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Mechanism of airway hyperresponsiveness to adenosine induced by allergen challenge in actively sensitized Brown Norway rats

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1 We have explored the role of allergen sensitization and challenge in defining the response of the airways of the Brown Norway (BN) rat to adenosine.

2 In naïve animals or in rats sensitized to ovalbumin (OA) adenosine induced only weak bronchoconstrictor responses. Challenge of sensitized animals with OA induced a marked airway hyperresponsiveness to adenosine which was not seen with methacholine or bradykinin.

3 The augmented bronchoconstrictor response to adenosine was not affected by acute bivagotomy or atropine nor mimicked by an i.v. injection of capsaicin. It was, however, blocked selectively by disodium cromoglycate methysergide or ketanserin and reduced in animals treated sub-chronically with compound 48/80.

4 The augmented response to adenosine was associated with increases in the plasma concentrations of both histamine and 5-hydroxytryptamine (5-HT), which were attenuated by pretreatment with disodium cromoglycate, and degranulation of mast cells in the lung.

5 Parenchymal strips from lungs removed from sensitized rats challenged with OA gave augmented bronchoconstrictor responses to adenosine relative to strips from sensitized animals challenged with saline. Responses were inhibited by methysergide and disodium cromoglycate.

6 These data demonstrate a marked augmentation of the bronchoconstrictor response to adenosine in actively sensitized BN rats challenged with OA. The augmented response is primarily a consequence of mast cell activation, leading to the release of 5-HT, which in turn induces bronchoconstriction. Our data further suggest the involvement of a discrete lung-based population of mast cells containing and releasing mainly 5-HT and brought into play by prior exposure to allergen.

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Abbreviations: BN, Brown Norway; DSCG, disodium cromoglycate; EDTA, ethylenediaminetetraacetic acid; HR, heart rate; 5-HT, 5-hydroxytryptamine; i.t., intratracheal; MABP, mean arterial blood pressure; OA, ovalbumin; R_L, airway resistance

Introduction

The heightened sensitivity of asthmatics to a range of stimuli (immunological, physical and/or pharmacological) that ordinarily are without effect in normal subjects is a defining characteristic of asthma (Woolcock, 1997). The phenomenon, termed bronchial (or airway) hyperresponsiveness, results in facilitation of bronchospasm and contributes to the airway obstruction characteristic of asthma (Cockcroft, 1997; Brusacco *et al*., 1998; Cheung *et al*., 1999; O'Connor *et al*., 1999). The phenomenon is particularly well exemplified by the bronchoconstrictor response to adenosine which is prominent in asthmatic patients and generally not present in control subjects (Cushley *et al*., 1983). The mechanism of adenosine-induced bronchoconstriction in asthmatic patients has been well explored and an intermediary role for the mast cell is established (Phillips & Holgate, 1995; Jacobson & Bai, 1997; Marquardt, 1997; Polosa & Holgate, 1997; Feoktistov *et al*., 1998; Forsythe & Ennis, 1999). Increasing evidence implicates the adenosine A_{2B} receptor as the site through which activation of the mast cell occurs (Feoktistov *et al*., 1998; Fozard & Hannon, 1999). The reason for the striking up-regulation of the response in the asthmatic airways remains, however, unknown.

Bronchoconstriction to adenosine has been demonstrated in a number of animal models. The mechanism varies widely between species involving activation of A_3 receptors in the guinea-pig (Thorne & Broadley, 1994; Kehoe & Broadley, 1996; Thorne *et al*., 1996), A_1 receptors on airway smooth muscle cells in the rabbit (Ali *et al*., 1994b; El-Hashim *et al*., 1996; Nyce & Metzger, 1997) and a combination of A_2, A_{2B} and A_3 receptors in the BDE rat (Pauwels & Van Der Straeten, 1987; Pauwels & Joos, 1995; Meade *et al*., 1996), the only strain which responds consistently to adenosine (Pauwels *et al*., 1995). For a variety of reasons, none of these models can be considered to reflect closely the clinical response to adenosine in asthmatics (Fozard & Hannon, 2000).

The aim of this study was to establish a new experimental animal model of bronchoconstriction to adenosine designed to mimic the effects seen in asthmatic subjects. Based on the

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marked up-regulation of the response to adenosine in allergic asthmatics, our strategy was to explore the role of allergen sensitization and subsequent challenge with allergen in defining the response of the airways to adenosine. The BN rat was chosen for these studies since this strain can be readily and reproducibly sensitized to allergen and manifests both early and late bronchoconstrictor responses to allergen challenge, the latter being associated with a selective pulmonary eosinophilic cell infiltrate (Pauwels *et al*., 1979; 1985; Elwood *et al*., 1992; 1993; Peers *et al*., 1993; Renzi *et al*., 1993; 1996; Schneider & Issekutz, 1996; Haczku *et al*., 1996; 1997). In the event, we were able to demonstrate a marked augmentation of the bronchoconstrictor response to adenosine in sensitized rats challenged with allergen. To define the mechanistic basis of this response was the aim of our studies.

A part of the results was presented to the Joint Meeting of the British and Portuguese Pharmacological Societies in April, 1999 (Hannon *et al*., 1999a,b).

Methods

Animals

Male BN rats weighing 200–300 g were supplied by Biological Research Laboratories (Füllinsdorf, Switzerland). They were kept at an ambient temperature of 22±2°C under a 12 h normal phase light-dark cycle and fed on NAFAG® pellets supplied by Nahr und Futtermittel AG, Gossau, Switzerland. Drinking water was freely available. All experiments were carried out with the approval of the Veterinary Authority of the City of Basel (Kantonales Veterinaeramt, Basel-Stadt).

Sensitization and challenge with allergen

The procedure is based on that described by Tarayre *et al*. (1992). OA (20 μg ml^{-1}) was mixed (30 min on ice) in a blender (Polytron, Kinematica Ltd.) with aluminium hydroxide (20 mg ml^{-1}) and injected (0.5 ml per animal s.c.) coincidentally with *Acullulare pertussis* adsorbat vaccine (0.2 ml per animal i.p.; diluted 1:4 with saline 0.9% w v^{-1}). Injection of OA, together with adjuvant, was repeated 14 and 21 days later. Sensitized animals were used in experiments between days 28 and 35.

For challenge with OA, animals were briefly anaesthetized (4% isofluran) in an anaesthetic chamber. OA or vehicle (saline, 0.2 ml per animal) was administered intratracheally and the animals allowed to recover.

Measurement of lung function

Animals were anaesthetized with sodium pentothal (70 mg kg^{-1} i.p.) and a tracheotomy performed. Heparinized polyethylene catheters were inserted into the left carotid artery for recording mean arterial blood pressure (MABP) and into the left jugular vein for drug administration. To suppress spontaneous respiration animals were given an intramuscular injection of vecuronium bromide (12 mg kg^{-1}). No experiment lasted longer than 90 min, during which time surgical anaesthesia was maintained without the need for supplementary anaesthesia. Body temperature was maintained at 37°C with a heated pad controlled by a rectal thermistor.

Animals were ventilated (7 ml kg^{-1}, 1 Hz) *via* the tracheal cannula with a mixture of air and oxygen (50:50, v v^{-1}). Ventilation was monitored at the trachea by a pneumotachograph (Fleisch 0000, Zabona, Switzerland) in line with the respiratory pump and connected to a differential pressure transducer (MP 4514871, Validyne, U.S.A.). Coincident pressure changes within the thorax were measured *via* an intrathoracic cannula, using a differential pressure transducer (MP 4524, Validyne, U.S.A.). From measurements of airflow and transpulmonary pressure, airway resistance (R_L, cm H_2O l^{-1} s^{-1}) was calculated after each respiratory cycle by use of a digital electronic pulmonary monitoring system (PMS, Mumed, London, U.K.). Mean arterial blood pressure and heart rate (HR) by derivation was recorded from the carotid artery by means of a pressure transducer (P23Dd, Gould, U.S.A.).

In vivo *studies: experimental protocols*

Bronchial hyperresponsiveness to adenosine induced by OA challenge in actively sensitized BN rats: Dose-response relationship, time course and selectivity with respect to methacholine and bradykinin Bronchoconstrictor responses to adenosine (0.3 and 1 mg kg^{-1} i.v.), methacholine (3 and 10 μg kg^{-1} i.v.) and bradykinin (30 and 100 μg kg^{-1} i.v.) were established sequentially in groups of actively sensitized animals challenged intratracheally with vehicle or OA. The interval between the two adenosine doses was 15 min. Fifteen minutes after the second adenosine dose, methacholine was administered with a 2 min interval between doses. Finally, after a further restabilization period of 15 min, bradykinin was given with a 15 min interval between doses. OA was given i.t. at doses of 0.03, 0.3 or 3 mg kg^{-1} and the sequence of bronchoconstrictor agents was started 3 or 24 h after OA challenge. Vehicle-challenged animals received saline (0.2 ml i.t.) 3 or 24 h before the start of the bronchoconstrictor agonist sequence.

Effects of bilateral vagotomy Bronchoconstrictor responses to adenosine (1 mg kg^{-1} i.v.), methacholine (3 and 10 μg kg^{-1} i.v.) and 5-HT (3 and 10 μg kg^{-1} i.v.) were established in groups of actively sensitized animals challenged with OA. Fifteen minutes after the single dose of adenosine, methacholine was administered with a 2 min interval between doses. After a further 15 min, 5-HT was given with a 2 min interval between doses. OA was given i.t., at a dose of 0.3 mg kg^{-1} and the sequence of bronchoconstrictor agents was started 3 h later. Bilateral vagotomy or a sham intervention was effected 10 min prior to the start of the agonist sequence.

Effects of atropine Bronchoconstrictor responses to adenosine (1 mg kg^{-1} i.v.), 5-HT (3–30 μg kg^{-1} i.v.) and methacholine (3–30 μg kg^{-1} i.v.) were established in groups of actively sensitized animals challenged with OA. Fifteen minutes after the single dose of adenosine, 5-HT was administered with a 2 min interval between doses. After a further 15 min, methacholine was given with a 2 min interval between doses. OA was given i.t., at a dose of 0.3 mg kg^{-1} and the sequence of bronchoconstrictor agents initiated 3 h later. Atropine (10 μg kg^{-1}) was given i.v. 5 min prior to the start of the agonist sequence.

Effect of capsaicin Bronchoconstrictor responses to adenosine (1 mg kg^{-1} i.v.) or capsaicin (100 μg kg^{-1} i.v.) and 5-HT (3–30 μg kg^{-1} i.v.) were established in groups of actively sensitized animals challenged with OA. Fifteen minutes after the single dose of adenosine or capsaicin, 5-HT was administered with a 2 min interval between doses. OA was given i.t., at a dose of 0.3 mg kg^{-1} and the sequence of bronchoconstrictor agents initiated 3 h later.

Effect of disodium cromoglycate Bronchoconstrictor responses to adenosine (1 mg kg^{-1} i.v.) and 5-HT (3–30 μg kg^{-1} i.v.) were established in groups of actively sensitized animals challenged with OA. Fifteen minutes after the single dose of adenosine, 5-HT was administered with a 2 min interval between doses. OA was given i.t., at a dose of 0.3 mg kg^{-1} and the sequence of bronchoconstrictor agents initiated 3 h later. Disodium cromoglycate (20 or 40 mg kg^{-1}) was given i.v. 5 min prior to the start of the agonist sequence.

Effects of methysergide and ketanserin Bronchoconstrictor responses to adenosine (1 mg kg^{-1} i.v.), methacholine (3–30 μg kg^{-1} i.v.) and 5-HT (3–30 μg kg^{-1} i.v.) were established in groups of actively sensitized animals challenged with OA. Fifteen minutes after the single dose of adenosine, methacholine was administered with a 2 min interval between doses. After a further 15 min, 5-HT was given with a 2 min interval between doses. OA was given i.t., at a dose of 0.3 mg kg^{-1} and the sequence of bronchoconstrictor agents initiated 3 h later. Methysergide (10 μg kg^{-1}) or ketanserin (50 μg kg^{-1}) was given i.v. 5 min prior to the start of the agonist sequence.

Depletion studies with compound 48/80 Actively sensitized BN rats were depleted of their resident mast cell mediator stores by repeated administration of compound 48/80 according to the schedule described by Reeves *et al.* (1997). Animals were given compound 48/80 at a dose of 1.2 mg kg^{-1} day^{-1} i.p. for 4 days and on the fifth day measurement of lung function performed. Control animals were given equivalent volumes of saline. On day 5, animals were challenged with OA (0.3 mg kg^{-1} i.t.) and 3 h later a sequence of bronchoconstrictor agonists initiated. Animals were given a bolus i.v. injection of adenosine (1 mg kg^{-1}) and 15 min later a dose-response curve to 5-HT (3–30 μg kg^{-1} i.v.) was performed, the interval between doses being 2 min. After a further 5 min, a dose-response curve to methacholine (3–30 μg kg^{-1} i.v; interval between doses 2 min) was obtained, followed 5 min later by a dose-response curve to compound 48/80 (0.1–3 mg kg^{-1} i.v; interval between doses 2 min).

Measurement of histamine and 5-HT in plasma

Animals were anaesthetized with sodium pentothal (70 mg kg^{-1} i.p.), and polyethylene catheters placed in both the left carotid artery (for blood collection) and right jugular vein (for drug administration). After set-up the animals were left for a stabilization period of at least 20 min. The details of the subsequent experimental interventions are given in the Results section. Blood samples (approximately 1 ml) were taken into 1.5 ml potassium ethylenediaminetetraacetate (EDTA) coated plastic collection tubes and chilled on ice. Samples were immediately centrifuged ($1700 \times g$, 30 min, 4°C; Omnifuge 2.0, Heraeus Sepratech, CH) and the overlying plasma aspirated and stored at −30°C prior to assay. The concentrations of histamine and 5-HT in the plasma were assessed by colorimetric assay using commercial kits (Immunotech).

Histology

Mast cell degranulation following adenosine administration Animals were anaesthetized with sodium pentothal (70 mg kg^{-1} i.p.) and a cannula inserted into the left jugular vein. After set-up the animals were left for a stabilization period of at least 10 min. The details of the experimental interventions are given in the Results section. Samples of lung tissue (left lobe) and skin were removed and placed in 10% phosphate-buffered neutral formalin. After 3 days fixation, the tissues were dehydrated and embedded in paraffin. The blocks were cut at 3 μm and each section stained with toluene blue. Mast cells were counted and the extent of degranulation scored blind according to the following scale: 0 = essentially intact mast cells with no, or only marginal, degranulation; 1 = mast cell showing unequivocal signs of degranulation; 2 = degranulated mast cell with no cell body visible (for full details, see Fozard *et al.*, 1996). The aim was to score a minimum of 100 mast cells per tissue section. However, in two of the 16 animals tested this was not possible and thus the relative percentages are presented.

In vitro *studies: lung parenchymal strip*

Naïve (non-sensitized) rats, or rats sensitized to OA, as described above, were challenged either with vehicle or OA, 0.3 mg kg^{-1} 3 h prior to death by exposure to carbon dioxide. The lungs were perfused *in situ via* a cannula inserted into the pulmonary artery with 60 ml of modified Krebs' solution of the following composition (mM): NaCl, 118; KCl, 4.8; $MgSO_4$, 1.2; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 11. The lungs were removed and four slices (10–12 mm long, 3 mm thick) were cut from the major lobe. Tissues were set up for recording isotonic tension in 10 ml organ baths containing modified Krebs' solution at 37°C bubbled with 95% O_2/5% CO_2. Resting tension was maintained at 1 g. After a stabilization period of 1 h, during which time the tissues were repeatedly washed, a supramaximal concentration of bethanechol (0.1 mM) was added. After repeated washing during 1–1.5 h, a single concentration of adenosine (0.1, 0.3 or 1 mM) was added to the bath followed 1 h later, after repeated washing, by establishment of a dose-response curve to 5-HT (100 nM–0.1 mM) and, a further 1 h later, a curve to bethanechol (100 nM–0.1 mM). Tension changes were expressed relative to the maximal response to bethanechol. In some experiments methysergide (10 nM), or disodium cromoglycate (0.03 mM) were included in the Krebs' solution from 15 min prior to establishment of the response to adenosine (0.1 mM) for the duration of the experiment.

Materials

Aluminium hydroxide was from Merck, Germany. *Acullulare pertussis* adsorbat vaccine was from the Vaccinal and Serotherapic Institute of Bern, Switzerland. Pentothal

(thiopentalum natricum) and Forene (isofluran, 100%) was obtained from Abbott, Switzerland. Norcuron (vecuronium bromide) was from Organon Teknika, Holland. Ovalbumin was obtained from Fluka, Switzerland. Bradykinin acetate was from Bachem, Switzerland. Bethanechol (carbamyl-β-methyl-choline chloride), capsaicin, ketanserin tartrate, methacholine chloride, disodium cromoglycate, compound 48/80 (condensation product of N-methyl-p-methoxyphenylethylamine with formaldehyde), 5-hydroxytryptamine creatinine sulphate and adenosine hemisulphate were obtained from Sigma, Switzerland. All compounds were made up freshly in 0.9% w v^{-1} NaCl on the day of the experiment.

Data analysis

All data are presented as means ± s.e.means. Statistical analysis was performed on raw data by means of Student's *t*-test for paired data or analysis of variance with *post hoc* pairwise multiple comparison procedures, using SigmaStat for Windows, version 2.03. A P value <0.05 was considered significant.

Results

Bronchoconstrictor and cardiovascular responses to adenosine in BN rats: Comparison between naïve animals and animals actively sensitized to OA with and without allergen challenge

The results are presented in Figure 1. Adenosine (0.1–10 mg kg^{-1} i.v., interval between doses 20 min) induced only weak bronchoconstrictor responses in both naïve and actively sensitized animals and there were only minor differences in responses between the two groups. In contrast, 3 h following intratracheal (i.t.) challenge of actively sensitized animals with OA (0.3 mg kg^{-1}), responses to adenosine were enhanced at all doses tested. Enhancement was selective for the airways since neither the fall in blood pressure nor the bradycardia induced by adenosine was increased in the challenged animals (Figure 1).

Bronchial hyperresponsiveness to adenosine induced by OA challenge in actively sensitized BN rats: Dose-response relationship, time course and selectivity with respect to methacholine and bradykinin

In these experiments, OA was given i.t. at doses of 0.03, 0.3 or 3 mg kg^{-1} and the sequence of bronchoconstrictor agents initiated 3 or 24 h after OA challenge. Vehicle-challenged animals received saline, 0.2 ml i.t., 3 or 24 h before the start of the bronchoconstrictor agonist sequence. There were no changes in basal airway resistance following any of the doses of OA or at either time point. The results are shown in Figure 2.

At the 3 h time point a marked dose-dependent augmentation of the response to adenosine was noted following each dose of allergen. Responses to methacholine and bradykinin were little changed although the responses to the smaller doses of both agents were significantly increased following the 3 mg kg^{-1} dose of OA.

At the 24 h time point the sensitivity of the airways to adenosine, was similar to that of control animals following each dose of OA. Responses to the higher dose of methacholine (10 μg kg^{-1}) were inhibited dose-dependently with increasing OA dose (significant at 3 mg kg^{-1} OA). Responses to bradykinin were little changed 24 h following OA (0.03 and 3 mg kg^{-1}) but were significantly augmented following 0.3 mg kg^{-1}.

From these studies, 0.3 mg kg^{-1} of OA given i.t., 3 h prior to testing was considered a suitable paradigm to explore the mechanism of the airway hyperresponsiveness to adenosine induced by allergen challenge in actively sensitized BN rats.

Effects of allergen challenge on bronchial responsiveness to adenosine administered locally to the airways in actively sensitized BN rats

The sensitivity of the airways to i.t. administration of adenosine was established in actively sensitized BN rats 3 h following challenge with OA (0.3 mg kg^{-1}, i.t.) or vehicle (saline, 0.2 ml, i.t.). A dose response curve to adenosine (3, 10 and 30 mg kg^{-1}) was constructed using one dose per animal

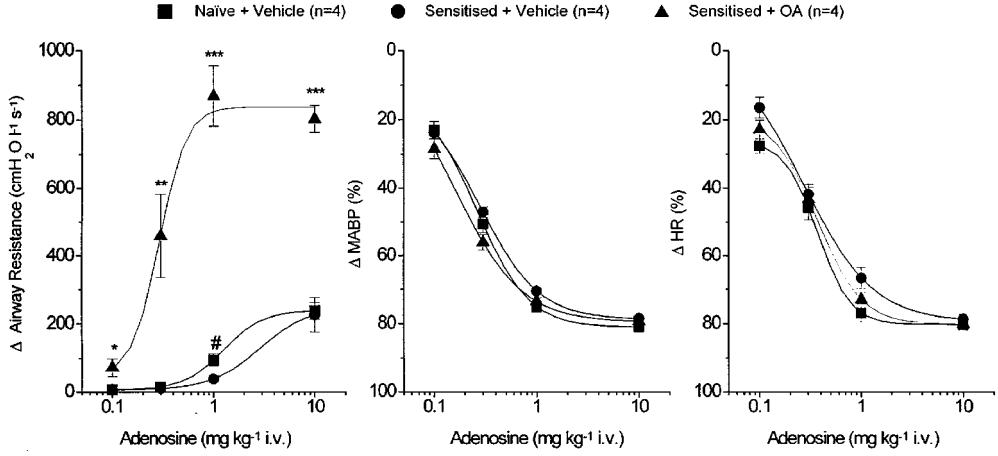

Figure 1 Comparison of the bronchoconstrictor and cardiovascular (mean arterial blood pressure, MABP; heart rate, HR) effects of adenosine in non-sensitized (naïve), Brown Norway rats challenged 3 h previously with vehicle (saline, 0.2 ml, i.t.) or animals, actively sensitized to ovalbumin (OA) challenged 3 h previously with vehicle (saline, 0.2 ml, i.t.) or OA (0.3 mg kg^{-1} i.t.). Results are expressed as means ± s.e.mean of the number (n) of animals shown in parentheses. $*P<0.05$, $**P<0.01$, $***P<0.001$ that the value is significantly different from the equivalent value in either the naïve or actively sensitized vehicle-challenged animals. $\#P<0.05$ that the value is significantly different from the equivalent value in the actively sensitized, vehicle-challenged animals.

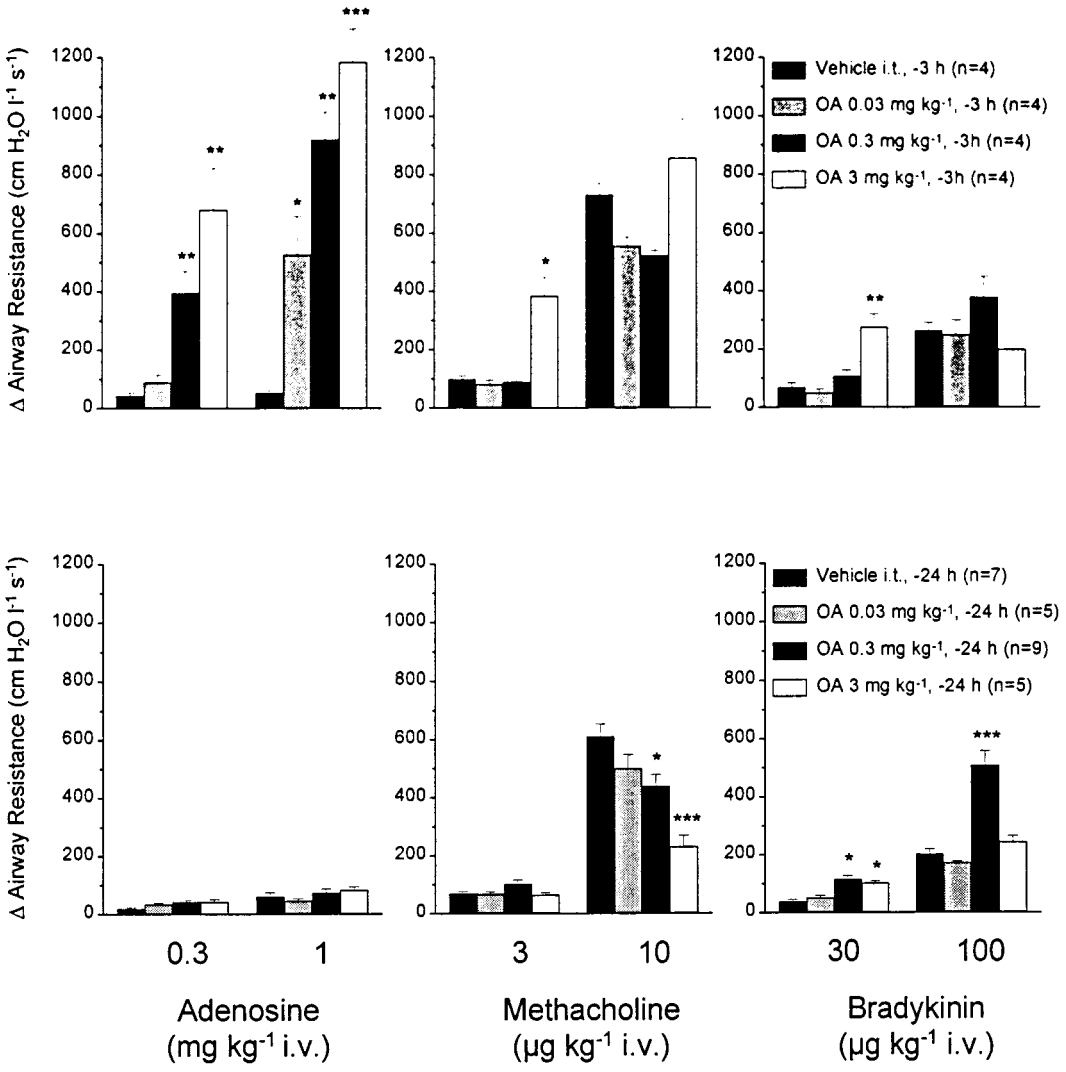

Figure 2 Increases in airway resistance induced by intravenous administration of adenosine, methacholine and bradykinin starting 3 h (upper panel) or 24 h (lower panel) post intratracheal instillation of ovalbumin (OA; 0.03, 0.3, or 3 mg kg^{-1}), or vehicle (saline, 0.2 ml), in actively sensitized Brown Norway rats. Results are expressed as means ± s.e.mean of the number (n) of animals shown in parentheses. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ indicates significant difference between the OA-treated and equivalent vehicle-treated group.

to avoid tachyphylaxis. The results are presented in Figure 3. They demonstrate a marked augmentation of the bronchoconstrictor response to adenosine following OA challenge and are thus qualitatively similar to those obtained when adenosine was given i.v. (see Figure 1).

Role of nerve activation in the augmented bronchoconstrictor response to adenosine induced by OA challenge in actively sensitized BN rats

Bilateral vagotomy The results are shown in Figure 4A. When carried out 10 min prior to starting the agonist sequence, bilateral vagal section had no effect on the bronchoconstrictor responsiveness to adenosine, methacholine or 5-HT.

Atropine The results are shown in Figure 4B. The dose of atropine used (10 $\mu g\ kg^{-1}$ i.v.) was defined in preliminary experiments as just supramaximal for blockade of the bronchoconstrictor responses to methacholine. Atropine was without effect on the bronchoconstrictor response to adenosine augmented following OA challenge. Similarly, responses to 5-HT were unchanged following atropine. In contrast, the bronchoconstrictor response to methacholine was markedly and significantly reduced following the antagonist.

Capsaicin Capsaicin releases tachykinins from sensory nerves (Holtzer, 1991; Fox, 1995). If activation of these neurones is important to the action of adenosine, capsaicin should mimic the effects of adenosine. The comparative effects of capsaicin at a high dose previously shown to activate strongly the bronchopulmonary sensory fibres (Yamawaki *et al*., 1993; Hong *et al*., 1998), and adenosine on the airways and cardiovascular system of sensitized BN rats challenged with OA are shown in Figure 5. In contrast to adenosine (1 mg kg^{-1} i.v.), which induced a substantial bronchoconstrictor response, a dose of 100 $\mu g\ kg^{-1}$, capsaicin had minimal effects on airway resistance. Capsaicin did, however, induce a biphasic change in blood pressure accompanied by an increase in heart rate consistent with the release of sensory neuropeptides.

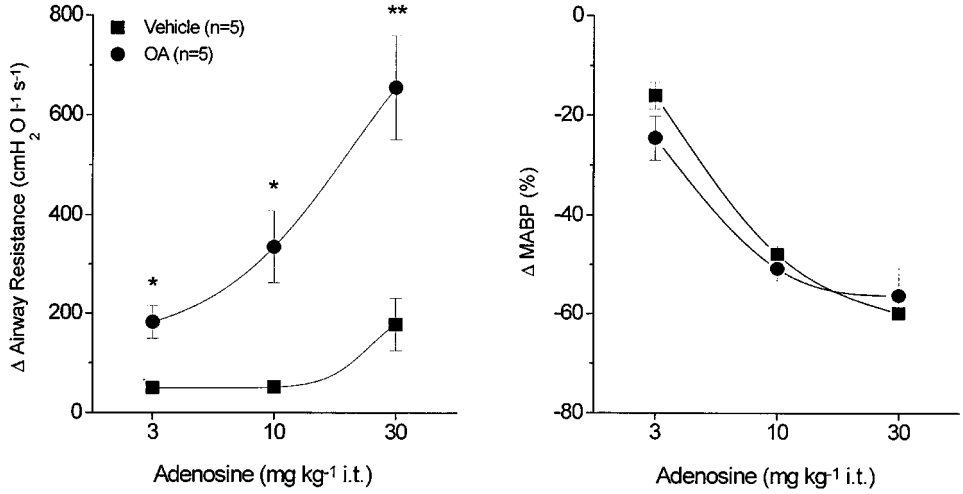

Figure 3 Changes in airway resistance and mean arterial blood pressure (MABP) induced by intratracheal (i.t.) administration of adenosine 3 h post i.t. instillation of ovalbumin (OA, 0.3 mg kg^{-1}) or vehicle (saline, 0.2 ml) in actively sensitized Brown Norway rats. Results are expressed as means ± s.e.mean of the number (n) of animals shown in parentheses. $^{*}P<0.05$, $^{**}P<0.01$ that the value is significantly different from the equivalent value in the vehicle-challenged groups.

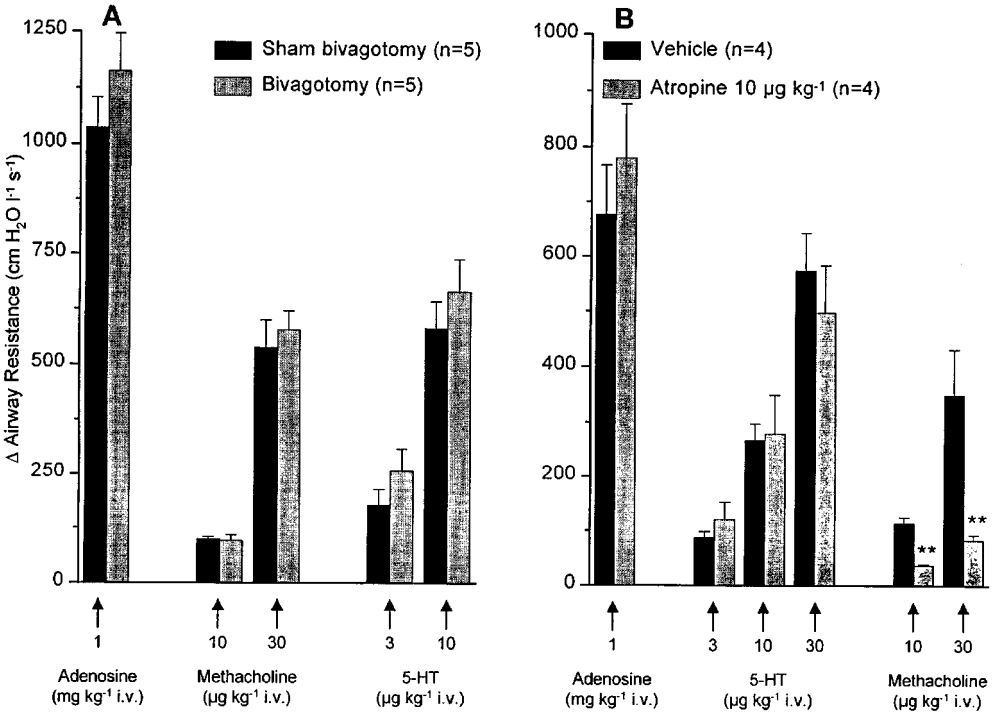

Figure 4 Effect of bilateral section of the vagus nerves (bivagotomy; A) and atropine (10 $\mu g\ kg^{-1}$; B) on bronchoconstrictor responses to adenosine, methacholine and 5-HT in actively sensitized Brown Norway rats challenged 3 h previously with ovalbumin (OA, 0.3 mg kg^{-1}). Bivagotomy was carried out 10 min before, and atropine was given i.v. 5 min prior to, the start of the injection sequence. Results are expressed as means ± s.e.mean of the number (n) of animals shown in parentheses. $^{**}P<0.01$, $^{***}P<0.001$ indicates significant difference between vehicle- and corresponding atropine-treated animals.

Role of the mast cell in the augmented bronchoconstrictor response to adenosine induced by OA challenge in actively sensitized BN rats

To evaluate the role of the mast cell in the bronchoconstrictor response to adenosine, the effects of the mast cell stabilizing agent, disodium cromoglycate, and the 5-HT_2 receptor antagonists, methysergide and ketanserin, were determined. The effects of adenosine in animals depleted of their mast cells by sub-chronic treatment with compound 48/80 were also evaluated.

Disodium cromoglycate The results are shown in Figure 6. Disodium cromoglycate, given i.v. 5 min prior to the start of the agonist sequence at doses previously shown to block mast

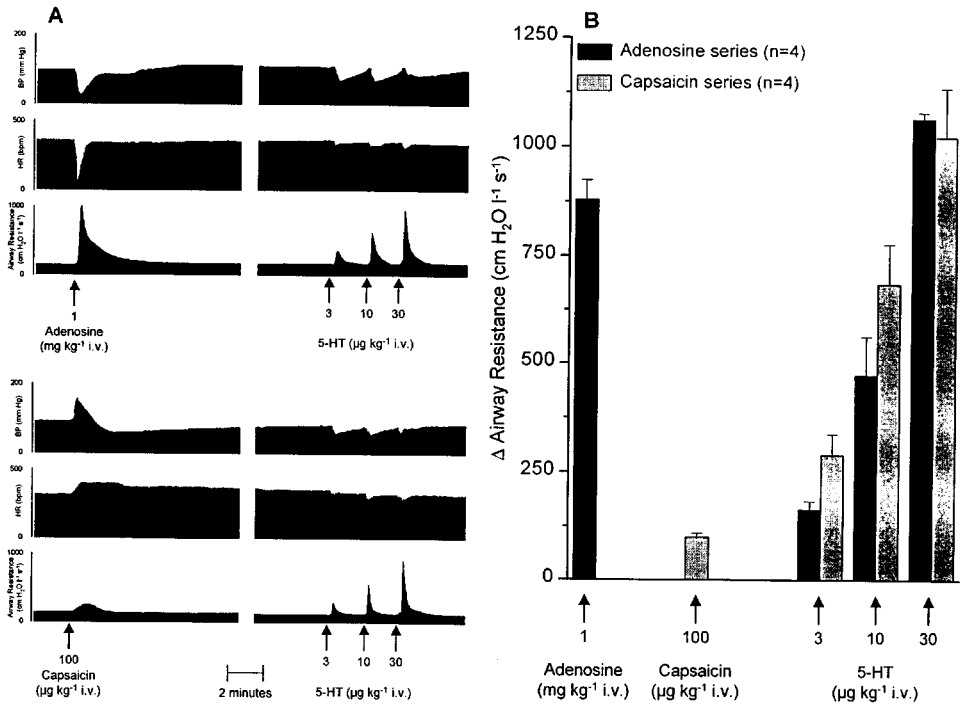

Figure 5 Bronchoconstrictor and cardiovascular effects of capsaicin (100 μg kg^{-1} i.v.) in actively sensitized Brown Norway rats challenged 3 h previously with ovalbumin (OA, 0.3 mg kg^{-1}): Comparison with adenosine (1 mg kg^{-1} i.v.). (A) Representative experimental records. (B) Bronchoconstrictor responses to capsaicin and adenosine expressed as means$\pm$s.e.mean of the number (n) of animals shown in parentheses.

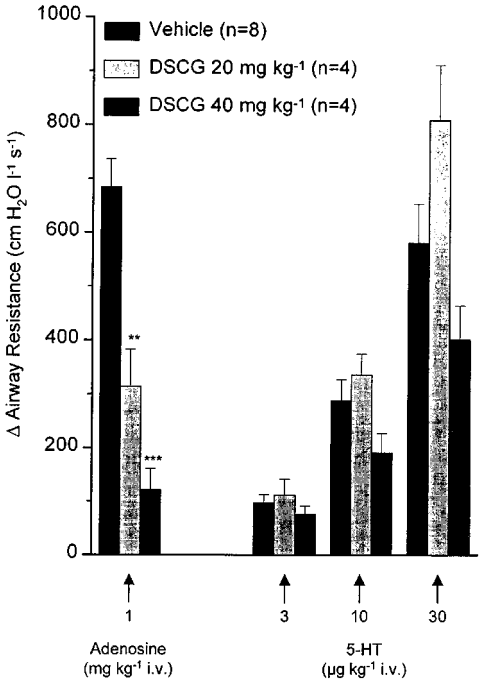

Figure 6 Effect of disodium cromoglycate (DSCG, 20–40 mg kg^{-1} given i.v. 5 min prior to the start of the injection sequence) on bronchoconstrictor responses to adenosine and 5-HT in actively sensitized Brown Norway rats challenged 3 h previously with ovalbumin (OA, 0.3 mg kg^{-1}). Results are expressed as means$\pm$ s.e.mean of the number (n) of animals shown in parentheses. $^{**}P<0.01$, $^{***}P<0.001$ indicates significant difference between vehicle- and corresponding disodium cromoglycate-treated animals.

cell degranulation in rats *in vivo* (20–40 mg kg^{-1}; Hannon *et al*., 1995), induced a marked and dose-dependent inhibition of the bronchoconstrictor response to adenosine. In contrast, neither the bradycardia nor the hypotension in response to adenosine was affected (data not illustrated). Bronchoconstrictor responses to 5-HT were not significantly altered by pretreatment with disodium cromoglycate (Figure 6).

Methysergide and ketanserin The results are shown in Figure 7. Both methysergide and ketanserin, given i.v. 5 min prior to the start of the agonist sequence at doses just supramaximal for blockade of 5-HT$_2$ receptors *in vivo* (10 and 50 μg kg^{-1}; Fozard & Leach, 1968; Fozard, 1982; respectively), induced essentially complete blockade of both the bronchoconstrictor response to adenosine and 5-HT, whereas responses to methacholine remained unchanged. The cardiovascular responses to adenosine and methacholine were not altered by pretreatment with either drug. Predictably, and consistent with the selectivity of methysergide and ketanserin for 5-HT$_2$ receptors, the hypotensive effects of 5-HT were increased following the antagonists (data not illustrated).

Sub-chronic treatment with compound 48/80 Compound 48/80 was administered i.p., to actively sensitized BN rats at a dose of 1.2 mg kg^{-1} day^{-1} on 4 consecutive days; the compound induced mild scratching and the animals appeared sedated in comparison to vehicle-treated animals. On day 5, animals were challenged with OA (0.3 mg kg^{-1} i.t.) and 3 h later a sequence of bronchoconstrictor agonists was started. The results are presented in Figure 8. Pretreatment with compound 48/80 resulted in a significant (*ca.* 65%)

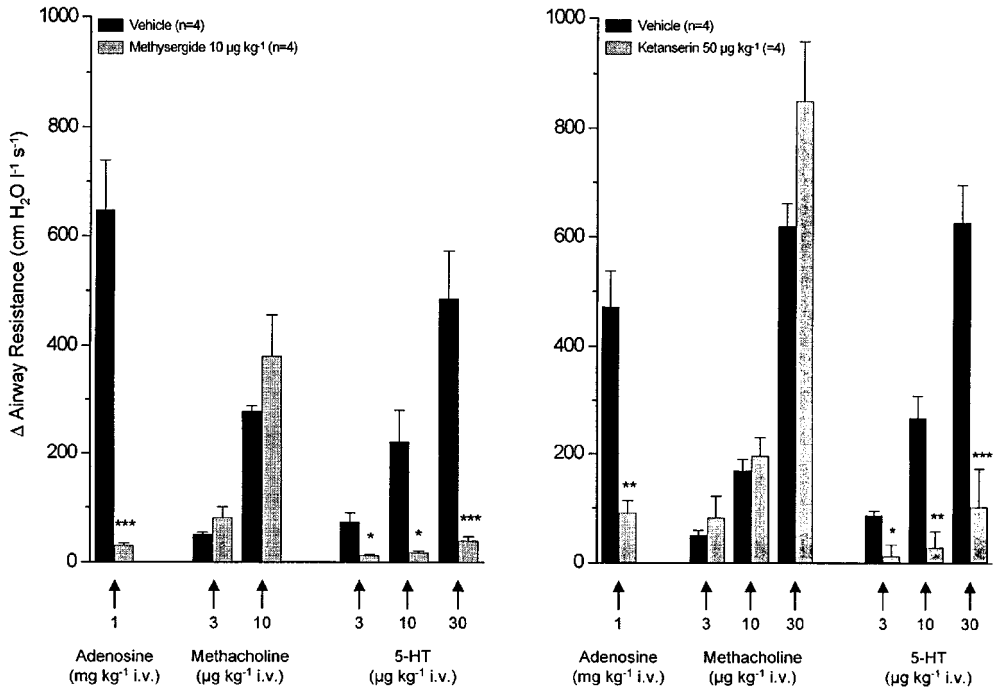

Figure 7 Effect of methysergide and ketanserin (10 and 50 μg kg^{-1}, respectively; given i.v. 5 min prior to the start of the injection sequence) on bronchoconstrictor responses to adenosine, methacholine and 5-HT in actively sensitized Brown Norway rats challenged 3 h previously with ovalbumin (OA, 0.3 mg kg^{-1}). Results are expressed as means$\pm$s.e.mean of the number (n) of animals shown in parentheses. *$P<0.05$, **$P<0.01$, ***$P<0.001$ indicates significant difference between vehicle- and the corresponding methysergide- or ketanserin-treated animals.

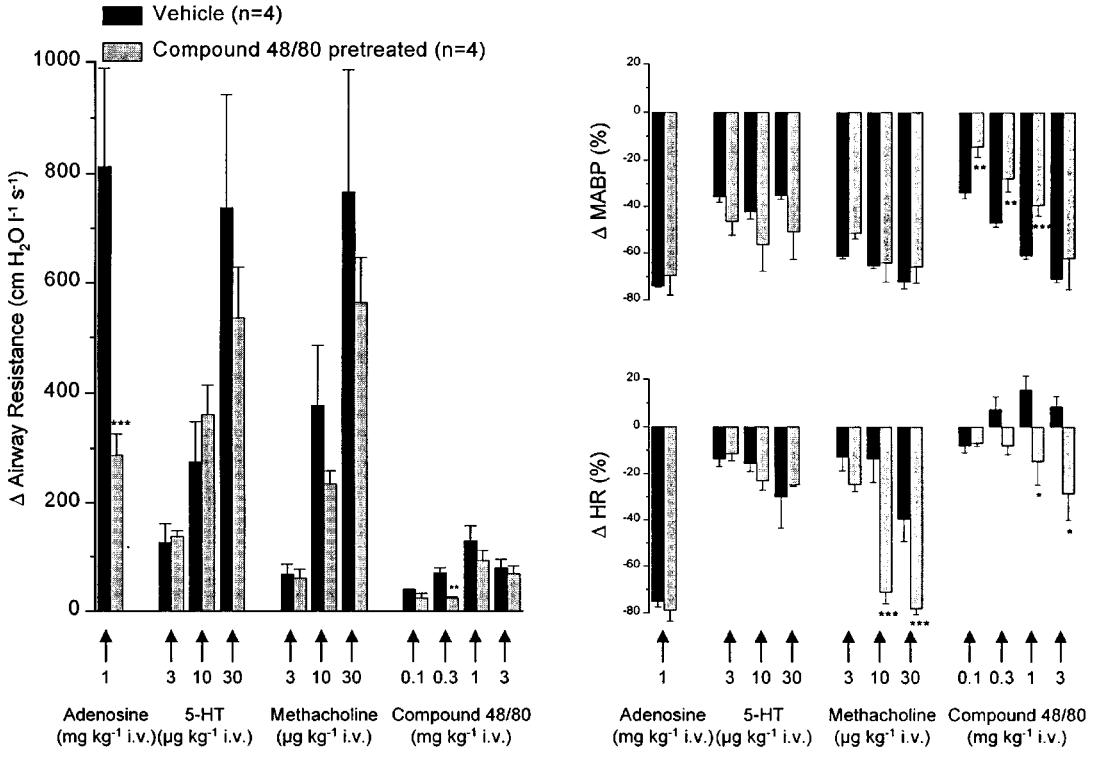

Figure 8 The effect of subacute treatment with compound 48/80 (1.2 mg kg^{-1} day^{-1} i.p., for four consecutive days) on responses to adenosine, 5-HT, methacholine and compound 48/80 with respect to airway resistance, mean arterial blood pressure (MABP) and heart rate (HR) elicited 3 h following ovalbumin (OA, 0.3 mg kg^{-1}) challenge in actively sensitized Brown Norway rats. Results are expressed as means$\pm$s.e.mean of the number (n) of animals shown in parentheses. *$P<0.05$, **$P<0.01$, ***$P<0.001$ indicates significant difference between vehicle- and compound 48/80- pretreated animals.

suppression of the bronchoconstrictor response to adenosine and to the 0.3 mg kg^{-1} dose of acutely administered compound 48/80; in contrast, airway responses to 5-HT or methacholine were not significantly affected. The falls in blood pressure induced by the three lowest doses of compound 48/80 were also inhibited significantly in the pretreated animals (Figure 8).

Plasma histamine and 5-HT concentrations following adenosine administration: Effects of disodium cromoglycate and methysergide

The experiments were carried out in actively sensitized animals challenged with vehicle (saline, 0.2 ml, i.t.) or OA (0.3 mg kg^{-1}, i.t.) 3 h prior to blood sampling. In each group a blood sample was taken from the carotid artery to give the baseline value. Five minutes later animals were given either adenosine (1 mg kg^{-1}) or vehicle (saline, 1 ml kg^{-1}) by bolus i.v. injection and 1 min later a second blood sample was taken. The results are presented in Figure 9.

Injection of vehicle had no effect on the plasma histamine concentrations in either group. In contrast, in actively sensitized animals challenged either with vehicle or OA, adenosine induced marked and significant increases in the plasma levels of histamine (>10 fold; $P<0.001$, respectively).

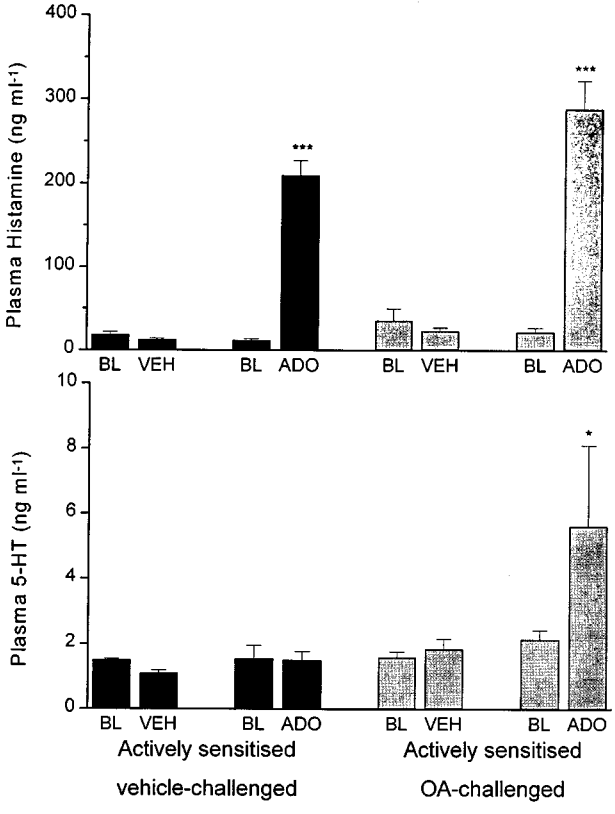

Figure 9 Changes in histamine (upper panel) or 5-HT (lower panel) plasma concentrations induced by intravenous injection of vehicle (VEH; saline, 0.2 ml) or adenosine (ADO, 1 mg kg^{-1}) in actively sensitized Brown Norway rats challenged 3 h previously with either vehicle (saline, 0.2 ml) or ovalbumin (OA, 0.3 mg kg^{-1}). Each column represents the means±s.e.mean of data from eight animals. *$P<0.05$, ***$P<0.001$ indicates significant difference between vehicle- and corresponding adenosine-treated animals.

Injection of vehicle did not change significantly the plasma 5-HT concentrations in either group. Following injection of adenosine the plasma 5-HT concentrations in the actively sensitized animals challenged with vehicle did not change significantly. In contrast, the plasma 5-HT concentrations in animals challenged with OA were increased significantly by 3.4-fold following injection of adenosine (Figure 9).

In a second study the effect of pretreatment with disodium cromoglycate or methysergide on the increases in plasma histamine and 5-HT concentrations induced by adenosine in actively sensitized animals challenged with OA was investigated. The results are presented in Figure 10. In confirmation of the data from the preceding experiment, adenosine (1 mg kg^{-1} i.v.) increased significantly the plasma histamine and 5-HT concentrations. The increases in both mediators were attenuated by treatment with disodium cromoglycate (40 mg kg^{-1} i.v.) given 5 min prior to adenosine. The net increases in plasma histamine concentrations (i.e. values post-

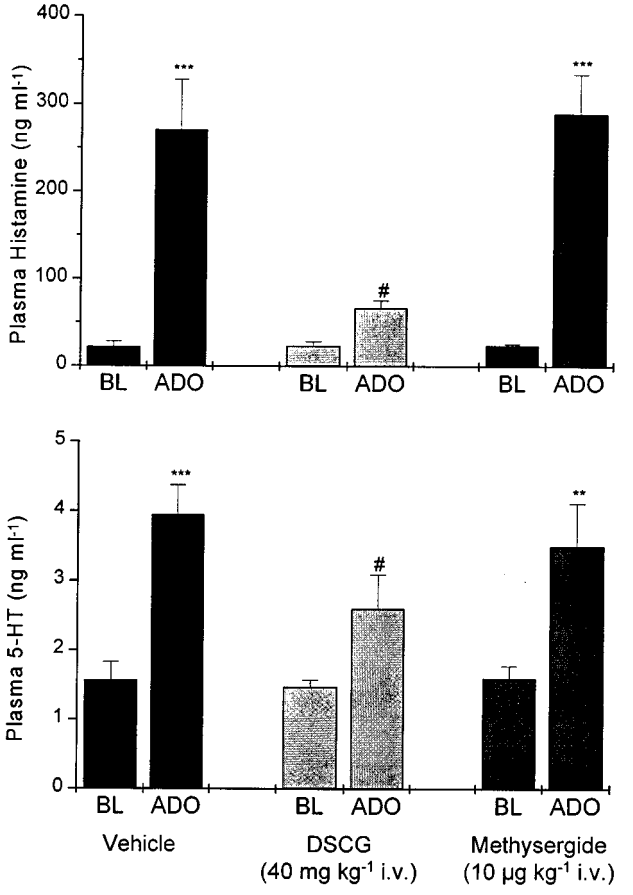

Figure 10 Changes in histamine (upper panel) and 5-HT (lower panel) plasma concentrations from baseline (BL) induced by adenosine (ADO; 1 mg kg^{-1} i.v.) in actively sensitized Brown Norway rats challenged intratracheally 3 h previously with ovalbumin (OA, 0.3 mg kg^{-1}). Animals were treated 5 min prior to adenosine administration with intravenous injections of vehicle (saline, 1 ml kg^{-1}), disodium cromoglycate (DSCG; 40 mg kg^{-1}) or methysergide (10 μg kg^{-1}). Each column represents the means±s.e.-mean of data from four animals. **$P<0.01$, ***$P<0.001$ indicates significant difference between vehicle and corresponding animals given adenosine. #$P<0.05$ indicates significant difference in histamine or 5-HT release induced by adenosine in vehicle-treated and DSCG-treated rats.

adenosine minus pre-adenosine baseline values) were: vehicle 248.7 ng ml^{-1}; disodium cromoglycate 43.8 ng ml^{-1}, $P<0.05$; net increases in plasma 5-HT concentrations: vehicle 2.4 ng ml^{-1}; disodium cromoglycate 1.1 ng ml^{-1}, $P<0.05$. In contrast, neither the increases in histamine nor 5-HT induced by adenosine were affected by pretreatment with methysergide (10 μg kg^{-1} i.v., given 5 min prior to adenosine) (Figure 10).

Mast cell degranulation induced by adenosine

The experiment was carried out in actively sensitized BN rats challenged with vehicle (saline, 0.2 ml, i.t.) or OA (0.3 mg kg^{-1}, i.t.). Three hours later, animals were anaesthetized and given adenosine (1 mg kg^{-1}) or vehicle (saline, 0.2 ml) by bolus intravenous injection. Three to 5 min later animals were killed and samples of lung and skin tissue removed for histological processing. The results are presented in Figure 11. Challenge with OA induced a small, but significant, increase in the proportion of partially or fully degranulated mast cells in lung, but not skin. Adenosine (1 mg kg^{-1} i.v.) administered to vehicle challenged animals had no effect on the degranulation status of mast cells in lung or skin. In contrast, adenosine induced a significant increase in the proportion of partially or fully degranulated mast cells in the lungs of actively sensitized animals challenged 3 h previously with OA; mast cells from skin were unaffected (Figure 11).

In vitro *studies on lung parenchymal strips*

In an attempt to provide further evidence for the mast cells of the lung being involved in the augmented response to adenosine after allergen challenge, the constrictor responses of lung parenchymal strips taken from sensitized animals challenged 3 h previously with OA, 0.3 mg kg^{-1}, were compared with those obtained in strips from sensitized or non-sensitized (naïve) rats challenged with vehicle (saline, 0.2 ml, i.t.). The results are shown in Figure 12. Tissues from naïve rats and sensitized rats challenged with vehicle responded only weakly to adenosine, despite showing robust responses to 5-HT and bethanechol. In contrast, strips taken from animals challenged with OA manifested concentration-dependent constrictor responses to adenosine which were significantly greater than those seen in either the naïve animals or sensitized animals challenged with vehicle. The response to 0.1 mM adenosine reached 40% of the maximum achieved by 5-HT at 0.1 mM; higher concentrations could not be used due to solubility constraints.

Inclusion of methysergide in the Krebs' solution at 10 nM, a concentration 3 fold higher than the K_D at 5-HT$_2$ receptors (Hoyer & Fozard, 1991), reduced markedly the constrictor response to adenosine and 5-HT. In contrast, in the presence of disodium cromoglycate (0.03 mM), a concentration which is just supramaximal for inhibition of mediator release from rat mast cells (Kusner *et al*., 1973), responses to adenosine were reduced significantly by 69% whereas those to 5-HT were not significantly altered (Figure 13).

Discussion

Our data show that the airways of naïve BN rats respond only weakly to adenosine. Low sensitivity of the airways to adenosine is a feature of a number of rat strains including the Fisher 344 and Sprague Dawley strains (Pauwels *et al*., 1979; 1993; Pauwels & Van Der Straeten, 1987). Naïve rabbits and guinea-pigs are also poorly responsive to adenosine (Ali *et*

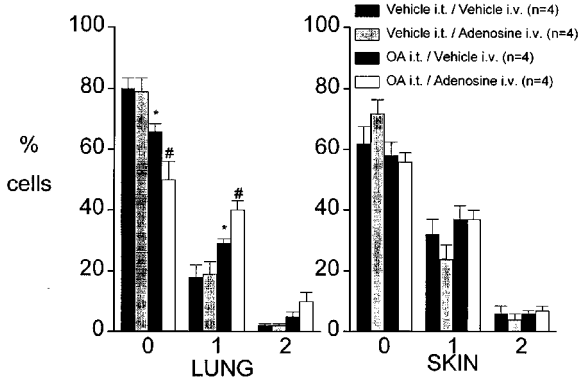

Figure 11 The degranulation status of mast cells in lung and skin 3–5 min following intravenous (i.v.) administration of adenosine (1 mg kg^{-1}) or vehicle (saline, 0.2 ml) to actively sensitized Brown Norway rats, 3 h following intratracheal (i.t.) administration of ovalbumin (OA, 0.3 mg kg^{-1}) or vehicle (saline, 0.2 ml). % cells = percentage of mast cell with a status defined according to the following scale: 0 = essentially intact mast cells with no, or only marginal, degranulation; 1 = mast cell showing unequivocal signs of degranulation; 2 = degranulated mast cell with no cell body visible. Results are expressed as means ± s.e.mean of the number (n) of animals shown in parentheses. $*P<0.05$ indicates significant difference between OA- treated animals and the corresponding vehicle treated controls. $\#P<0.05$ indicates significant difference between adenosine-treated animals and the corresponding vehicle-treated controls.

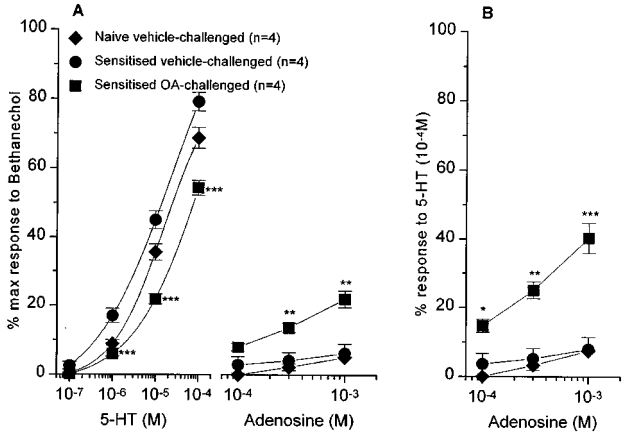

Figure 12 Contractile effects of 5-HT and adenosine on parenchymal strips prepared from lungs removed from non-sensitized (naïve), Brown Norway rats challenged 3 h previously with vehicle (saline, 0.2 ml, i.t.) or animals, actively sensitized to ovalbumin (OA) challenged 3 h previously with vehicle (saline, 0.2 ml, i.t.) or OA (0.3 mg kg^{-1} i.t.). Responses to adenosine are expressed relative to both the maximal response to bethanechol (A) and, to take into account the sensitivity of tissues from the three groups to 5-HT, relative to the response to 5-HT, 10^{-4} M (B). Results are expressed as means ± s.e.mean of the number (n) of tissues shown in parentheses. $*P<0.05$, $**P<0.01$, $***P<0.001$ that the value is significantly different from the equivalent value in the actively sensitized vehicle-challenged animals.

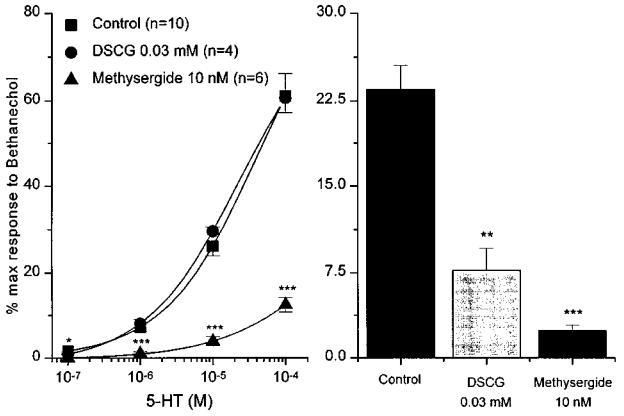

Figure 13 Effect of disodium cromoglycate (DSCG, 0.03 mM) and methysergide (10 nM) on contractile responses to 5-HT and adenosine on parenchymal strips prepared from lungs removed from Brown Norway rats actively sensitized to ovalbumin (OA) and challenged 3 h previously with OA (0.3 mg kg^{-1} i.t.). Results are expressed as means ± s.e.mean of the number (n) of tissues shown in parentheses. $*P<0.05$, $**P<0.01$, $***P<0.001$ that the value is significantly different from the equivalent value in the control group.

al., 1994a,b; Thorne & Broadley, 1994; El-Hashim *et al*., 1996). These observations contrast with the findings in BDE rats where adenosine was demonstrated to induce consistent, though relatively weak, bronchoconstrictor responses (Pauwels & Van Der Straeten, 1987). This may be a reflection of the fact that rats of the BDE strain are innately atopic (Jacobson & Bai, 1997).

Sensitization *per se* did not increase the sensitivity of the airways of BN rats to adenosine. This contrasts with the increase in responsiveness of the airways to adenosine recorded in guinea-pigs sensitized to OA (Thorne & Broadley, 1994). Moreover, rabbits sensitized from birth with either ragweed allergen (Ali *et al*., 1994a) or *Alternia tenuis* allergen (El-Hashim *et al*., 1996) demonstrated significant upregulation of the response to adenosine or its analogue, cyclopentyladenosine, relative to non-immunized controls. On the other hand, the discrepancy between atopic asthmatics (which respond well to adenosine) and the sensitized BN rat (which does not) may be more apparent than real. Thus, asthmatics presumably undergo regular exposure to allergen, a situation which would resemble more that of the sensitized challenged animal than that of animals sensitized but not exposed to allergen.

Challenge of actively sensitized BN rats with allergen induced profound changes in the responsiveness of the airways to adenosine, methacholine and bradykinin. The effects were clearly related to the dose of allergen, the spasmogen used to induce bronchoconstriction and the time after challenge that the spasmogens were administered. Somewhat unexpectedly, the sensitivity of the airways to methacholine was reduced 24 h after challenge with the higher doses of allergen. Generally, responses to cholinergic agonists are increased 16–24 h following allergen challenge in man (Aalbers *et al*., 1991) and BN rats (Elwood *et al*., 1992; 1993; Underwood *et al*., 1995) and hence the findings with methacholine in the present studies do not agree with the literature. At present, we have no convincing explanation for the discrepancy, although it may reflect differences in the dose and procedures used to effect allergen challenge which are generally 'optimized' to give strong responses for a particular endpoint analysis.

The most consistent change was in the response to adenosine which was augmented at each time point and/or following the different doses of allergen. Since the object of these studies was to define the optimal conditions inducing a marked and consistent change in the response to adenosine, OA, 0.3 mg kg^{-1} given 3 h prior to testing was chosen for further detailed evaluation.

Throughout these studies adenosine was given i.v. and it was considered important to demonstrate a similar effect of allergen challenge following local administration of adenosine to the airways. In the event, i.t. administration of adenosine gave a qualitatively similar result to that obtained following i.v. administration; although the doses required were significantly higher. The reason for this is unclear, although it may reflect a more efficient delivery of adenosine to the mast cells believed to be involved in the bronchoconstrictor response to adenosine (*vide infra*) following i.v. rather than i.t. administration. A striking finding in these experiments was that the associated cardiovascular effects of adenosine, were similar in both OA- and vehicle-challenged animals irrespective of the route of administration. The fact that hyperresponsiveness does not extend to the cardiovascular effects of adenosine, rules out a pharmacokinetic interaction as an explanation for the augmented bronchoconstrictor response to adenosine following allergen challenge and implies a fundamentally different mechanism of action of adenosine at the level of the airways and the cardiovascular system.

It is self-evident that the airway smooth muscle must mediate the augmented bronchoconstrictor response to adenosine. However, a general increase in smooth muscle reactivity cannot be the basis of the phenomenon since active sensitization and subsequent exposure to allergen had minimal effects on the bronchoconstrictor response to methacholine (present studies) or 5-HT (Fozard & Hannon, 2000). This suggests that the bronchoconstrictor effect of adenosine may result from an upregulation of an indirect mechanism of action involving the release of bronchoconstrictor mediators from one or more intermediary cell types. The principal candidates for such a role are the airway innervation (Pauwels & Joos, 1995; Hong *et al*., 1998; Tamaoki *et al*., 1999) and mast cells (Pauwels & Joos, 1995; Fozard *et al*., 1996).

Our data suggest that neither the airway cholinergic nerves nor capsaicin-sensitive afferent neurones play a role in the bronchoconstrictor response to adenosine augmented following allergen challenge. Thus, the response was not antagonized by atropine at a dose which ablated responses of the airways to the selective muscarine receptor agonist, methacholine, nor by acute vagal nerve section. Reflex or direct activation of the vagal cholinergic innervation cannot, therefore, underlie the bronchoconstrictor response to adenosine. Further, the response to adenosine could not be mimicked by acute intravenous administration of a high dose of capsaicin adequate to activate the sensory afferent nerve fibres of the rat airways (Yamawaki *et al*., 1993; Hong *et al*., 1998). The cardiovascular effects seen with capsaicin provides direct evidence of sensory neurotransmitter release under the conditions of the present experiments. Despite this, bronchoconstrictor responses to capsaicin were small and simply not

comparable to those produced by adenosine. It bears emphasis in this context that it is the A_1 adenosine receptor which mediates activation of the bronchopulmonary sensory afferent nerves in the rat (Hong *et al*., 1998). In our hands, the bronchoconstrictor response to adenosine was resistant to blockade by the potent and selective A_1 receptor antagonist, dipropylcyclopentylxanthine (DPCPX; Hannon *et al*., 1999b). It is difficult to reconcile these findings with a significant contribution from the sensory afferent innervation to the bronchoconstrictor response to adenosine.

Since previous studies have implicated the mast cell in the bronchoconstrictor response to adenosine and its analogues in the BDE rat (Pauwels & Van Der Straeten, 1987; Pauwels & Joos, 1995; Meade *et al*., 1996), this particular inflammatory cell was the obvious candidate for a role in the response to adenosine augmented following allergen challenge. Our analysis provides pharmacological, biochemical and histological data strongly supportive of the concept.

First, the response to adenosine was markedly suppressed by low doses of the 5-HT_2 receptor antagonists, methysergide or ketanserin, which also blocked the bronchoconstrictor responses to 5-HT but did not affect the responses to methacholine. The lack of effect of these agents on methacholine indicates no generalized suppression of smooth muscle reactivity. Moreover, at the same dose used to suppress the bronchoconstrictor response to adenosine, methysergide did not suppress the increase in histamine and 5-HT concentrations induced by adenosine. Both histamine and 5-HT are contained within rat mast cells and are released upon degranulation (Purcell & Hanahoe, 1990a,b; Meade *et al*., 1996). However, because the rat airways are relatively insensitive to histamine (Lulich & Patterson, 1980; Hannon *et al*., 1995) it is the 5-HT which results in bronchoconstriction (Nagase *et al*., 1996). 5-HT contracts rat airways smooth muscle by an action at 5-HT_2 receptors (Cohen *et al*., 1985; Cohen, 1989; Nagase *et al*., 1996). Methysergide and ketanserin have in common potent 5-HT_2 receptor antagonist activity (Fozard, 1982; Mylecharane, 1990; Hoyer & Fozard, 1991). Blockade by these agents is thus consistent with an intermediatory role for the mast cell in the bronchoconstrictor response to adenosine. A similar conclusion was drawn by Nordstroem & Delbro (1986) with respect to contractile responses to adenosine on rat trachea *in vitro*.

Second, the response to adenosine was blocked selectively by disodium cromoglycate, a compound recognized as an inhibitor of mediator release from mast cells (Bernstein & Bernstein, 1997). The fact that responses to 5-HT were unaltered following disodium cromoglycate indicates an effect other than to suppress bronchial reactivity in general or the bronchoconstrictor effects of 5-HT in particular. The data are therefore consistent with mast cell involvement in the response to adenosine. In support of this, the increased plasma histamine and 5-HT concentrations associated with the bronchoconstrictor response to adenosine were inhibited by treatment with disodium cromoglycate. Coupled with the fact that the pulmonary nerves appear not to be involved in the response to adenosine (see above), the data render it unnecessary to invoke a role for the several other pharmacological properties of disodium cromoglycate which could theoretically play a role in the observed blockade of adenosine (Page, 1994; Bernstein & Bernstein, 1997; Eady & Norris, 1997).

Third, the response to adenosine augmented following allergen challenge was suppressed in animals treated with compound 48/80, a mast cell degranulating agent (Paton, 1951). Repeated treatment with compound 48/80 to achieve depletion of mast cell-derived mediators has been a successful strategy in defining a role of the mast cell in a variety of experimental paradigms (Banks *et al*., 1990; Hannon *et al*., 1995; Meade *et al*., 1996; Reeves *et al*., 1997). We used a pretreatment schedule which resulted in extensive depletion of skin mast cells as quantified by inhibition of plasma protein extravasation (Reeves *et al*., 1997). Evidence of mast cell depletion in the present studies was inferred by the inhibition of both the bronchoconstrictor response and the fall in blood pressure induced by compound 48/80 administered acutely in the treated animals. Using this paradigm, significant blockade of the response to adenosine was observed with no suppressant effects on either the cardiovascular or airway responsivity to 5-HT or methacholine. Again, the data support an intermediary role for the mast cell in the bronchoconstrictor response to adenosine augmented following allergen challenge.

It is of interest to note in passing that the bronchoconstrictor responses to acutely administered compound 48/80 were weak relative to adenosine. One possible explanation is that since compound 48/80 was injected cumulatively there may have been desensitization by the lower doses of responses to the higher doses. The finding may also reflect a relatively weak effect on the subpopulation of mast cells present in the lung on which adenosine is suggested to act (*vide infra*). We have previously documented the tissue heterogeneity of the mast cell degranulation response to compound 48/80 in the rat (Fozard *et al*., 1996). Such an explanation would fit with the observation that blockade of adenosine was incomplete (*ca*. 60% inhibition) in animals treated sub-chronically with compound 48/80.

Fourth, adenosine induced increases in the plasma concentrations of the mast cell mediators, histamine and 5-HT. At a qualitative level these data are fully supportive of mast cell degranulation being involved in the response to adenosine. However, closer examination of the data revealed some interesting aspects to the results in this series of experiments. Thus, marked increases in plasma histamine concentrations were seen following adenosine in both actively sensitized animals challenged with OA and in those challenged with vehicle. Thus, adenosine appears to activate mast cells under both these experimental circumstances to a broadly similar extent. The fact that bronchoconstrictor effects to adenosine are minimal in actively sensitized, vehicle-challenged animals may reflect the insensitivity of the rat airways to histamine (Hannon *et al*., 1995) and the fact that in these animals there was no evidence from the plasma 5-HT levels of 5-HT release following adenosine.

Thus, the marked augmentation of the bronchoconstrictor response to adenosine following OA challenge is associated with the appearance of 5-HT, in addition to histamine, in the plasma tempting speculation that exposure to OA makes available to adenosine a source of 5-HT not available in vehicle-challenged animals. The concentrations of 5-HT in the plasma are low relative to those of histamine which, given the evidence discussed above for an involvement of mast cells in the response to adenosine, suggests that the 5-HT may arise from a relatively small proportion of the total mast cell population.

Since the functional correlate is bronchoconstriction it is reasonable to speculate that the tissue location of this subpopulation of mast cells is the lung. Such a scenario might not be unexpected given that mast cells contain both histamine and 5-HT (Benditt *et al*., 1955; Purcell *et al*., 1989a,b; Purcell & Hanahoe 1990a,b), that there is differential storage of histamine and 5-HT in different anatomical locations (Purcell *et al*., 1989a) and that secretion patterns of histamine and 5-HT vary in mast cells from different locations or in response to different secretagogues (Purcell *et al*., 1989a; Purcell & Hanahoe, 1990a). Importantly, the concept receives support from two further experimental observations.

First, histological examination revealed mast cell degranulation in the lung but not skin following i.v. injection of 1 mg kg^{-1} adenosine to sensitized BN rats challenged with OA; no degranulation was seen following adenosine in animals challenged with vehicle. Although statistically significant, the proportion of the mast cells resident in lung tissue which were degranulated following adenosine was relatively small. Clearly, this would not preclude the release of sufficient 5-HT to induce bronchoconstriction. Moreover, a partial degranulation would, perhaps, be expected since repeated bronchoconstrictor responses can be elicited at the dose of adenosine used (Fozard & Hannon, 2000). The observation provides direct evidence that mast cells of the lung are involved in the bronchoconstrictor response to adenosine.

Second, the augmentation of the bronchoconstrictor response to adenosine by allergen challenge in sensitized animals can be reproduced *ex vivo* in the lung parenchymal strip. As in the *in vivo* studies, the response can be inhibited by methysergide and disodium cromoglycate implicating mediator release from mast cells in the response. Since the parenchymal preparations are taken from lungs washed free of platelets or basophils, these data provide further strong evidence that the mast cells of the lung are the intermediary cells involved in the augmented response to adenosine following allergen challenge.

In summary, our data demonstrate a marked and selective augmentation of the bronchoconstrictor response to adenosine in actively sensitized BN rats challenged with OA. The augmented response is primarily, if not exclusively, a consequence of mast cell activation leading to the release of 5-HT which in turn induces a direct constrictor effect on the bronchial smooth muscle. Our data further suggest the involvement of a discrete lung-based population of mast cells containing and releasing mainly 5-HT and brought into play by exposure to allergen. Further definition as to why these particular cells are sensitised to the effects of adenosine following allergen challenge and the mechanisms underlying the phenomenon must await further experimentation. Nevertheless, upregulation by allergic activation and mast cell dependency represent striking similarities between the bronchoconstrictor response to adenosine seen following allergen challenge in the BN rat and that on the airways of asthmatics.

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