Effect of allergen challenge on airway responsiveness to histamine and sodium metabisulphite in mild asthma

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Abstract

Background – Airway responsiveness to histamine and methacholine, direct smooth muscle spasmogens, is increased following inhalation of allergen. Although the aetiology of this phenomenon is unclear, increased cellular or neural activity may be involved since allergen also induces increases in airway responsiveness to the mast cell stimulus adenosine 5'-monophosphate (AMP) and the neural stimulus bradykinin.

Methods – To explore this further, the airway responsiveness to sodium metabisulphite (MBS), an indirect neural stimulus with similar characteristics to bradykinin, was compared in 18 mild steroid-naïve asthmatic subjects with the airway responsiveness to histamine before and after allergen challenge with extracts of house dust mite, grass pollen, or cat. All subjects inhaled doubling increments of histamine and MBS until the concentration provoking a 20% fall in forced expiratory volume in one second (PC₂₀) was reached before and three hours after allergen challenge. Twelve of the subjects had additional challenges at 24 hours after the allergen.

Results – Following allergen challenge all subjects showed an early response and 14 also had a late asthmatic response. For histamine there was a significant increase in airway responsiveness at both three and 24 hours compared with values before the allergen (0.89 (0.25) and 1.53 (0.52) doubling dose changes, respectively). In contrast, airway responsiveness to MBS was unaltered by allergen challenge (0.29 (0.27) and –0.33 (0.28) doubling dose changes compared with pre-allergen values at three and 24 hours, respectively).

Conclusion – These data suggest that activation of airway sensory nerves is unlikely to contribute to the increase in airway responsiveness following inhalation of allergen. The previously observed allergen induced increase in airway responsiveness to bradykinin and AMP may involve non-neural pathways.

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bronchial hyperresponsiveness correlates with disease severity and the need for treatment.² Exposure to allergen provokes increased airway responsiveness to several bronchoconstrictor stimuli.^{3,4} Airway responsiveness to histamine or methacholine, both of which act directly on airway smooth muscle, is increased following allergen challenge and may persist for more than 72 hours.^{5,6} Changes in airway responsiveness to indirect acting bronchoconstrictor stimuli are less predictable. Airway responsiveness to bradykinin, a neural stimulus, is increased and sustained following allergen challenge,⁷⁻⁹ whereas bronchial reactivity to adenosine 5'-monophosphate (AMP), a mast cell stimulus, is increased at three but not at 24 hours after allergen challenge.⁶

The pathogenesis of increased airway responsiveness is unclear, in particular the relationship between airway responsiveness and airway inflammation induced by allergen exposure.¹⁰ The development of the late asthmatic response (LAR) following allergen challenge is dependent upon a complex series of changes in humoral and cellular immune mechanisms resulting in mucosal damage and oedema with infiltration of eosinophils and lymphocytes.^{3,4} These cells release mediators of inflammation such as cytokines and leukotrienes which not only perpetuate the inflammatory process by recruiting further inflammatory cells but also have direct effects on resident airway cells, mast cells, smooth muscle, and airway nerves that may underlie the changes in airway responsiveness. Thus, allergen induced airway inflammation may result in increased airway responsiveness to both direct and indirect stimuli.

Indirect bronchoconstriction following bradykinin and AMP appears to be specific for asthma and may be a more relevant measurement of airway responsiveness in asthmatic patients than non-specific challenge with histamine or methacholine.¹¹ The greater increases in airway responsiveness to bradykinin than methacholine following allergen challenge suggest that upregulation of neural reflexes may occur as a consequence of allergen induced inflammation.⁸ To examine this further we have compared the effect of allergen challenge on airway responsiveness to another indirect spasmogen, metabisulphite (MBS), with airway responsiveness to histamine, a direct spasmogen. MBS provokes cough and bronchoconstriction, suggesting an effect via airway sensory nerves, and exhibits similar characteristics to bradykinin.¹² This study was de-

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Increased airway responsiveness is a characteristic feature of asthma.¹ The degree of

Table 1 Patient details

	Patient no.	Age (years)	FEV ₁ (% predicted)	Allergen	Max % change FEV ₁ (EAR)	Max % change FEV ₁ (LAR)
Phase 1 (all LAR)	1	23	94	Cat	19	—
	2	29	90	House dust mite	43	—
	3	32	97	House dust mite	36	—
	4	28	102	Cat	29	—
	5	23	94	House dust mite	33	—
	6	30	105	Grass pollen	41	—
	Mean				33.5 (3.6)	—
Phase 2 (LAR)	7	30	101	Grass pollen	62	30
	8	29	103	Grass pollen	49	31
	9	24	93	House dust mite	17	19
	10	29	114	Grass pollen	29	17
	11	29	91	Cat	30	19
	12	24	103	Grass pollen	28	37
	13	19	87	House dust mite	24	29
	14	21	91	Cat	22	20
	Mean		97.5 (2.0)		32.6 (5.4)	25.3 (2.6)
Phase 2 (no LAR)	15	25	100	House dust mite	24	13
	16	24	99	House dust mite	36	6
	17	25	89	House dust mite	38	7
	18	32	94	Cat	31	10
	Mean		95.5 (2.5)		32.3 (3.1)	9.0 (1.6)

FEV₁ = forced expiratory volume in one second; EAR = early asthmatic response; LAR = late asthmatic response.

signed to explore the involvement of airway neural mechanisms following allergen challenge using MBS as a measure of airway responsiveness.

Methods

SUBJECTS

Eighteen non-smoking subjects (12 men) with mild asthma (as defined by documented peak flow variability and episodic wheeze relieved by β_2 agonists) gave written informed consent to participate in the study which was approved by the Royal Brompton and National Heart Hospitals ethics committee. All subjects were admitted to the Clinical Studies Unit of the Royal Brompton Hospital. All were atopic as defined by skin prick testing to common aero-allergens (*Dermatophagoides pteronyssinus*, mixed grass pollen, cat hair). None had suffered an exacerbation of wheeze nor a respiratory infection within the previous six weeks. Each subject had clinical features of asthma which were controlled with β_2 adrenoceptor agonists alone, and had a baseline forced expiratory volume in one second (FEV₁) in excess of 80% of their predicted value (individual characteristics summarised in table 1). Inhaled sympathomimetics and caffeinated beverages were withheld for at least eight hours before each study.

STUDY DESIGN

The study was conducted in two phases: phase 1 involved a crossover study measuring airway responsiveness to histamine and MBS before and after both allergen and methacholine control challenges and phase 2 involved measurements of airway responsiveness to histamine and MBS before and after allergen challenge alone (fig 1).

Phase 1

Six subjects attended for two study days, each of 11 hours maximum duration and separated by a period of 21 days. On each occasion they were challenged with either allergen or methacholine (which served as a physiological control). Measurements of airway responsiveness to MBS and histamine were made before and after these challenges. The time points for the measurements of airway responsiveness were at two and one hours before and three and four hours after the allergen/control challenge. The order of the study days (that is, allergen or methacholine) was randomised for all subjects. The sequence of MBS and histamine inhalation was the same for each subject on both study days. Each individual was therefore randomised to one of two groups determining the order of the stimuli. Three patients received histamine challenges before MBS – that is, two hours before and three hours after the challenge – and the remaining three patients received MBS challenge first at each time point. Both before and after allergen challenge histamine and MBS challenges were only performed if the FEV₁ had recovered to within 10% of the baseline value.

Control and allergen challenges were administered in a single blind manner. Histamine and MBS challenges were performed by an independent operator (LJC) who remained blinded to allergen or control challenges.

On both study days measurements of FEV₁ were recorded for a maximum of eight hours after allergen/control challenges. Before leaving the unit all subjects received nebulised budesonide 2 mg and, if required, nebulised salbutamol 2.5 mg.

Phase 1

Phase 2

Figure 1 Study protocol; AR = airway responsiveness.

Phase 2

During phase 2 a further 12 subjects attended for one study day and were admitted overnight to the unit for at least 28 hours. Measurements of airway responsiveness to MBS and histamine were made at two and one hours before and at three and four hours after allergen challenge in a similar manner to phase 1 and, additionally, at 24 and 25 hours after allergen challenge. As in phase 1, each individual was randomly allocated to one of two groups defining the sequence in which histamine and MBS challenges were done. Allergen challenges were administered by the attending physician in an open manner who also administered the pre-allergen histamine and MBS challenges. The challenges at three and four hours and 24 and 25 hours after allergen were performed by an independent observer (LJC) who remained blinded to the pre-allergen PC₂₀ data. As in phase 1, at all time points histamine and MBS challenges were only performed if the FEV₁ had recovered to within 10% of the baseline value.

Measurements of FEV₁ were made half hourly for 4–10 hours and hourly thereafter prior to undergoing further airway responsiveness measurements at 24 and 25 hours after allergen challenge. After the final challenge, before leaving the unit, all subjects received nebulised budesonide 2 mg and nebulised salbutamol 2.5 mg.

MATERIALS

On each study day fresh solutions of methacholine, histamine, MBS, (Sigma Chemicals, Poole, UK) and allergen (Aquagen SQ, ALK, Horsholm, Denmark) were made in 0.9% saline. The concentration ranges for methacholine and histamine were 0.03–32 mg/ml and for MBS 0.063–160 mg/ml. Freeze dried allergen extracts were used to make dilutions from a stock solution of 100 000 IU/ml to give final concentrations of 200, 1000, 2500, 5000, 12 500, 25 000, and 50 000 IU/ml. The allergen used for each subject was predicted by the response to skin prick testing.

MEASUREMENT OF PULMONARY FUNCTION AND PC₂₀

Pulmonary function was measured with a dry wedge spirometer (Vitalograph, Buckingham, UK). A standard challenge protocol was used for both MBS and histamine provocation tests. On arrival in the laboratory each subject rested for 15 minutes before three measurements of FEV₁ were taken at one minute intervals, the best of which was taken as the baseline. Subjects then inhaled five breaths of saline control by inspiring slowly from functional residual capacity (FRC) to total lung capacity (TLC) followed by breath holding for five seconds. FEV₁ was measured two minutes after inhalation of the saline. Unless a fall in FEV₁ of >10% was observed after saline, subjects inhaled five breaths of serially doubling increments of either histamine or MBS at three minute intervals until a 20% fall in FEV₁ was recorded from

the post-saline value. A log dose response curve was constructed for each agonist and the provocative concentration causing a 20% fall in FEV₁ (log₁₀PC₂₀) was calculated by linear interpolation.

ALLERGEN CHALLENGE

Allergen challenge was commenced one hour after the second pre-allergen PC₂₀ bronchial challenge and was administered as five nebulisations from a dosimeter (MB3) similar to the other airway challenges. The initial dose for the allergen inhalation test was 200 IU/ml and FEV₁ was measured five and 10 minutes after administration of the allergen. Serially increasing doses of allergen were inhaled and the cumulative dosage resulting in a fall of at least 15% in FEV₁ after saline diluent within 10 minutes was recorded and constituted an adequate challenge and the early asthmatic response (EAR). The FEV₁ was recorded every 15 minutes for the first hour and half hourly thereafter. The LAR was defined as a fall in FEV₁ of 15% from the post-saline FEV₁ between five and eight hours on at least two occasions (phase 1) and between five and 10 hours on at least three occasions (phase 2).

CONTROL CHALLENGE (PHASE 1 ONLY)

In a similar manner to the allergen challenge, measurements of FEV₁ were recorded five and 10 minutes after inhalation of serial increments of methacholine. The challenge was terminated when a fall in FEV₁ of ≥25% from the post-saline value was observed. To maintain subject blindness, methacholine was administered in fourfold increments to provide challenge conditions similar to the allergen day in terms of both the number of doses delivered and the degree of bronchoconstriction experienced.

ANALYSIS OF DATA

All airway data are expressed as means (SE) except the PC₂₀ data which were expressed as geometric means. The extent of the LAR was assessed as the maximal fall in FEV₁ from baseline and expressed as percentage change. All PC₂₀ values were log transformed for analysis.

The effect of allergen on airway responsiveness to both histamine and MBS was expressed in terms of doubling dose changes and calculated using the formula:

$$[\log_{10}PC_{20}(\text{pre-allergen}) - \log_{10}PC_{20}(\text{post-allergen})]/\log_{10}2$$

where the post-allergen PC₂₀ was either three or 24 hours after challenge.

All data were analysed using the Student's *t* test for matched pairs, a *p* value of ≤0.05 being considered significant.

Table 2 Individual airway responsiveness to bronchial challenges at three and 24 hours after allergen challenge

Patient no.	log PC ₂₀ histamine			log PC ₂₀ metabisulphite			Maximum % fall FEV ₁ (LAR)
	Pre	3 hours post	24 hours post	Pre	3 hours post	24 hours post	
1	0.73	0.44	—	1.22	1.15	—	—
2	−0.72	−1.18	—	0.53	0.52	—	—
3	0.57	0.43	—	0.82	1.06	—	—
4	−0.02	−0.21	—	1.18	1.10	—	—
5	0.10	0.15	—	0.81	0.67	—	—
6	0.18	0.15	—	0.75	0.81	—	—
7	0.43	−0.01	−0.79	0.73	0.95	0.96	30
8	−0.19	−0.42	−0.73	0.76	0.78	0.79	31
9	−0.01	−0.27	−0.51	1.20	0.33	0.91	19
10	0.88	0.78	0.62	0.89	1.12	1.25	17
11	−0.20	−0.26	−0.30	0.77	0.96	1.09	19
12	−1.04	—	−1.12	0.58	—	0.50	37
13	−1.17	−1.52	—	0.1	—	—	29
14	0.71	−0.45	−0.56	0.50	0.37	0.81	20
15	—	—	—	0.84	0.59	—	13
16	−0.01	−0.03	−0.19	0.71	0.85	0.96	7
17	0.32	−0.24	−0.42	1.05	0.27	0.67	10
18	0.28	0.28	0.55	0.90	0.75	1.14	6
Mean (SE)	0.05 (0.14)	−0.15 (0.15)	−0.34 (0.18)	0.80 (0.06)	0.77 (0.07)	0.91 (0.07)	
Geometric mean (mg/ml)	1.11	0.70	0.45	6.25	5.84	8.09	

Results

RESPONSE TO ALLERGEN CHALLENGE/BASELINE LUNG FUNCTION

The mean percentage predicted FEV₁ for all subjects was 97 (1.6)%. There was no difference in baseline lung function on the study days nor was there any significant difference in baseline lung function between those showing either single or dual response to allergen (LAR, mean FEV₁ 97.5 (2.0)% predicted; non-LAR mean FEV₁ 95.5 (2.5)% predicted; table 1).

In both phases all 18 subjects tolerated the acute allergen response well and demonstrated an EAR (mean maximum fall in FEV₁ of 32.8 (3.9)%). The mean maximum percentage fall in FEV₁ for EAR was similar for single and dual responders (table 1).

In phase 1 all six subjects experienced equivalent acute bronchoconstriction following allergen and control challenge; after allergen the mean maximum fall in FEV₁ was 33.5 (3.6)%; after methacholine the mean maximum fall in FEV₁ was 36.1 (4.5)%. All subjects showed an LAR after allergen challenge. In phase 1 the maximum fall in the FEV₁ during the LAR is not defined as each subject received a bronchodilator once they fulfilled the criteria for the late response. On the control day no subject experienced bronchoconstriction between five and eight hours.

In phase 2 eight of the 12 subjects experienced an LAR with a mean maximum fall in FEV₁ of 25.3 (2.6)%. The four subjects who did not show an LAR had a mean maximum fall in FEV₁ of 9.0 (1.6)% 4–10 hours after allergen challenge (table 1).

AIRWAY RESPONSIVENESS AFTER ALLERGEN

In phase 1 all six subjects completed the protocol. In phase 2 nine of the 12 subjects completed the protocol in full.

From phase 2 incomplete data are presented on two subjects showing LAR. Subject 12 had a prolonged EAR and was not within 10% of baseline FEV₁ at 3–4 hours. Subject 13 had not recovered to within 10% of baseline FEV₁ for a three hour post-allergen MBS challenge and had a profound LAR precluding any chal-

lenge at 24 and 25 hours. One of the four subjects without an LAR (no. 15) failed to recover to within 10% of baseline FEV₁ following MBS challenge and therefore did not undergo histamine challenges at any time point. In addition, he developed a profound LAR and was not challenged at 24–25 hours.

In the two phases combined evaluable data were available on 16 subjects (nos 1–11, 13, 14, and 16–18) for a comparison of airway responsiveness to histamine before and three hours after allergen, and also on 16 subjects (nos 1–11 and 14–18) to compare airway responsiveness to MBS. In phase 2 data were available on 10 subjects to compare airway responsiveness to histamine (nos 7–12, 14, 16–18) and MBS (nos 7–12, 14, 16–18) before and 24 hours after allergen (table 2).

For the group as a whole there was a significant increase in airway responsiveness to histamine challenge at both three and 24 hours following allergen challenge compared with the pre-allergen value (change in mean doubling dose 0.89 (0.25) at three hours (n=16) and 1.53 (0.52) at 24 hours (n=10); p<0.05 at both time points; table 2, fig 2). For subjects with an LAR the change in mean doubling dose was 0.95 (0.28) at three hours (n=13) and 1.88 (0.62) at 24 hours (n=7); p<0.05 at both time points (table 2). The absence of an LAR did not significantly affect the result as

Figure 2 Doubling dose shift from baseline in airway responsiveness to histamine and sodium metabisulphite at three and 24 hours after allergen challenge.

Figure 3 Individual data showing log PC₂₀ for six subjects undergoing histamine and sodium metabisulphite challenges before and three hours after allergen challenge and control challenge (methacholine).

two of the three subjects without an LAR who completed the protocol showed an increase in airway responsiveness to histamine at both three and 24 hours (table 2).

For MBS no significant changes in airway responsiveness were found compared with pre-allergen values at either three or 24 hours for the group as a whole (change in mean doubling dose 0.29 (0.27) at three hours ($n=16$) and -0.33 (0.28) at 24 hours ($n=10$); table 2, fig 2). Similarly, for subjects with an LAR the change in mean doubling dose of 0.09 (0.29) at three hours ($n=12$) and -0.40 (0.31) at 24 hours ($n=7$) was not significant (table 2).

For the six subjects in phase 1 airway responsiveness on the control day was unchanged to either histamine or MBS following bronchial challenge with methacholine. The individual data for these subjects for airway responsiveness to both control and allergen are shown in fig 3.

In both phases the sequence of histamine and MBS challenges did not affect the results – that is, there were no differences in the results between the group receiving histamine first at each time point compared with those receiving MBS first.

Discussion

In this study we have shown that, in subjects with mild asthma, allergen challenge provoked an increase in airway responsiveness to the direct smooth muscle stimulus histamine but did not alter airway responsiveness to the indirect neural stimulus MBS. An effect on MBS was not seen at three hours despite the presence of an LAR in 12 subjects, nor at 24 hours in seven subjects. The absence of a change in airway responsiveness to either stimulus following methacholine induced bronchoconstriction (equivalent to that induced by allergen) confirms that this is an allergen related

effect rather than simply a consequence of airway calibre alone.

Our observed increase in airway responsiveness to histamine is compatible with other data on direct bronchoconstrictor challenges after allergen.^{3–7} The mechanisms underlying this are unclear but may be due to a number of inflammatory changes in the airway.^{3,4} The change in airway responsiveness may reflect increased activity or sensitivity of cells, nerves, or smooth muscle as a consequence of this inflammation.¹³ Airway smooth muscle is under the influence of inflammatory mediators released during the LAR which may be responsible for increased smooth muscle responsiveness.^{3,13} Only a part of the observed reduction in PC₂₀ can be attributed to changes in airway calibre alone.¹⁴ Also, it is possible that damage to the airway epithelium during the inflammatory response to allergen will facilitate passage of the bronchoconstricting stimulus to its target site.¹⁵ All of these factors would predict an increase in airway responsiveness irrespective of the underlying stimulus. Thus, our failure to demonstrate an effect of allergen on MBS challenge is surprising. We expected that allergen would provoke an increase in airway responsiveness to MBS at least comparable to that of histamine.

The aim of our study was to assess the contribution of neural pathways to increased airway responsiveness following allergen using MBS as an indirect neural stimulus. Previous studies have shown increased airway responsiveness to indirect challenge with AMP similar to that of methacholine three hours after allergen,⁶ but a greater and more prolonged increase to bradykinin than to methacholine.^{7–9} This effect on airway responsiveness to bradykinin persists for up to three weeks and has been attributed to upregulation of neural reflexes.^{7,8} Thus, the results of our study are even harder to explain as the constrictor effects of MBS and bradykinin have a similar pharmacological profile. Airway responsiveness to both bradykinin and MBS is attenuated by anticholinergic agents,^{16,17} cromoglycate,^{18,19} and by bradykinin β_2 receptor antagonists.²⁰ This evidence suggests that similar neural mechanisms underlie the constrictor action of these two agents. If upregulation of neural reflexes was implicated in the increase in airway responsiveness to bradykinin after allergen, it might be expected that airway responsiveness to MBS would also increase after allergen. Our results suggest that the shared mechanisms of bronchoconstriction of these agents do not contribute to the pathophysiology of increased airway responsiveness following allergen challenge.

It is possible that differences in the bronchoconstrictor action of MBS and bradykinin may explain our results and also account for some of the processes involved in changes in airway responsiveness after allergen challenge. Inhibition of the cyclo-oxygenase pathway with flurbiprofen significantly attenuated airway responsiveness to MBS.²¹ By contrast, airway responsiveness to bradykinin was unaffected by either flurbiprofen²² or aspirin.¹⁶ Cyclo-oxygenase metabolites may therefore be involved

in airway responsiveness to MBS but not to bradykinin. This distinction could be important if prostanoids modulate airway responses to allergen. Previous data are conflicting. A single dose of indomethacin attenuated the LAR but not the EAR to allergen,²³ whereas twice daily dosing for two days reduced airway responsiveness to histamine at 24 hours without an effect on either the EAR or LAR.²⁴ It is tempting to speculate that bronchoconstrictor prostanoids are released during the LAR resulting in a depletion of stores at 24 hours leading to partial refractoriness to MBS. This hypothesis can only be sustained if airway responsiveness to MBS is mediated at least in part by bronchoconstrictor prostanoids.²¹ As the EAR is unaffected and may even be increased by indomethacin,²⁵ this does not explain our results on airway responsiveness to MBS at three hours. Indeed, the role of prostaglandins in MBS induced bronchoconstriction is far from clear as inhaled PGE₂ protects against MBS challenge²⁶ and indomethacin failed to inhibit the response to initial challenge but reduced refractoriness to subsequent challenge with MBS.²⁷ Thus, although cyclo-oxygenase products may contribute to allergen induced late responses, they are a complex family of inflammatory mediators with both bronchoconstrictor and bronchoprotective airway effects. We cannot therefore propose prostanoid involvement as an explanation for the lack of an effect on airway responsiveness to MBS following allergen challenge.

Indirect challenge with MBS and AMP may share similar neural pathways, at least in part, as airway responsiveness to both spasmogens is inhibited to the same degree by frusemide²⁸ and nedocromil sodium or cromoglycate.^{19,29} As with bradykinin, this putative shared mechanism cannot explain our results at three hours after allergen challenge. Allergen provoked an increase in airway responsiveness to AMP which is predominantly a mast cell stimulus at three but not at 24 hours.⁶ The absence of an effect on both challenges at 24 hours is either further indirect evidence that neural pathways are not upregulated by allergen or that the mechanism of MBS is in question.

Repeated MBS challenge at hourly intervals results in refractoriness and may explain our findings.²⁷ In the present study MBS challenges were separated by five and 21 hours, respectively. Our previous observation that three serial MBS challenges at three hourly intervals did not exhibit refractoriness makes this unlikely.²¹ Cross refractoriness may exist between allergen and MBS. It is possible that allergen challenge may cause depletion of preformed neurotransmitters that participate in the action of MBS. The timing of challenges renders this unlikely. Furthermore, cross refractoriness may occur between histamine and MBS as each challenge was separated by an interval of one hour. Our study design allowed for this by balancing the sequence of challenges between subjects. Moreover, we established that cross refractoriness does not occur between histamine and MBS in a previous pilot study in our unit. Finally, we cannot account for the

contrast in effect on airway responsiveness to histamine and MBS. We might reasonably have expected enhanced smooth muscle responsiveness irrespective of the stimulus as a consequence of allergic inflammation.^{3,13,14} Indeed, anti-inflammatory treatment with 14 days treatment of budesonide reduced airway responsiveness to methacholine and MBS by an equivalent one doubling dose, implying an inflammatory influence on smooth muscle responsiveness independent of an indirect airway action of MBS.³⁰

The results of our study may raise questions about the relative importance of neural factors in the aetiology of increased airway responsiveness following allergen challenge. Furthermore, we have demonstrated a clear distinction in airway responsiveness to two spasmogens with different airway actions, supporting the view that several mechanisms are involved in the pathogenesis of an allergen induced increase in airway responsiveness. Further studies comparing challenges with separate bronchoconstrictor actions may help to unravel these complex mechanisms.

- 1 Barnes PJ. New concepts in the pathogenesis of bronchial hyperresponsiveness and asthma. *J Allergy Clin Immunol* 1989;83:1013-26.
- 2 Hargreave FE, O'Byrne PM, Ramsdale EH. Mediators, airway responsiveness and asthma. *J Allergy Clin Immunol* 1985;65:76:272-6.
- 3 Durham SR. Late asthmatic responses. *Respir Med* 1990;84:263-8.
- 4 Cockcroft DE, Ruffin RE, Dolovich S, Hargreave FE. Allergen-induced increase in non-allergic bronchial reactivity. *Clin Allergy* 1977;7:503-13.
- 5 Cockcroft DW, Murdock KY. Changes in bronchial responsiveness to histamine at intervals after allergen challenge. *Thorax* 1987;42:302-8.
- 6 Aalbers R, Kauffman HK, Koeter GH, Postma DS, De Vries K, De Monchy JGR. Dissimilarity in methacholine and adenosine 5'-monophosphate 3 and 24 hours after allergen challenge. *Am Rev Respir Dis* 1991;144:352-7.
- 7 Djukanovic R, Polosa R, Holgate ST. The effect of bronchial allergen challenge on methacholine and bradykinin airway responsiveness. *Eur Respir J* 1991;340s:584A.
- 8 Djukanovic R. Is airways hyperreactivity selective or non-selective? *Agents Actions* 1993;43:231-9.
- 9 Berman AR, Togias AG, Skloot G, Proud D. Allergen challenge of atopic asthmatics enhances bronchial reactivity to bradykinin more than that to methacholine. *Am J Respir Crit Care Med* 1994;149:A1042.
- 10 Chung KF. Role played by inflammation in the hyper-reactivity of the airways in asthma. *Thorax* 1986;42:657-62.
- 11 Rogers DF, O'Connor BJ. Airway hyperresponsiveness: relation to asthma and inflammation. *Thorax* 1993;48:1095-6.
- 12 Barnes PJ. Effect of nedocromil sodium on airway sensory nerves. *J Allergy Clin Immunol* 1993;92:182-6.
- 13 Cockcroft DW. Bronchial hyperresponsiveness. In: Mygind N, Pipkorn U, Dahl R, eds. *Rhinitis and asthma*. Copenhagen: Munksgaard, 1990:172-87.
- 14 Cartier A, Thompson NC, Frith PA, Roberts R, Hargreave FE. Allergen-induced increase in bronchial responsiveness to histamine; relationship to the late asthmatic response and change in airway calibre. *J Allergy Clin Immunol* 1982;70:170-7.
- 15 Boucher RC, Parte PD, Hogg JC. Relationship between airway hyperreactivity and hyperpermeability in Ascaris-sensitive monkeys. *J Allergy Clin Immunol* 1979;64:197-9.
- 16 Fuller RW, Dixon CM, Cuss FM, Barnes PJ. Bradykinin-induced bronchoconstriction in humans. Mode of action. *Am Rev Respir Dis* 1987;135:176-80.
- 17 Bellingham GJ, Dixon CM, Ind PW. Inhibition of inhaled metabisulphite-induced bronchoconstriction by inhaled frusemide and ipratropium bromide. *Br J Clin Pharmacol* 1992;34:71-4.
- 18 Dixon CM, Barnes PJ. Bradykinin-induced bronchoconstriction: inhibition by nedocromil sodium and sodium cromoglycate. *Br J Clin Pharmacol* 1989;27:831-6.
- 19 Dixon CM, Ind PW. Inhaled sodium metabisulphite induced bronchoconstriction: inhibition by nedocromil sodium and sodium cromoglycate. *Br J Clin Pharmacol* 1990;30:371-6.
- 20 Mansour E, Ahmed A, Cortes A, Caplan J, Burch RM, Abraham WM. Mechanisms of metabisulphite-induced bronchoconstriction: evidence for bradykinin β_2 -receptor stimulation. *J Appl Physiol* 1992;72:1831-7.

- 21 O'Connor BJ, Barnes PJ, Chung KF. Inhibition of sodium metabisulphite induced bronchoconstriction by frusemide in asthma; role of cyclooxygenase products. *Thorax* 1994; **49**:307–11.
- 22 Polosa R, Phillips GD, Lai CKW, Holgate ST. Contribution of histamine and prostanooids to bronchoconstriction provoked by inhaled bradykinin in atopic asthma. *Allergy* 1990; **45**:174–82.
- 23 Fairfax AJ, Hanson JM, Morley J. The late reaction following bronchial provocation with house dust mite allergen. Dependence on arachidonic acid metabolism. *Clin Exp Allergy* 1983; **52**:393–8.
- 24 Kirby JG, Hargreave FE, Cockcroft DW, O'Byrne PM. Effect of indomethacin on allergen-induced asthmatic responses. *J Appl Physiol* 1989; **66**:578–83.
- 25 Fish JE, Ankin MG, Adkinson NF, Peterman VI. Indomethacin modification of immediate-type immunologic airway responses in allergic asthmatic and non-asthmatic subjects: evidence for altered arachidonic acid metabolism in asthma. *Am Rev Respir Dis* 1981; **123**:609–14.
- 26 Pavord ID, Wisniewski A, Mathur R, Wahedna I, Knox AJ, Tattersfield AE. Effect of prostaglandin E2 on bronchial reactivity to sodium metabisulphite and methacholine in patients with asthma. *Thorax* 1991; **46**:633–7.
- 27 Pavord ID, Wisniewski A, Tattersfield AE. Refractoriness to inhaled sodium metabisulphite in subjects with mild asthma. *Eur Respir J* 1994; **7**:50–4.
- 28 O'Connor BJ, Chung KF, Chen-Worsdell YM, Fuller RW, Barnes PJ. Effect of inhaled furosemide and bumetanide on adenosine 5'-monophosphate- and sodium metabisulphite-induced bronchoconstriction in asthmatic subjects. *Am Rev Respir Dis* 1991; **143**:1329–33.
- 29 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.
- 30 O'Connor BJ, Ridge SM, Barnes PJ, Fuller RW. Greater effect of inhaled budesonide on adenosine 5'-monophosphate-induced bronchoconstriction in asthma. *Am Rev Respir Dis* 1992; **146**:560–4.