

Early- and late-phase bronchoconstriction, airway hyper-reactivity and cell influx into the lungs, after 5'-adenosine monophosphate inhalation: comparison with ovalbumin

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Summary

Background Antigen inhalation in atopic asthmatic patients results in an early asthmatic response (EAR), accompanied by a late asthmatic response (LAR) in 60% of patients. Inhaled 5'-adenosine monophosphate (5'-AMP) causes immediate bronchoconstriction in asthmatics but not in normal subjects.

Objectives The aims of this study were to investigate whether 5'-AMP can produce a LAR, airway hyper-reactivity (AHR) and cell influx to the lungs, in a sensitized guinea-pig model of asthma, and to compare with the profile of activity after ovalbumin (OVA) inhalation.

Methods Airway responses to inhaled OVA (10 µg/mL) and 5'-AMP (3 and 300 mM) of actively sensitized, conscious guinea-pigs were determined by whole body plethysmography as the change in specific airway conductance (sG_{aw}). Inhaled histamine (1 mM) was used to investigate AHR, and cell influx was determined by bronchoalveolar lavage (BAL).

Results Exposure to OVA revealed an EAR, and LAR at 6 h post-challenge. AHR to histamine occurred 24 h after challenge together with a significant increase in total and differential (eosinophils and macrophages) cell counts. Low dose 5'-AMP (3 mM) produced an EAR, LAR at 6 h after challenge, and AHR to histamine 12 h post-challenge. No AHR occurred 24 h after inhalation. Total and macrophage cell counts were increased significantly 6, 12 and 24 h after exposure. Bronchodilatation followed high dose 5'-AMP (300 mM), followed by a LAR at 6 h. AHR to histamine occurred 12 h after challenge, but not at 24 h. A significant increase in total and differential (eosinophils and macrophages) cell counts occurred 6, 12 and 24 h post-exposure. No changes were observed in non-sensitized guinea-pigs.

Conclusion OVA challenge revealed an EAR, LAR, cell influx and AHR in a guinea-pig model of asthma. This study demonstrated for the first time that a LAR and AHR to histamine can be revealed following 5'-AMP inhalation, in sensitized but not unsensitized guinea-pigs. Cell influx at 6, 12 and 24 h post-challenge suggests that it may be associated with the LAR and AHR.

Keywords adenosine (5'-AMP), bronchoconstriction, conscious guinea-pigs, inflammatory cells, late asthmatic response, ovalbumin

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Introduction

Adenosine is produced constitutively by cells under normal conditions and is generated in high concentrations in hypoxic environments such as that found in the inflamed lung in asthmatic patients [1]. Bronchoalveolar lavage (BAL) fluid (BALF) from asthmatic patients has revealed increased adenosine levels compared with normal control patients [2], and raised histamine levels, indicating mast cell degranulation. Minutes after allergen challenge, allergic asthmatics showed higher adenosine concentrations in plasma, which were not seen in the plasma of normal subjects [3]. Inhaled

adenosine causes a marked bronchoconstriction in asthmatics and allergic non-asthmatics compared with normal subjects [4] and potently constricts the asthmatic airways *in vitro* [5] but produces little or no contraction of bronchi from non-asthmatics. H₁ receptor antihistamines, such as terfenadine and astemizole, almost totally inhibited the bronchoconstrictor response to adenosine monophosphate (AMP) inhalation in asthmatic and atopic subjects [6, 7]. Following a 5'-AMP challenge in sensitized but not unsensitized guinea-pigs, there is an immediate bronchoconstriction and dose-dependent cellular infiltration of leucocytes, particularly macrophages and eosinophils, into the BALF of these animals [8].

Ovalbumin (OVA) causes an early asthmatic response (EAR), late asthmatic response (LAR), airway hyper-reactivity (AHR) and cellular infiltration to the lungs in sensitized animals [9, 10]. The question therefore posed by the

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present study, was whether 5'-AMP also causes a LAR and AHR, in common with an OVA challenge. Only one previous study exists on whether 5'-AMP inhalation in asthmatic subjects induces AHR and a LAR, which failed to detect such responses [11], but this has not been confirmed subsequently.

The present study therefore examined the effect(s) of 5'-AMP inhalation on airway function in our animal model of asthma, in order to establish whether a reproducible LAR and AHR can be revealed, along with cellular infiltration into the lungs. Sensitized guinea-pigs were challenged with 5'-AMP (3 or 300 mM) and airway function monitored for up to 24 h post-challenge. These two doses have been used previously in sensitized guinea-pigs [8], but the animals were monitored for only 15 min after exposure. The findings were compared with inhalation challenge with the allergen, OVA, since the sensitization and challenge protocols with OVA were different from those used previously in these laboratories [8, 10].

Methods

Animal husbandry

Male Dunkin–Hartley guinea-pigs (Harlan) (300–350 g) were used. Animal welfare and experimental procedures were undertaken in accordance with the Animal Scientific Procedures Act 1986 under Home Office personal and project licenses. Sensitized or non-sensitized guinea-pigs were used.

Sensitization

Guinea-pigs were actively sensitized by intraperitoneal (i.p.) injections of OVA (100 µg) and aluminium hydroxide (100 mg) in 1 mL of normal saline. Guinea-pigs were administered a booster injection of the same solution on day 5. All procedures were carried out 12–16 days later. This sensitization technique differs from previous studies from these laboratories [8, 12] in that an extra booster injection of the solution was administered on day 5.

Ovalbumin exposures

Sensitized animals were exposed, in a stainless steel circular metal chamber (70 cm diameter, 16 cm depth) to a nebulized solution of OVA (10 µg/mL in saline), generated by a Wright nebulizer supplied with air at a pressure of 20 lb p.s.i. at 0.3 mL/min. Animals were exposed for 1 h, after the chamber was saturated with nebulized OVA. Respiratory function was measured before, and at intervals up to 12 h and at 24 h post-challenge. Controls were exposed to saline.

5'-adenosine monophosphate exposures

Animals were exposed in a sealed wooden exposure chamber (620 × 30 × 420 mm) with a perspex window in the lid, to a nebulized solution of 5'-AMP (3 or 300 mM in saline), generated by a Wright nebulizer. Animals were exposed for 1 min after the box was saturated with the nebulized 5'-AMP. Respiratory function was measured before and at intervals up to 12 h and at 24 h post-challenge. Controls were exposed to saline. Different chambers were used for 5'-AMP and OVA challenge as 5'-AMP required administration for only 1 min, and so in a smaller chamber, saturation of the nebulized

compound could be achieved quicker. No animals displayed respiratory distress with OVA or 5'-AMP exposures or failed to complete their exposures.

Airway reactivity to histamine

Basal specific airway conductance (sG_{aw}) was recorded before any challenge. Airway reactivity to histamine delivered by a Wright nebulizer (1 mm, in saline, nose only, for 20 s) was assessed 24 h before OVA, 5'-AMP or saline exposures and at 12 or 24 h afterwards. sG_{aw} was measured at 0, 5 and 10 min after histamine exposure. This dose was selected because it produces a threshold bronchoconstriction and therefore permits measurement of bronchial hyper-reactivity as an enhanced response. A higher concentration of histamine (3 mM) caused a substantial bronchoconstriction in this model and in one group sG_{aw} was monitored for 12 h after this histamine exposure to determine whether it caused a LAR.

Non-invasive measurement of specific airway conductance

Airway function was recorded as sG_{aw} using whole body plethysmography in unanaesthetized spontaneously breathing guinea-pigs as described previously [12, 13]. Pressure transducers (UP1, Lectromed, Letchworth, Hertfordshire, UK) attached to the pneumotachograph and plethysmograph chamber, respectively, measured changes in respiratory flow and box pressure, by means of an AcqKnowledge[®] software Biopac[®] data acquisition system [10], which replaced the original oscilloscope and angle resolver as used by Griffiths-Johnson et al. [14].

Guinea-pigs are obligatory nose breathers [15] and it is possible that changes in sG_{aw} may also reflect nasal resistance, for example, arising from vascular changes in the nasal mucosa. However, the nasal component in the responses to a range of spasmogens including OVA have been shown to be negligible compared with the lower airways [15].

Bronchoalveolar lavage

Within 20 min of assessing airway reactivity to histamine at 24 h after OVA, saline or 5'-AMP challenge, guinea-pigs were overdosed with pentobarbitone sodium (Euthatal[®] 400 mg/kg i.p.). A polypropylene cannula was inserted into the trachea and normal saline solution (1 mL 100 g⁻¹ guinea-pig weight) was passed into the lungs using a 5 mL syringe and was recovered 3 min later [8]. This procedure was repeated and the total cell count (cells/mL) in the pooled lavage fluid was determined using a Neubauer haemocytometer (Fisher Scientific International Inc., Loughborough, UK). Differential cell counts were made after samples of the BAL fluid (100 µL) were centrifuged using a Shandon Cytospin (Cytospin3, Thermo Electron Bioscience Technologies, Runcorn, UK), onto glass microscope slides, at 1000 r.p.m. for 7 min. The resulting cytospin smear was differentially stained with 1.5% Leischman's stain (in 100% methanol), and a minimum of 500 cells were counted and the proportion of macrophages, eosinophils and neutrophils were calculated. Lavage was also performed at 6 and 12 h after 5'-AMP or saline challenge in separate groups of guinea-pigs, after any determination of reactivity to inhaled histamine and at 24 h after histamine (3 mM) challenge.

Data analysis

To reduce intersubject variability, sG_{aw} was expressed as the percentage change from the basal value taken immediately before an exposure. The mean values ($\pm$ SEM) for n animals are represented at each time-point. Responses to histamine were compared at the mean peak change in sG_{aw} by paired Student's t -tests. BAL cell counts ($\pm$ SEM) were compared using analysis of variance (ANOVA), followed by an unpaired Student's t -test. Changes in airway function were compared using ANOVA, followed by the Bonferroni multiple comparisons test. All significant differences observed with ANOVA were also found when a non-parametric Mann-Whitney U -test was applied. Differences were considered statistically significant when $P < 0.05$.

Materials

OVA was purchased from BDH Laboratory Supplies (Poole, UK). Aluminium hydroxide was purchased from Prolabo (Paris, France), 5'-AMP and histamine were both purchased from Sigma (Poole, Dorset, UK). OVA, aluminium hydroxide, 5'-AMP and histamine were all dissolved in 0.9% saline (Baxter, Thetford, UK).

Results

Effect of ovalbumin on airway function and cell infiltration

Figure 1 shows the mean time course for the changes in sG_{aw} of conscious OVA-sensitized guinea-pigs challenged with OVA (10 μ g/mL in saline). An EAR was seen as an immediate bronchoconstriction with a maximum fall in sG_{aw} of $-63.4 \pm 2.8\%$ ($P < 0.05$), which resolved after 6 h. Each animal displayed a late-phase bronchoconstrictor response (LAR), the greatest bronchoconstrictor response occurring at 7 h, with a maximum late-phase peak fall in sG_{aw} of

$-32 \pm 3.2\%$. Non-sensitized animals challenged with OVA and saline-challenged sensitized animals showed no EAR or LAR (Fig. 1).

A BAL revealed a significant increase in total cells, eosinophils and macrophages, when compared with non-sensitized animals challenged with OVA, and sensitized animals challenged with saline (Fig. 2). Neutrophils did not appear after OVA challenge of sensitized guinea-pigs.

Effect of 5'-adenosine monophosphate on airway function

Figure 3 shows changes in sG_{aw} following exposure to 3 mM 5'-AMP. An EAR peaked at 0 min, with a significant reduction in sG_{aw} of $-40.6 \pm 6.2\%$ ($P < 0.05$). Each animal then displayed a late asthmatic bronchoconstrictor response at 6 h, with a significant fall in sG_{aw} of $-37.3 \pm 3.5\%$. Non-sensitized guinea-pigs challenged with 5'-AMP (3 mM) and saline challenged, sensitized animals failed to show either an EAR or LAR (Fig. 3).

Figure 4 shows changes in sG_{aw} following exposure to the high dose (300 mM) of 5'-AMP. Following this challenge in sensitized animals, an immediate bronchodilator effect was produced, with a significant maximum peak increase in sG_{aw} of $24.9 \pm 6.8\%$ ($P < 0.05$), occurring at 15 min after challenge. Each animal displayed a LAR at 6 h, with a significant mean maximum reduction in sG_{aw} of $-30.5 \pm 3.7\%$ ($P < 0.05$). Non-sensitized guinea-pigs exposed to 5'-AMP (300 mM) and saline challenged, sensitized animals failed to show the early bronchodilator or LAR.

Effect of 5'-adenosine monophosphate on cell infiltration

Bronchoalveolar lavage following both 5'-AMP challenges (3 and 300 mM) revealed a significant increase in both total cell and macrophage counts (Fig. 5) at 6, 12 and 24 h post-challenge, compared with saline challenge. The levels of cells after saline challenge were similar to that in non-sensitized

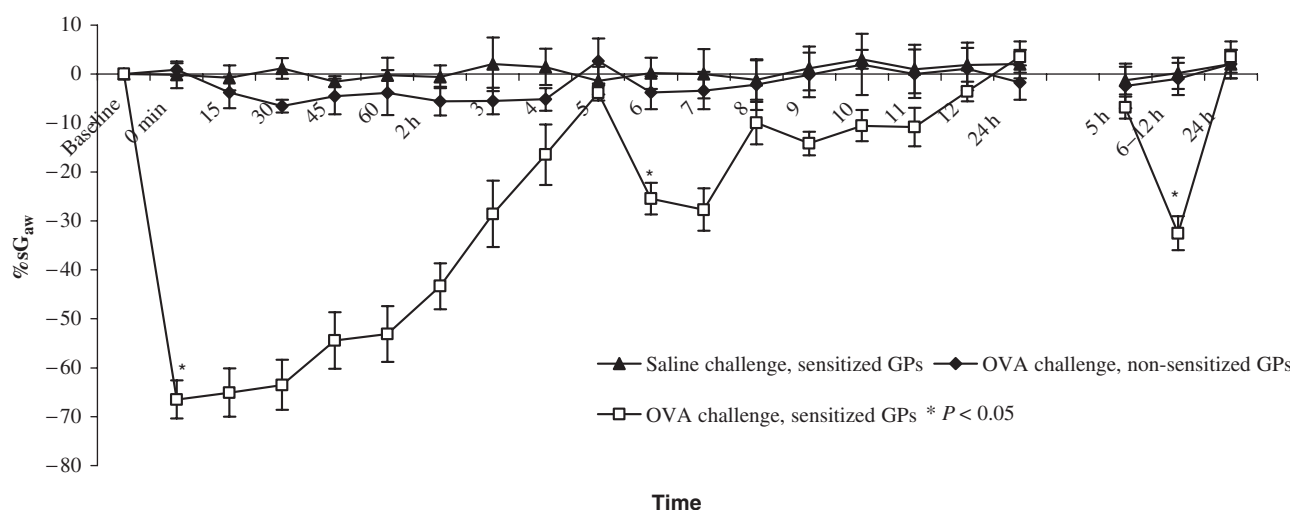


Fig. 1. Effect of ovalbumin (OVA) and saline exposures on the airway function of conscious sensitized and non-sensitized guinea-pigs. OVA or saline was inhaled for 1 h and specific airway conductance (sG_{aw}) values were measured before and at intervals up to 12 and 24 h after exposure. Lung function recovered to baseline values by 24 h, to allow accurate assessment of airway reactivity to histamine at this time-point. Each time-point represents the mean $\pm$ SEM ($n = 6$) change in sG_{aw} expressed as a percentage of the baseline sG_{aw} values. Negative values represent bronchoconstriction. The mean $\pm$ SEM of the peak fall in sG_{aw} between 6 and 12 h is also shown. Significance of differences between OVA exposure in sensitized, non-sensitized and saline-challenged guinea-pigs was determined by analysis of variance, followed by Student's unpaired t -test.

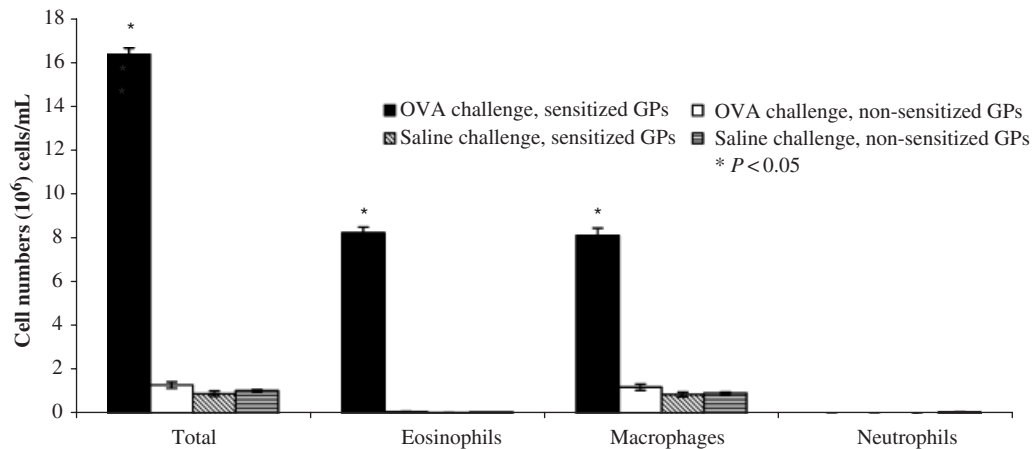


Fig. 2. Effect of ovalbumin (OVA) and saline challenges of sensitized and non-sensitized guinea-pigs on cellular infiltration into the airways 24 h after exposure. Bronchoalveolar lavage was carried out at this time-point, and total cell count, macrophages, eosinophils and neutrophils were counted. Results are expressed as the mean $\pm$ SEM. * $P < 0.05$ represents significant differences compared with non-sensitized and saline-challenged animals.

animals 24 h after 5'-AMP (3 mM) challenge. Following inhalation of 300 mM 5'-AMP, eosinophil infiltration into the BALF was significant at 6, 12 and 24 h after challenge, when compared with saline challenged and non-sensitized control groups. No significant eosinophilia was observed at any of the time-points following a 3 mM 5'-AMP challenge.

Effects of ovalbumin and 5'-adenosine monophosphate on airway reactivity to histamine

No guinea-pigs (sensitized and non-sensitized) displayed a bronchoconstrictor response to histamine 24 h before challenge (Fig. 6). Twenty-four hours after exposure to OVA, a histamine challenge produced a significant bronchoconstriction, with a mean reduction in sG_{aw} of $-30.5 \pm 3.4\%$ (Fig. 6a), in sensitized guinea-pigs, but not in non-sensitized or saline-challenged sensitized or non-sensitized guinea-pigs.

Guinea-pigs exposed to 5'-AMP (3 or 300 mM), produced a significant bronchoconstrictor response to histamine 12 h post-challenge (Fig. 6b), with maximum peak reductions in sG_{aw} of $-40.6 \pm 6.6\%$ and $-32.0 \pm 1.7\%$, respectively, but not 24 h post-challenge, and only in sensitized guinea-pigs.

Effect of histamine exposure on lung function and cell influx

Histamine (3 mM) exposure in non-sensitized guinea-pigs produced an immediate bronchoconstriction, the peak reduction in sG_{aw} being $-42.31 \pm 6.1\%$ (Fig. 7). No LAR was observed during recording up to 12 h. BAL at 24 h after challenge revealed no significant influx of inflammatory cells; total cells, macrophages and eosinophils were identical to control levels after saline challenge (Fig. 7).

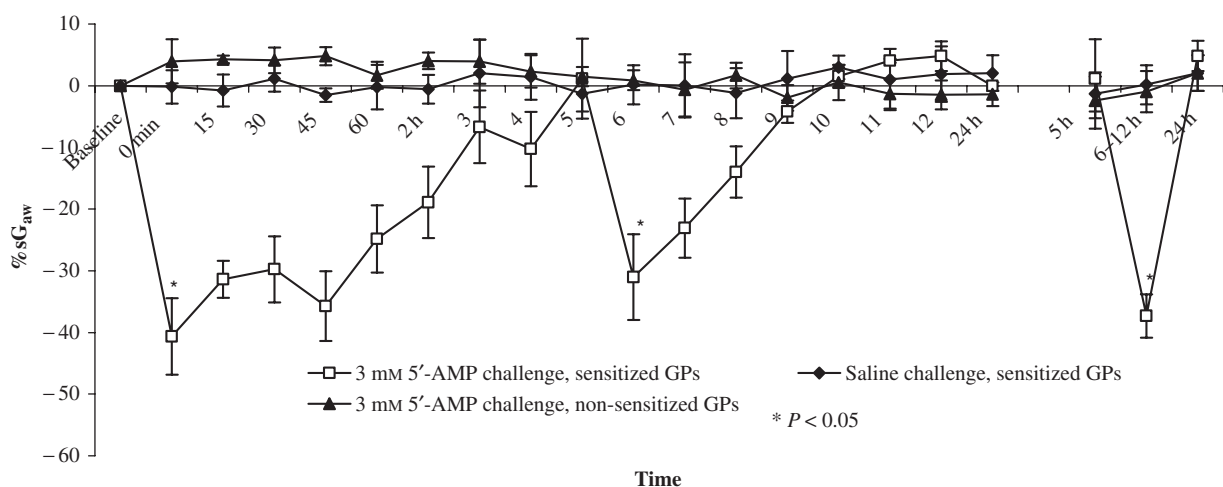


Fig. 3. Effect of 5'-adenosine monophosphate (5'-AMP) (3 mM) and saline challenges on the airway function of conscious sensitized and non-sensitized guinea-pigs. 5'-AMP or saline was inhaled for 1 min following box saturation and specific airway conductance (sG_{aw}) values were measured before and at intervals up to 12 and at 24 h after exposure. Lung function recovered to baseline values by 24 h to ensure an accurate assessment of airway reactivity to histamine at this time-point. Each time-point represents the mean $\pm$ SEM ($n = 6$) change in sG_{aw} expressed as a percentage of the baseline sG_{aw} values. Negative values represent bronchoconstriction. The mean $\pm$ SEM of the peak fall in sG_{aw} between 6 and 12 h is also shown. Significance of differences between 5'-AMP exposure in sensitized, non-sensitized and saline-challenged guinea-pigs was determined by analysis of variance, followed by Student's unpaired t -test.

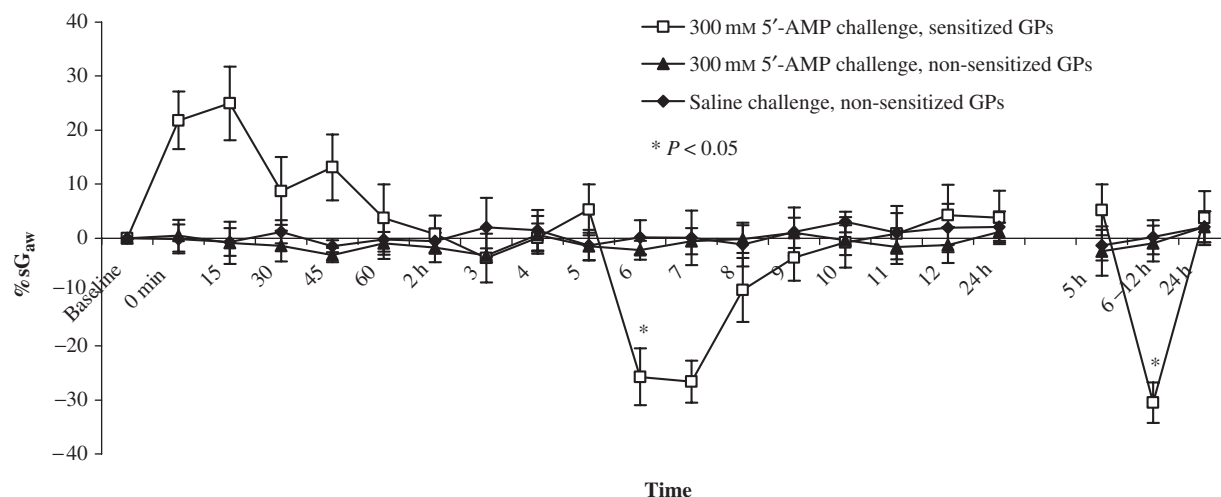


Fig. 4. Effect of 5'-adenosine monophosphate (5'-AMP) (300 mM) and saline challenges on the airway function of conscious sensitized and non-sensitized guinea-pigs. 5'-AMP or saline was inhaled for 1 min following box saturation and sG_{aw} values were measured before and at intervals up to 12 and at 24 h after exposure. Lung function recovered to baseline values by 24 h to ensure an accurate assessment of airway reactivity to histamine at this time-point. Each time-point represents the mean $\pm$ SEM ($n=6$) change in baseline sG_{aw} expressed as a percentage of the baseline sG_{aw} values. Negative values represent bronchoconstriction. The mean $\pm$ SEM of the peak fall in sG_{aw} between 6 and 12 h is also shown. Significance of differences between 5'-AMP exposure in sensitized, non-sensitized and saline-challenged guinea-pigs was determined by analysis of variance, followed by Student's unpaired *t*-test.

Discussion

This study has demonstrated both immediate and late bronchoconstrictor responses to an OVA challenge in actively sensitized guinea-pigs, the LAR occurring 7 h after the initial challenge. The early and LARs were more pronounced and reproducible than observed in earlier studies from these laboratories which used a single injection of OVA for sensitization [12]. In sensitized guinea-pigs, an antigen

challenge can initiate the release of pharmacological mediators such as histamine, tachykinins, leukotrienes and prostaglandins from cells such as macrophages and mast cells, which act on smooth muscle to cause bronchospasm [9, 16, 17]. This is seen as the early bronchoconstrictor response. T cells can also be activated by the allergen to release cytokines and tumour necrosis factor, resulting in the recruitment of eosinophils to the airways and their subsequent activation [18].

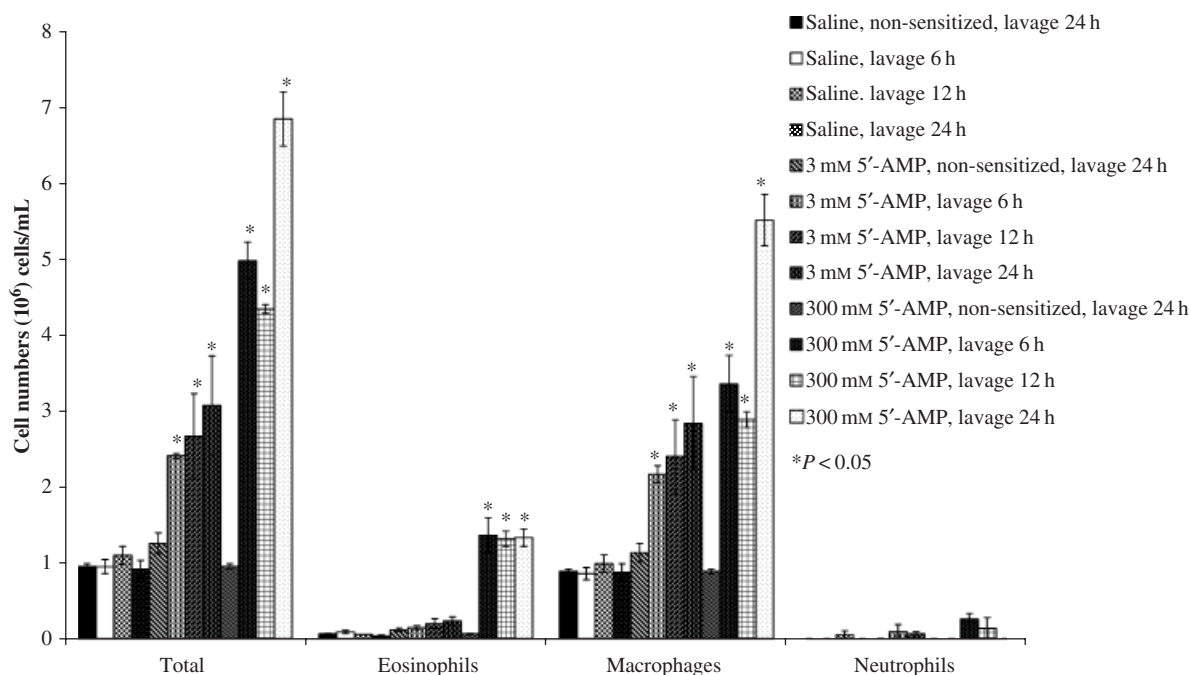


Fig. 5. Effects of 5'-adenosine monophosphate (5'-AMP) (3 or 300 mM) and saline challenges of sensitized and non-sensitized guinea-pigs on cellular infiltration into the airways at 6, 12 or 24 h after exposure. Bronchoalveolar lavage was carried out at these time-points, and total cell count, macrophages, eosinophils and neutrophils were counted. Results are expressed as the mean $\pm$ SEM. * $P<0.05$ represents significant differences compared with saline-challenged animals' cell counts at the corresponding time-points.

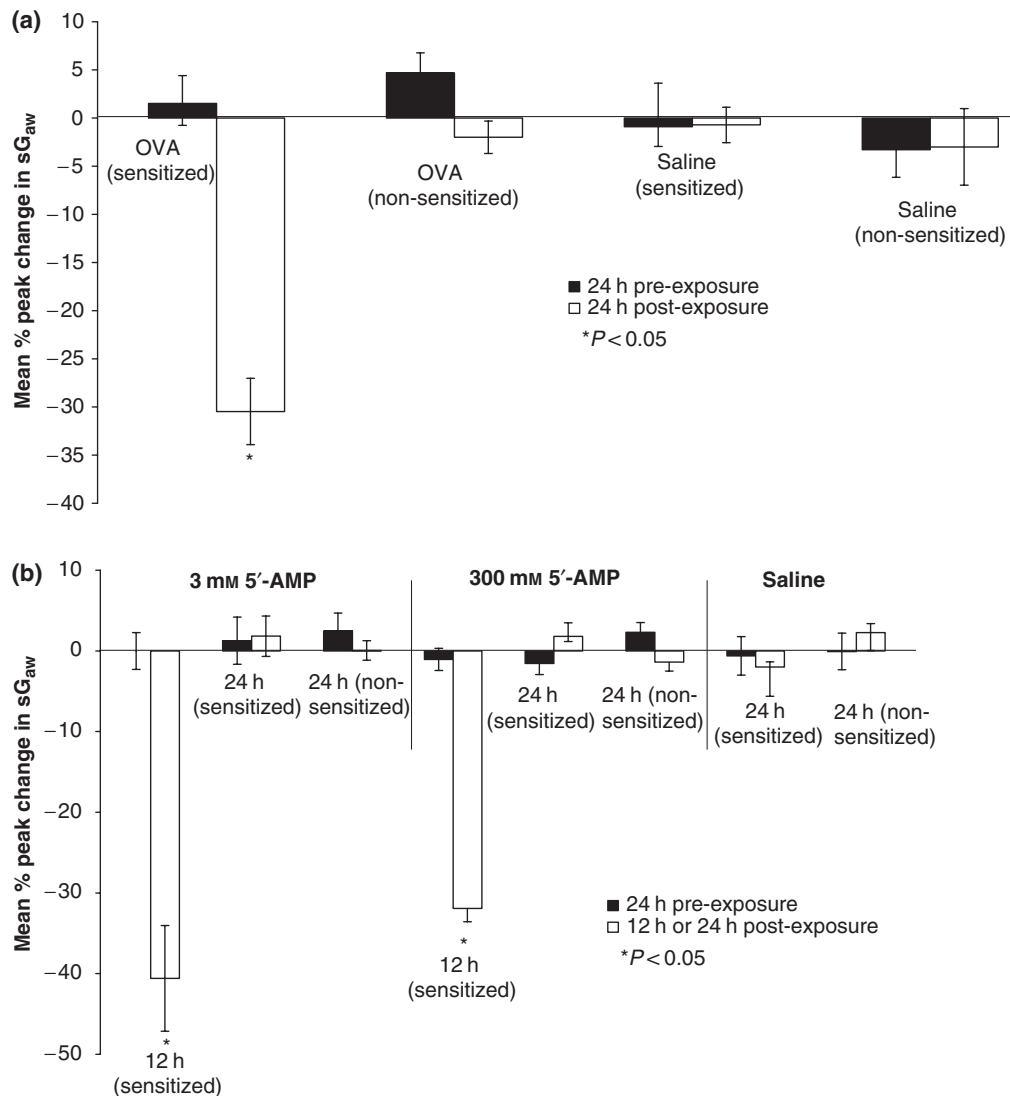


Fig. 6. Peak responses of the airways to nose-only exposures to histamine (1 mm, 20 s) 24 h before and (a) 24 h after 1 h ovalbumin (OVA) (10 µg/mL) or saline challenges, and (b) 12 or 24 h after 5'-adenosine monophosphate (5'-AMP) (3 or 300 mm) or saline challenges, in sensitized and non-sensitized conscious guinea-pigs. Each point represents the mean $\pm$ SEM ($n = 6$) of the individual peak change in specific airway conductance (sG_{aw}) from baseline sG_{aw} values expressed as a percentage of the baseline before exposure to histamine. Negative values represent bronchoconstriction. Differences between before and after exposure to histamine were determined by analysis of variance followed by Student's *t*-test and denoted as * $P < 0.05$.

Eosinophils can release platelet-activating factor (PAF) and leukotrienes that cause bronchial inflammation, possibly associated with the late bronchoconstrictor response [19]. As seen in previous studies from this laboratory [10, 12], eosinophils were found to accumulate in the airways, as measured by their increased appearance into the BALF 24 h after exposure to OVA. Many studies have suggested that eosinophils and their mediators are involved in the development of the LAR following allergen challenge [18, 19]. Significant eosinophilia was found in the BALF of patients who developed a LAR, in comparison with patients who did not produce a LAR [19]. The present study supports this claim, as the LAR was accompanied by an increase in eosinophilia to the airways.

Hutson et al. [20] found that after depletion of neutrophils by injection of a polyclonal antibody against guinea-pig neutrophils, the animals still produced a normal late-phase

response after OVA challenge, suggesting that neutrophils do not participate in the LAR. The present study has also confirmed that the levels of this polymorphonuclear leucocyte do not increase after OVA challenge, and it is therefore unlikely to play a crucial role in the pathophysiologic processes that give rise to the late-phase response.

AHR to chemical, physical and pharmacological stimuli, including histamine and methacholine, is a hallmark of allergic asthma [21]. In sensitized guinea-pigs, AHR can develop following the LAR [9, 10, 12], and this is also true for allergic asthmatics [22]. Airway hyperresponsiveness is probably acquired during life as a result of airway reactions to various stimuli, although genetic factors such as atopy are likely to predispose the person to develop hyperresponsiveness. It has been shown that allergen-induced AHR in asthmatic subjects occurs in association with the LAR [22]. This was confirmed in the present study, as the LAR was

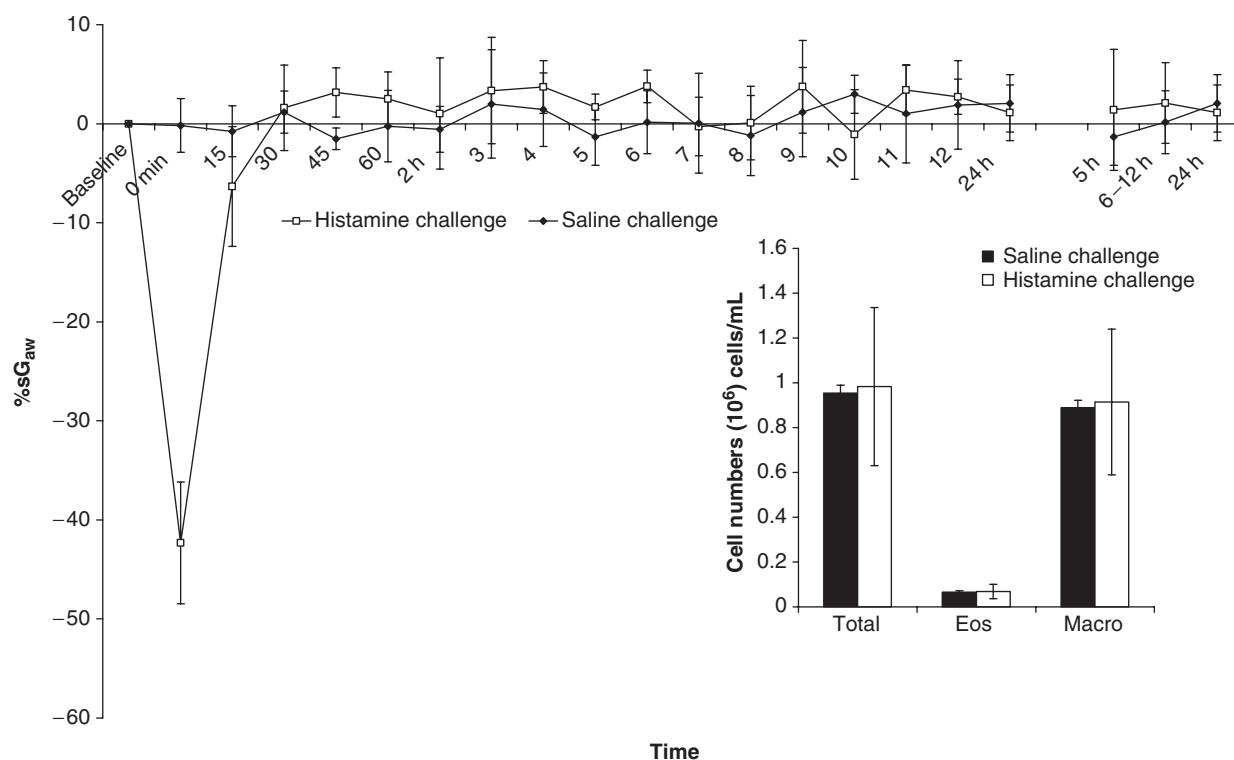


Fig. 7. Effect of histamine (3 mM) and saline challenges (20 s, nose only) on the airway function and (inset)-cellular infiltration to the lungs, of conscious non-sensitized guinea-pigs. specific airway conductance (sG_{aw}) values were measured before and up to 24 h after exposure. Each time-point represents the mean $\pm$ SEM ($n = 6$) change in sG_{aw} expressed as a percentage of the baseline sG_{aw} values. Negative values represent bronchoconstriction. BAL was recovered 24 h after challenge.

followed by an increased responsiveness to histamine, and previous studies from this laboratory have shown that AHR does not occur in the absence of the LAR [23].

This study has shown that a bronchoconstrictor response can be produced following the low dose 5'-AMP challenge in sensitized guinea-pigs but not in unsensitized animals. This is consistent with previous findings from Cushley et al. [4], who found that inhaled adenosine caused a marked bronchoconstriction in asthmatics, when compared with normal subjects. Thorne and Broadley [24] and Spruntulis and Broadley [8] have shown that inhaled adenosine can produce a bronchoconstrictor response in guinea-pigs sensitized to OVA, but not in unsensitized animals. As far as we are aware, however, we have shown for the first time that a 1 min inhalation of 5'-AMP in sensitized guinea-pigs can produce both an early and LAR. Exposure to the lower dose (3 mM) of 5'-AMP resulted in a significant bronchoconstrictor response immediately after the challenge, as found by Spruntulis and Broadley [8]. This bronchoconstrictor effect resolved within 4 h, and a LAR followed at 6 h. This is the first time that the bronchoconstrictor effect to inhaled adenosine has been monitored for longer than 15 min in an animal model. It has been suggested that inhaled 5'-AMP does not produce a LAR in asthmatic patients [11]. However, this has only been reported by one group and they used a single dose to produce a 20% fall in forced expiratory volume in 1 s (FEV_1) from baseline (PC_{20}). It is possible that a higher dose may have induced a late-phase. This does not appear to have been examined again since 1988 and the phenomenon therefore deserves further investigation in human asthmatics. Comparisons between the

levels of 5'-AMP used in the present study and the clinical situation are difficult because of the different modes of administration. In human studies the subjects inhaled the 5'-AMP as an aerosol directly via the mouth, the PD_{20} for FEV_1 being 5.1 μ mol [25]. In our study the aerosol was delivered into the exposure chamber. Comparisons with endogenous levels of adenosine of 198 μ M at the epithelial lining [2] are also difficult since we do not know how much of our doses of 5'-AMP (3 mM and 300 mM) is broken down to adenosine.

Sensitized guinea-pigs exposed to the 100-fold higher dose of 5'-AMP (300 mM) for 1 min displayed an immediate bronchodilator response, which is consistent with previous findings [8]. This was also followed by a LAR at 6 h after exposure, although it was no larger than with the lower dose, indicating that perhaps it had reached its maximum effect. In contrast to an OVA challenge, no AHR to histamine was observed 24 h after either 5'-AMP challenges, suggesting that mediators responsible for AHR, such as nitric oxide (NO) and superoxide anions (O_2^-) [21], tachykinins [22] and leukotrienes [22], are absent, or present in smaller quantities following exposure to this agent. This supports previous work by Spruntulis & Broadley [8] who reported no hyperactivity to histamine 24 h after a 5'-AMP challenge (3 mM and 300 mM). Sensitized animals challenged with both concentrations of 5'-AMP were however hyperresponsive to inhaled histamine 12 h post-challenge. It can therefore be concluded that AHR to inhaled histamine is short lived and is lost between 12 and 24 h after challenge.

Comparisons between the OVA and 300 mM 5'-AMP challenges show that both challenges result in very similar

LARs, in terms of severity and time course. It is believed that eosinophils are associated with the LAR following an OVA challenge [9], as one does not occur without the other. It was therefore speculated that eosinophilia would also be associated with the LAR following the 5'-AMP challenge. However, on examination of the BALF, it was found that the eosinophilia was significantly less than after OVA challenge. Therefore, following a 5'-AMP challenge, AHR and a LAR are evident with a lower level of eosinophilia in the airways. This could possibly be because of activation of another pathway/receptor which may mask or inhibit eosinophilia in the BALF, without affecting the late bronchoconstrictor response.

As a positive control for the generation of a LAR by any agent that causes an EAR, non-sensitized guinea-pigs received a histamine challenge using a dose that caused a substantial bronchoconstriction. This failed to induce a LAR. It might be expected that a vasoactive agent like histamine which increases vascular permeability [26] may therefore cause infiltration of inflammatory cells into the lungs. However, there was no change in macrophages or eosinophils at 24 h after the histamine challenge.

It is known that the immediate bronchoconstrictor response (EAR) to adenosine is mast cell dependent, and both A_{2b} and A_3 receptors may mediate mast cell activation, depending on the species [27, 28]. Human mast cells from the MHC-1 cell line and BALF express the A_{2b} receptor, but not the A_3 receptor, suggesting that the A_{2b} receptor may mediate the bronchoconstrictor response to adenosine [29]. It can therefore be suggested that clinically, adenosine may act on A_{2b} receptors on mast cells, resulting in the release of histamine, which could initiate the EAR. The release of proinflammatory mediators from mast cells could initiate a cascade of events that then go on to initiate the LAR, 6 h after the initial challenge.

The bronchodilator effect following exposure to 300 mM 5'-AMP has been previously reported [8]. Bronchial relaxation via A_2 receptors is the dominant response of isolated tracheal preparations to adenosine analogues [30, 31]. This has more recently been assigned to the A_{2A} subtype [32] and suggests the response observed here is also because of activation of A_{2A} receptors on the airway smooth muscle. The reason for a lack of bronchodilator action with the high dose 5'-AMP in non-sensitized guinea-pigs is not clear. It is possible that there is a certain degree of bronchial tone in sensitized animals only which allows bronchorelaxation to be evident.

In summary, we have shown that following an OVA challenge in guinea-pigs sensitized with the same allergen, animals display an EAR, LAR, AHR to histamine and cellular infiltration to the airways. We confirmed that eosinophilia is associated with the LAR, and neutrophils have no involvement in the pathophysiological processes that give rise to the LAR, following an OVA challenge in sensitized guinea-pigs. We have also shown that airway hyperresponsiveness to histamine occurs in association with the LAR.

This study has demonstrated for the first time that 5'-AMP inhalation can produce both early and late bronchoconstrictor responses, AHR and leucocyte infiltration to the lung, in sensitized guinea-pigs. The atopic status is required for all four responses. Cell influx in the BALF at 6, 12 and 24 h after

5'-AMP challenge suggests that cellular infiltration may be associated with the LAR and AHR. This study has shown very close similarities in the responses of guinea-pigs to the antigen and 5'-AMP, the main difference being that AHR after 5'-AMP dose not persist until 24 h after challenge.

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References

- 1 Barnes PJ, Chung KF, Page CP. Inflammatory mediators of asthma: an update. *Pharmacol Rev* 1998; 50:515–96.
- 2 Driver AG, Kukloy CA, Ali S, Mustafa SJ. Adenosine in bronchoalveolar lavage fluid in asthma. *Am Rev Respir Dis* 1993; 148:91–7.
- 3 Mann JS, Holgate ST, Renwick AG, Cushley MJ. Airway effects of purine nucleosides and release with bronchial provocation in asthma. *J Appl Physiol* 1986; 61:1667–76.
- 4 Cushley MJ, Tattersfield AE, Holgate ST. Inhaled adenosine and guanosine on airway resistance in normal and asthmatic subjects. *Br J Clin Pharmacol* 1983; 15:161–5.
- 5 Björck T, Gustafsson LE, Dahlen SE. Isolated bronchi from asthmatics are hyperresponsive to adenosine, which apparently acts indirectly by liberation of leukotrienes and histamine. *Am Rev Respir Dis* 1992; 145:1087–91.
- 6 Phillips GD, Rafferty P, Beasley R, Holgate ST. Effect of oral terfenadine on the bronchoconstrictor responses to inhaled histamine and adenosine 5' monophosphate in non-atopic asthma. *Thorax* 1987; 42:939–45.
- 7 Rafferty P, Beasley R, Southgate P, Holgate ST. The role of histamine and adenosine-induced bronchoconstriction. *Int Arch Allergy Appl Immunol* 1987; 82:292–4.
- 8 Spruntulis LM, Broadley KJ. A_3 receptors mediate rapid inflammatory cell influx into the lungs of sensitized guinea pigs. *Clin Exp Allergy* 2001; 31:943–51.
- 9 Santing RE, Olymulder CG, Zaagsma J, Meurs H. Relationships among allergen-induced early and late phase airway obstructions, bronchial hyperreactivity, and inflammation in conscious un restrained guinea pigs. *J Allergy Clin Immunol* 1994; 193:1021–30.
- 10 Danahay H, Broadley KJ. Effects of inhibitors of phosphodiesterase, on antigen-induced bronchial hyperreactivity in conscious sensitized guinea pigs and airway leukocyte infiltration. *Br J Pharmacol* 1997; 120:289–97.
- 11 Phillips GD, Holgate ST. Absence of a late-phase response or increase in histamine responsiveness after bronchial provocation with adenosine 5'-monophosphate in atopic and non-atopic asthma. *Clin Sci* 1988; 75:429–36.
- 12 Toward TJ, Broadley KJ. Early and late bronchoconstrictions, airway hyper reactivity, leucocyte influx and lung histamine and nitric oxide after inhaled antigen: effects of dexamethasone and rolapram. *Clin Exp Allergy* 2004; 34:91–102.
- 13 Toward TJ, Broadley KJ. Airway reactivity, inflammatory cell influx and nitric oxide in guinea pig airways after lippolysaccharide inhalation. *Br J Pharmacol* 2000; 131:271–81.
- 14 Griffiths-Johnson DA, Nicholls PJ, McDermott M. Measurement of specific airways conductance in guinea pigs: a non-invasive method. *J Pharmacol Methods* 1988; 19:233–48.

- 15 Finney NJ, Forsberg KI. Quantification of nasal involvement in a guinea pig plethysmograph. *J Appl Physiol* 1994; 76: 1432–8.
- 16 Schuiling M, Zuidhof AB, Meurs H, Zaagsma J. Role of tachykinin NK2-receptor activation in the allergen-induced late asthmatic reaction, airway hyperreactivity and airway inflammatory cell influx in conscious, unrestrained guinea-pigs. *Br J Pharmacol* 1999; 127:1030–8.
- 17 Santing RE, Schraa EO, Wachters A, Olymulder CG, Zaagsma J, Meurs H. Role of histamine in allergen-induced asthmatic reactions, bronchial hyperreactivity and inflammation in unrestrained guinea-pigs. *Eur J Pharmacol* 1994; 254:49–57.
- 18 Corrigan CJ, Kay AB. T cells and eosinophils in the pathogenesis of asthma. *Immunol Today* 1992; 13:501–6.
- 19 Durham SR, Kay AB. Eosinophils, bronchial hyperreactivity and late phase asthmatic reactions. *Clin Allergy* 1985; 15:411–8.
- 20 Hutson PA, Church MK, Miller TP, Holgate ST. Early and late phase bronchoconstriction after allergen challenge of non-anesthetised guinea pigs. The association of disordered airway physiology to leukocyte infiltration. *Am Rev Respir Dis* 1988; 137:548–57.
- 21 De Boer J, Meurs H, Flendrig L, Koopal M, Zaagsma J. Role of nitric oxide and superoxide in allergen induced hyperreactivity after the late asthmatic reaction in guinea-pigs. *Br J Pharmacol* 2001; 133:1235–342.
- 22 Hargreave FE, Dolovich J, O'Byrne PM, Ramsdale EH, Daniel EE. The origin of airway hyperresponsiveness. *J Allergy Clin Immunol* 1986; 78:825–32.
- 23 Smith N, Broadley KJ. Optimization of sensitisation protocols for asthmatic responses to ovalbumin in guinea pigs. *Proc Br Pharmacol Soc*, pA₂ online 2004; 2:131p.
- 24 Thorne JR, Broadley KJ. Adenosine-induced bronchoconstriction of isolated lung and trachea from sensitised guinea-pigs. *Br J Pharmacol* 1992; 106:978–85.
- 25 Phillips GD, Scott VL, Richards R, Holgate ST. Effect of nedocromil sodium and sodium cromoglycate against bronchoconstriction induced by inhaled adenosine-5'-monophosphate. *Eur Respir J* 1989; 2:210–7.
- 26 Handley DA, Anderson AJ, Koester J, Snider ME. New millennium bronchodilators for asthma: single-isomer beta agonists. *Curr Opin Pulm Med* 2000; 6:43–9.
- 27 Ezeamuzie CI, Philips E. Adenosine A3 receptors on human eosinophils mediate inhibition of degranulation and superoxide anion release. *Br J Pharmacol* 1999; 27:188–94.
- 28 Feoktistov I, Biaggioni I. Adenosine A2B receptors. *Pharmacol Rev* 1997; 49:381–402.
- 29 Marquardt DL, Walker LL, Wasserman SI. Cromolyn inhibition of mediator release in mast cells derived from mouse bone marrow. *Am Rev Respir Dis* 1986; 133:1105–8.
- 30 Ghai G, Zimmerman MB, Hopkins MF. Evidence for A1 and A2 adenosine receptors in guinea pig trachea. *Life Sci* 1987; 41: 1215–24.
- 31 Losinski A, Alexander SPH. Adenosine receptor-mediated relaxation of guinea-pig precontracted isolated trachea. *Br J Pharmacol* 1995; 116:2425–8.
- 32 Fozard JR, Ellis KM, Dantas MFV, Tigani B, Mazzoni L. Effects of CGS 21680, a selective A (2A) receptor agonist, on allergic airways inflammation in the rat. *Eur J Pharmacol* 2002; 438:183–8.