of significant interest was the fact that the contraction by R-PIA in both non-sensitized and sensitized tracheae was not antagonized by 8-PT, indicating that, like the response to adenosine, it was not mediated *via* A_1 or A_2 receptors. This is

contrary to the literature cited above, which shows that contractile responses to R-PIA are blocked, in guinea-pig isolated tracheal muscle, by 8-PT (Farmer *et al*., 1988). An A_1 receptor involvement in the contractile responses of guinea-pig actively sensitized lungs, to adenosine at least, is unlikely since the response was not inhibited by the selective A_1 antagonist, 8-cyclopentyltheophylline (Thorne *et al*., 1996). Further studies with a more selective A_1 receptor agonist such as N^6-cyclopentyladenosine are, however, warranted.

In conclusion, this study has demonstrated that passive sensitization of isolated tracheal preparations reveals a constrictor response to adenosine which is not present in non-sensitized tissues. This is comparable to active sensitization which also exposed a constrictor response (Thorne & Broadley, 1992; Thorne *et al*., 1996). The insensitivity of this response in both actively and passively sensitized tracheae to antagonism by the methylxanthine, 8-PT, the fact that the A_3 receptor agonist (IB-MECA) also causes a contractile response, albeit rather small, and the blockade of adenosine by the A_3 receptor antagonist, MRS-1220, suggest that the adenosine response is mediated *via* A_3 receptors. A contractile response to R-PIA in non-sensitized as well as in passively and actively sensitized tissues throws into doubt the adenosine receptor agonist profile of this analogue.

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