Contribution of a cholinergic reflex mechanism to allergen-induced bronchial hyperreactivity in permanently instrumented, unrestrained guinea-pigs

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1 In conscious, permanently instrumented, unrestrained, ovalbumin-sensitized guinea-pigs the development of allergen-induced bronchial hyperreactivity to histamine- and methacholine-inhalation was investigated after the early as well as after the late asthmatic response.

2 The allergen-induced increase in bronchial reactivity to histamine was significantly higher than to methacholine.

3 The muscarinic receptor antagonist, ipratropium bromide (1.0 mM, 3 min inhalation), blocked methacholine-induced bronchoconstriction and caused a significant 1.7 fold inhibition of the histamine-induced bronchoconstriction of control animals.

4 A lower dose of ipratropium bromide (0.1 mM, 3 min inhalation) had no significant effect on histamine-induced bronchoconstriction in control animals, but significantly reduced the allergen-induced increase in bronchial reactivity to histamine between the early and late asthmatic response. At 1.0 mM ipratropium bromide, no further reduction was observed.

5 These results clearly indicate that an exaggerated cholinergic reflex mechanism contributes to allergen-induced bronchial hyperreactivity to histamine.

Keywords: Bronchial hyperreactivity; vagal reflex mechanism; allergic guinea-pigs; ipratropium bromide; histamine; methacholine

Introduction

Bronchial hyperreactivity (BHR) is a common feature of allergic asthma, characterized by an increased airway narrowing in response to various bronchoconstrictor stimuli, including pharmacological agents, such as histamine and methacholine, and chemical irritants such as SO₂. The increased bronchoconstriction to SO₂ can be reversed by administration of the muscarinic receptor antagonist, atropine (Simonsson *et al.* 1967; Alanko & Poppius, 1974), suggesting an increased cholinergic reflex activity in these patients. In man as well as in animals, histamine has been shown to induce bronchoconstriction by acting directly on airway smooth muscle, but also by stimulating afferent vagal sensory nerve endings just beneath the airway epithelium, leading to cholinergic reflex bronchoconstriction by the release of acetylcholine from efferent postganglionic vagal nerve endings that project on airway smooth muscle. Cholinoceptor agonists appear not to evoke this vagal reflex mechanism (Vidruk *et al.* 1977; Holtzman *et al.* 1980).

BHR in allergic asthmatic patients is invariably associated with an influx of inflammatory cells, predominantly eosinophils and neutrophils, into the airways (Chung, 1986; Wardlaw *et al.* 1988). Cytotoxic mediators derived from these inflammatory cells may damage the airway epithelium, therewith exposing sensory nerve endings beneath the airway epithelium (Frigas & Gleich, 1986). In addition, these mediators may inactivate presynaptically located, autoinhibitory muscarinic M₂-receptors, thus causing enhanced acetylcholine release from efferent vagal nerve terminals after stimulation of the reflex arc (Jacoby *et al.* 1993). Evidence for a reduced M₂-muscarinic receptor function contributing to enhanced cholinergic reflex bronchoconstriction in asthmatics was recently presented by Minette *et al.* (1989).

In animal studies, this M₂-receptor dysfunction has been observed *in vivo* after repeated allergen provocation (Fryer &

Wills-Karp, 1991). In a recently developed guinea-pig model of allergic asthma, characterized by allergen-induced early (EAR) and late (LAR) phase asthmatic responses, bronchial hyperreactivity and airway inflammation (Santing *et al.* 1992; 1994), a similar M₂-receptor dysfunction was already present between the early and late asthmatic reaction after a single allergen provocation. Loss of M₂-receptor function was indicated by a strongly decreased potentiation by selective M₂-receptor antagonists of the cholinergic twitch contraction of tracheal smooth muscle induced by electrical field stimulation that causes neurotransmitter release from the vagal nerve terminals (Ten Berge *et al.*, 1992).

In the present study we investigated the severity of allergen-induced BHR towards histamine and methacholine and the contribution of a cholinergic reflex bronchoconstriction to the allergen-induced BHR towards histamine *in vivo*.

Methods

Animals

Specific pathogen-free guinea-pigs (Charles River SAVO, Kiszlegg, Germany), were used in this study. All animals were sensitized to ovalbumin at 4 weeks of age, weighing approximately 250 g. A shift to immunoglobulin E (IgE) class antibodies was obtained by using an allergen solution containing 100 µg ovalbumin and 100 mg Al(OH)₃ per ml saline (Andersson, 1980). The allergen solution was gently rotated for 60 min to obtain an alu-gel, and 0.5 ml was injected i.p., while another 0.5 ml was divided over seven s.c. injection sites in the proximity of lymphnodes in the paws, lumbar regions and neck (Van Amsterdam *et al.*, 1991). The animals were operated on 4 weeks after sensitization and used experimentally in weeks 5 to 7. The animals were

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housed in individual cages in climate-controlled animal quarters and were given water and food *ad libitum*. All protocols described were approved by the University of Groningen Animal Health Committee, which is responsible for the care and proper use of experimental animals.

Measurement of airway function

Airway function was assessed by measurement of pleural pressure (P_{pl}) as described previously (Santing *et al.*, 1992). In brief, a small latex balloon, connected to a saline-filled cannula, was surgically implanted inside the thoracic cavity. The free end of the cannula was driven subcutaneously to the neck of the animal, where it was exposed and attached permanently. Via an external fluid-filled cannula the pleural balloon was connected to a pressure transducer (Gould P23ID, Gould Medical BV, Bilthoven, The Netherlands). P_{pl} (in cmH_2O) was continuously measured by feeding the signals into an A/D converter interfaced to an Atari 1040 ST computer. P_{pl} data were sampled for 10 s every minute. Breath by breath variation was normally less than 15%. Incidental large variations caused by sudden movements or deep sighs were rejected from the calculation.

Using a combination of flow, measured with a pneumotachograph implanted inside the trachea, and pleural pressure, measured with the latex balloon, we have previously shown that changes in P_{pl} are linearly related to changes in airway resistance and hence can be used as a sensitive index for both allergic and non-allergic bronchoconstriction (Santing *et al.*, 1992). Baseline P_{pl} -measurement remained stable for several weeks, and no signs of inflammation were observed at the sites of surgery for at least five weeks after the operation. Airway function can be registered repeatedly and continuously, for periods of at least 24 h, while the animals are unaware of the measurements being taken.

Provocation procedures

Ovalbumin, histamine and methacholine provocations were performed by inhalation of aerosolized solutions. These provocations were carried out in a specially designed animal cage with a volume of 9 l, in which the guinea-pigs could move freely (Santing *et al.*, 1992). A DeVilbiss nebulizer (type 646, DeVilbiss, Somerset, PA, U.S.A.) driven by an airflow of 81 min^{-1} , provided the aerosol with an output of 0.43 ml min^{-1} . The animals were habituated to the experimental conditions and the provocation procedure on two sequential days at least one week after operation, when preoperative weight was restored. On the first day, the animals were placed inside the provocation cage unconnected to the transducers. Three consecutive inhalations with saline were performed, lasting 3 min each and separated by a 7 min interval. The next day, this protocol was repeated with the animals connected to the measurement system.

On the experimental days after the habituation protocol, allergen, histamine, methacholine, and saline provocations were performed as indicated below. All provocations were preceded by an adaptation period of at least 30 min, followed by two consecutive control inhalations with saline as described above. A baseline P_{pl} -value was calculated by averaging the P_{pl} -values from the last 20 min of the adaptation period. Histamine and methacholine provocations were performed starting with a $25 \mu\text{g ml}^{-1}$ solution in saline, followed by increasing dosage steps of $25 \mu\text{g ml}^{-1}$. Identical to the preceding saline inhalations, each provocation lasted 3 min and was separated from the previous provocation by a 7 min interval. Animals were challenged until the P_{pl} increased by more than 100% above baseline for at least three consecutive min. The concentration causing a 100% increase in P_{pl} (PC_{100}) was calculated by linear interpolation of the concentration- P_{pl} response curve. After the histamine and

methacholine provocations, P_{pl} returned to baseline within 30 min.

Allergen provocations were performed by inhalation of increasing concentrations of 1.0, 3.0 and 5.0 mg ml^{-1} ovalbumin in saline for 3 min each, separated by 7 min intervals, until an increase in P_{pl} of more than 100% was observed. Under these conditions none of the animals developed anaphylactic shock after allergen provocation.

Experimental protocol

Allergen-induced BHR towards histamine and methacholine

In a first group of animals, allergen provocations were performed 24 h after a PC_{100} -measurement with either histamine or methacholine. At 6 h (between the early and late asthmatic reaction) and at 24 h (after the late asthmatic reaction) after allergen provocation, the PC_{100} -values were established again to assess allergen-induced BHR for histamine or methacholine, respectively. On the allergen provocation day, airway function was continuously measured during the whole procedure for the quantitative assessment of early (usually between 0 and 5 h after allergen provocation (Santing *et al.*, 1994)) and late (usually between 8 and 24 h after allergen provocation (Santing *et al.*, 1994)) phase asthmatic responses. Between the measurements of histamine and methacholine PC_{100} values at 6 h and 24 h, the animals were placed in a larger cage, where they could move around freely and eat and drink *ad libitum*. During this transfer the animals remained connected to the measurement system.

Effect of ipratropium bromide on histamine- and methacholine-induced bronchoconstriction In another group of animals, the effects of three increasing concentrations of ipratropium bromide or saline on histamine- or methacholine-induced bronchoconstriction were tested. Thirty min after the assessment of histamine or methacholine PC_{100} , the first concentration of ipratropium (or saline) was inhaled for 3 min. Ten min afterwards the next PC_{100} -measurement was started by inhalation of histamine or methacholine at 2 concentration steps below the maximal concentration of the preceding PC_{100} -measurement. In this way, histamine- or methacholine-induced bronchoconstriction occurred approximately 30 min after ipratropium bromide inhalation. This procedure was repeated 2 times, using 10 fold higher concentrations of ipratropium bromide or saline. For histamine, a concentration range of 0.1, 1.0 and 10.0 mM ipratropium bromide was used, and for methacholine this range was 0.01, 0.1, and 1.0 mM ipratropium bromide.

Effect of ipratropium bromide on allergen-induced BHR towards histamine At least 7 days after testing the effects of ipratropium bromide on histamine- or methacholine-induced bronchoconstriction, the PC_{100} towards histamine was (re-) established. Allergen provocation was performed 24 h thereafter. At 5 h after allergen provocation the PC_{100} towards histamine was measured again to assess allergen-induced BHR. Identical to the procedure described above, ipratropium bromide (0.1 or 1.0 mM) was inhaled for 3 min at 5.5 h after allergen provocation. Subsequently, histamine PC_{100} -values were determined at 6 h and 24 h after allergen provocation.

In addition, two control groups were used. A first control group received saline instead of allergen and 0.1 mM ipratropium bromide at 5.5 h after saline inhalation. The second control group received saline instead of ipratropium bromide at 5.5 h after allergen provocation. During the experimental period, airway function was continuously measured for the quantitative assessment of early and late phase asthmatic responses.

Data analysis

The magnitudes of the allergen-induced early and late asthmatic responses were expressed as the area under the P_{pl}

time-response curve (AUC) between 0 and 6 h after allergen provocation for the early response, and between 8 and 24 h after provocation for the late response. P_{50} was expressed as percentage change from baseline and AUC was determined by trapezoid integration over discrete (5 min) time-periods. Based on AUC-values from saline control provocations, threshold values (mean $\pm 2 \times$ s.d.; 99% confidence interval) of 1185% 5 min for a positive early response and 2790% 5 min for a positive late response were defined (Santing *et al.*, 1994). Using these criteria, animals were characterized as single early responders and dual responders (i.e. animals expressing an early as well as late asthmatic response).

Changes in allergen-induced asthmatic reactions as well as in airway reactivity towards histamine or methacholine were analysed by Student's *t* test for paired or unpaired data as indicated. Differences were considered to be statistically significant when $P < 0.05$. All data are presented as average $\pm$ s.e. mean.

Chemicals

Histamine hydrochloride, ovalbumin (grade III), and aluminium hydroxide were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and methacholine chloride from Aldrich (Milwaukee, WI, U.S.A.). Ipratropium bromide was a gift from Boehringer Ingelheim GmbH (Ingelheim am Rhein, Germany).

Results

Allergen-induced BHR towards histamine or methacholine

Allergen-induced increases in bronchial reactivity towards histamine and methacholine inhalation are presented in Table 1. Only dual responding animals (16 out of 32) were used for this evaluation, because it was previously found that the bronchial reactivity to histamine at 6 h and 24 h after allergen provocation was significantly increased in dual responding animals only (Santing *et al.* 1994). The early asthmatic response averaged $4,839 \pm 958\%$ 5 min in the histamine challenged animals and $5,140 \pm 1,458\%$ 5 min in the methacholine challenged animals. For the late asthmatic response these values were $8,460 \pm 1,593\%$ 5 min and $7,945 \pm 1,281\%$ 5 min, respectively. No significant differences were observed between histamine and methacholine-challenged animals. Bronchial reactivity was significantly enhanced after the early asthmatic response (6 h after provocation) as well as after the late asthmatic response (24 h after provocation) (Table 1), while the former was significantly larger, when tested with histamine ($P < 0.001$; $n = 15$) as well as with methacholine ($P < 0.05$; $n = 11$). Comparison of the developed BHR towards histamine and methacholine showed that the BHR for histamine was significantly larger than for methacholine, at 6 h ($P < 0.05$) as well as 24 h after allergen provocation ($P < 0.001$; unpaired analysis) (Table 1).

Effect of ipratropium bromide on histamine- and methacholine-induced bronchoconstriction

Bronchial reactivity for histamine (Figure 1a) and methacholine (Figure 1b) was assessed four times per animal with an interval of approximately 1 h. In the control group, saline inhalations had no effect on the subsequent PC_{100} -values for histamine, while a small but significant decrease in PC_{100} -values for methacholine was observed between the first and second determination ($P < 0.01$), which was unaltered during subsequent PC_{100} -measurements. Ipratropium bromide, in a concentration of 0.1 mM, reduced the sensitivity to methacholine 2.5 fold ($P < 0.05$; Figure 1b), but had no significant effect on histamine-induced bronchoconstriction (Figure 1a). However, 1.0 mM ipratropium, a concen-

Table 1 Allergen-induced changes in bronchial reactivity to histamine and methacholine in dual responding guinea-pigs between the early and late asthmatic response (6 h) and after the late asthmatic response (24 h)

Compound applied	n	Ratio PC_{100} (pre-post-allergen provocation)	
		6 h	24 h
Histamine	15	$3.04 \pm 0.32^{***}$	$1.68 \pm 0.10^{***}$
Methacholine	11	$1.96 \pm 0.31^{*/\dagger}$	$1.17 \pm 0.05^{**/\dagger\dagger}$

Data represent mean values $\pm$ s.e.mean. Mean PC_{100} before allergen provocation for histamine: 137.7 ± 16.9 and for methacholine 96.7 ± 9.0 . Statistical analysis: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, Student's paired *t* test compared to before allergen provocation; $\dagger P < 0.05$, $\dagger\dagger P < 0.001$, Student's unpaired *t* test compared to BHR for histamine.

Figure 1 Effect of ipratropium bromide (●: 0.01 to 1.0 mM; ■: 0.1 to 10 mM) or saline inhalation (○) on histamine (a) and methacholine (b) PC_{100} . Subsequent PC_{100} measurements were performed with an interval of approximately 1 h, with ipratropium bromide or saline being administered 30 min after the preceding PC_{100} measurement. Data represent the average $\pm$ s.e. mean of 5–7 animals. Statistical analysis: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, Student's paired *t* test compared to first measurement.

tration that caused a 50 fold reduction in the response to methacholine inhalation ($P < 0.001$; Figure 1b), decreased the sensitivity to histamine 1.7 fold ($P < 0.05$; Figure 1a). Increasing the concentration of ipratropium to 10 mM caused a 2.0 fold decrease in histamine PC_{100} ($P < 0.05$; Figure 1a). However, this value was not significantly different from that obtained in the presence of 1.0 mM ipratropium.

Effect of ipratropium bromide on allergen-induced BHR towards histamine

Saline inhalation did not affect the reactivity of the airways to histamine-aerosol measured 5 h later. Moreover, 0.1 mM ipratropium, administered 5.5 h after saline inhalation, did not significantly affect the PC₁₀₀-value for histamine at 6 h and 24 h after saline inhalation (Figure 2). A second group of animals was treated with saline instead of ipratropium at 5.5 h after allergen provocation. In these animals the bronchial reactivity to histamine was significantly increased at 5 h ($P < 0.01$) as well as at 6 h ($P < 0.01$) and 24 h ($P < 0.05$) after allergen provocation, compared to the PC₁₀₀ before allergen provocation. No significant difference in the PC₁₀₀ for histamine was measured between 5 h and 6 h after allergen provocation (Figure 2).

The PC₁₀₀ for histamine at 5 h after allergen provocation was similarly decreased in all groups (Figure 2). Treatment with 0.1 mM ipratropium bromide at 5.5 h after allergen provocation significantly reduced the BHR to histamine at 6 h as compared to 5 h after the provocation ($P < 0.01$; $n = 5$; Figure 2). A 10 fold higher ipratropium concentration applied at 5.5 h after allergen provocation did not further increase the PC₁₀₀ for histamine (Figure 2).

Bronchial reactivity to histamine at 24 h after allergen provocation was significantly increased compared to prechallenge values in all three allergen provocation groups ($P < 0.05$, each). Moreover, ipratropium bromide inhalations (0.1 mM or 1.0 mM) applied at 5.5 h after allergen provocation did not significantly affect the histamine PC₁₀₀ at 24 h after allergen provocation as measured in the absence of ipratropium bromide. No significant differences were observed in the severity of the early as well as the late asthmatic response between the different allergen-challenged groups (not shown).

Discussion

In this study, allergen-induced BHR to histamine in permanently instrumented, unrestrained guinea-pigs was significantly higher than with methacholine. Acute methacholine-induced bronchoconstriction is thought to act primarily through direct stimulation of the airway smooth muscle (Vidruk *et al.*, 1977; Holtzman *et al.*, 1980). By contrast, part of the histamine-induced bronchoconstriction may also be mediated by afferent vagal nerve endings located beneath the airway epithelium (Vidruk *et al.*, 1977; Holtzman *et al.*, 1980).

The increased BHR towards histamine as compared to methacholine would suggest that this vagal reflex mechanism may contribute to the allergen-induced BHR towards histamine. In control animals, it was found that inhalation of 1 mM ipratropium bromide strongly inhibited the methacholine response and significantly reduced the histamine-induced bronchoconstriction. A 10 fold increase in ipratropium concentration did not further reduce the histamine PC₁₀₀-value. These results clearly demonstrate that acetylcholine released from the efferent vagus nerve endings indeed contributes to histamine-induced bronchoconstriction in this guinea-pig model. This mechanism presumably involves stimulation of histamine H₁-receptors on myelinated afferent nerve endings beneath the airway epithelium (Vidruk *et al.*, 1977).

To determine the vagal contribution to allergen-induced BHR towards histamine between the early and late asthmatic reaction, BHR was measured at 5 h and 6 h after allergen provocation, with ipratropium bromide applied in between. While saline did not significantly affect the severity of BHR, ipratropium bromide markedly inhibited the allergen-induced increase in bronchial reactivity towards histamine.

The clear inhibition of histamine-hyperreactivity after allergen provocation by an ipratropium concentration as low

Figure 2 Effect of ipratropium inhalation on allergen-induced bronchial hyperreactivity to histamine. Saline (●), or ipratropium (■, 0.1 mM; ◆, 1 mM) inhalation was performed at 5.5 h after allergen provocation. Open symbols represent data from a control inhalation with saline instead of allergen. In these animals, 0.1 mM ipratropium was administered at 5.5 h after saline inhalation. Data represent the average $\pm$ s.e. mean of 5–6 animals. Statistical analysis: * $P < 0.05$, ** $P < 0.01$, Student's paired t test compared to 24 h prechallenge (before): # $P < 0.05$; ## $P < 0.01$, Student's paired t test compared to 5 h value.

as 0.1 mM, which had virtually no effect on non-allergen provoked animals, clearly indicates that histamine-induced vagal reflex bronchoconstriction is increased after allergen provocation. A 10 fold increase of the ipratropium concentration did not further reduce the hyperreactivity for histamine, indicating that in addition to the cholinergic component, other mechanisms are also involved. This is also illustrated by the hyperreactivity observed with methacholine as the agonist, which does not involve a vagal reflex mechanism.

Both concentrations of ipratropium bromide did not affect the magnitude of the late asthmatic reaction as well as the BHR to histamine after this reaction. This finding may, however, be due to the washout of ipratropium bromide during the late reaction. Several mechanisms may account for the enhanced bronchial reactivity after allergen provocation. Firstly, disruption of the airway epithelium by cytotoxic mediators released from inflammatory cells (Frigas & Gleich, 1986) or opening of tight junctions between the airway epithelial cells by endogenous histamine released after allergen provocation (Braude *et al.*, 1984), may facilitate the access of various bronchoconstrictor compounds to the airway smooth muscle.

Secondly, a dysfunction of autoinhibitory muscarinic M₂-receptors, located presynaptically on airway smooth muscle, has recently been postulated in asthmatic patients (Minette *et al.*, 1989) as well as in allergic guinea-pigs (Fryer & Wills-Karp, 1991). In our guinea-pig model, a similar M₂-dysfunction has been observed *in vitro*, 6 h after allergen provocation (Ten Berge *et al.*, 1992). The pathological mechanisms causing this M₂-dysfunction presumably involve cationic proteins, released from activated eosinophils, including major basic protein (MBP), eosinophilic cationic protein (ECP) and eosinophil peroxidase (EPO) (Fryer & Jacoby, 1992). Very recently, Jacoby and coworkers (1993) observed that MBP and EPO, but not ECP displaces [³H]-N-methylscopolamine from M₂-receptors in heart membranes via allosteric antagonism. It is therefore possible that allergen-induced eosinophilic infiltration of the airways leads to M₂-receptor dysfunction, thereby eliminating the negative feedback control on acetylcholine release. This may potentiate vagally mediated bronchoconstriction, as indicated by the enhanced BHR for histamine in the present study.

Thirdly, increased mucus secretion and oedema formation, resulting in a reduced airway calibre, may amplify airway narrowing due to smooth muscle contraction. However, in the present study this is unlikely because airway function (P_{pi}) assessed between the early and late asthmatic response, as well as after the late response, had returned to baseline value.

In conclusion, we have shown that in this new guinea-pig model of asthma, the allergen-induced increase in bronchial hyperreactivity to histamine was significantly higher than to methacholine. Ipratropium bromide inhalation in a concentration (0.1 mM), which had no significant effect on histamine-induced bronchoconstriction in control animals, significantly

reduced histamine-induced bronchoconstriction at 6 h after allergen provocation, indicating that the cholinergic reflex bronchoconstriction which contributes to histamine-induced bronchoconstriction is increased after allergen provocation. This allergen-induced exaggerated cholinergic contribution to histamine-induced bronchoconstriction may at least partly explain the markedly enhanced bronchial reactivity to histamine after allergen exposure.

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References

- ALANKO, K. & POPPIUS, H. (1974). Anticholinergic blocking of prostaglandin-induced bronchoconstriction. *Br. Med. J.*, **1**, 294.
- ANDERSSON, P. (1980). Antigen-induced bronchial anaphylaxis in actively sensitized guinea pigs. *Allergy*, **35**, 65–71.
- BRAUDE, S., ROYSTON, D., COE, C. & BARNES, P.J. (1984). Histamine increased lung permeability by an H_2 -receptor antagonism. *Lancet*, **ii**, 372–374.
- CHUNG, K.F. (1986). Role of inflammation in the hyperreactivity of the airways in asthma. *Thorax*, **41**, 657–662.
- FRIGAS, E. & GLEICH, G.J. (1986). The eosinophil and the pathophysiology of asthma. *J. Allergy Clin. Immunol.*, **77**, 527–537.
- FRYER, A.D. & JACOBY, D.B. (1992). Function of pulmonary M_2 muscarinic receptors in antigen-challenged guinea pigs is restored by heparin and poly-L-glutamate. *J. Clin. Invest.*, **90**, 2292–2298.
- FRYER, A.D. & WILLS-KARP, M. (1991). Dysfunction of M_2 -muscarinic receptors in pulmonary parasympathetic nerves after antigen challenge. *J. Appl. Physiol.*, **71**, 2255–2261.
- HOLTZMAN, M.J., SELLER, J.R., DIMEO, M., NADEL, J.A. & BOUSHEY, H.A. (1980). Effect of ganglionic blockade on bronchial reactivity in atopic subjects. *Am. Rev. Respir. Dis.*, **122**, 17–25.
- JACOBY, D.B., GLEICH, G.J. & FRYER, A.D. (1993). Human eosinophil major basic protein is an endogenous allosteric antagonist at the inhibitor muscarinic M_2 receptor. *J. Clin. Invest.*, **91**, 1314–1318.
- MINETTE, P.A.H., LAMMERS, J.-W.J., DIXON, C.M.S., MCCUSKER, M.T. & BARNES, P.J. (1989). A muscarinic agonist inhibits reflex bronchoconstriction in normal but not in asthmatic subjects. *J. Appl. Physiol.*, **67**, 2461–2465.
- SANTING, R.E., MEURS, H., VAN DER MARK, TH.W., REMIE, R., OOSTEROM, W.C., BROUWER, F. & ZAAGSMA, J. (1992). A novel method to assess airway function parameters in chronically instrumented, unrestrained guinea pigs. *Pulm. Pharmacol.*, **5**, 265–272.
- SANTING, R.E., OLYMULDER, C.G., ZAAGSMA, J. & MEURS, H. (1994). Relationships between allergen-induced early and late phase airway obstructions, bronchial hyperreactivity and inflammation in conscious, unrestrained guinea pigs. *J. Allergy Clin. Immunol.*, **93**, 1021–1030.
- SIMONSSON, B.G., JACOBS, F.M. & NADEL, J.A. (1967). Role of the autonomic nervous system and the cough reflex in the increased responsiveness of airways in patients with obstructive airway disease. *J. Clin. Invest.*, **46**, 1812–1818.
- TEN BERGE, R.E.J., ROFFEL, A.F., VAN DER ZANDE, J., SANTING, R.E. & ZAAGSMA, J. (1992). Dysfunction of prejunctional M_2 -muscarinic receptors in tracheal preparations of IgE-sensitized guinea pigs after the early allergic reaction. *Am. Rev. Respir. Dis.*, **145**, A437.
- VAN AMSTERDAM, R.G.M., SANTING, R.E., DE BOER, J., BROUWER, F. & ZAAGSMA, J. (1991). IgG- and IgE-mediated histamine release from superfused guinea-pig airway tissues. *J. Pharm. Pharmacol.*, **10**, 694–698.
- VIDRUK, E.H., HAHN, H.L., NADEL, J.A. & SAMPSON, S.R. (1977). Mechanisms by which histamine stimulates rapidly adapting receptors in dog lungs. *J. Appl. Physiol.*, **43**, 397–402.
- WARDLAW, A.J., DUNETTE, S., GLEICH, G.J., COLLINS, J.V. & KAY, A.B. (1988). Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma: relationship to bronchial hyperreactivity. *Am. Rev. Respir. Dis.*, **137**, 62–69.

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