The influence of ipratropium bromide and sodium cromoglycate on benzalkonium chloride-induced bronchoconstriction in asthma

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1 Benzalkonium chloride, an antibacterial preservative that is added to nebuliser solutions, has been shown to cause bronchoconstriction when inhaled by asthmatic subjects.

2 To investigate the potential role of reflex and mast cell-dependent mechanisms in the pathogenesis of bronchoconstriction produced by benzalkonium chloride we examined the effects of ipratropium bromide and sodium cromoglycate on this response in both concentration-response and time-course studies in nine asthmatic subjects.

3 Pretreatment with inhaled ipratropium bromide (1 mg) and sodium cromoglycate (40 mg) displaced the benzalkonium chloride concentration-response curves to the right by a mean 2.2 fold and 3.1 fold respectively.

4 Ipratropium bromide and sodium cromoglycate markedly attenuated the airway response to benzalkonium chloride throughout the 45 min time course period, inhibiting the overall response by 56% and 78% respectively.

5 We conclude that benzalkonium chloride provokes bronchoconstriction in asthmatic subjects through a combination of mast cell activation and stimulation of peripheral and central neural pathways.

Keywords ipratropium bromide sodium cromoglycate benzalkonium chloride bronchoconstriction asthma

Introduction

Benzalkonium chloride (BC), a bacteriocidal preservative present in a number of nebuliser solutions, is a bronchoconstrictor agonist when inhaled by asthmatic subjects (Beasley *et al.*, 1987a). The observations that BC causes the release of preformed mediators such as histamine (Read & Kiefer, 1979; Read *et al.*, 1982) and 5-hydroxytryptamine (Coleman *et al.*, 1981) from rodent mast cells *in vitro*, suggested that this airways response may occur following the activa-

tion of mast cells within the bronchial mucosa. Because histamine is the only mediator with bronchoconstrictor properties that has been identified within the granules of human pulmonary mast cells, we proceeded to investigate the effect of the terfenadine and astemizole, two potent and specific H₁-histamine receptor antagonists on bronchoconstriction provoked by BC. Although H₁-receptor blockade significantly attenuated bronchoconstriction during the first 5

min of the challenge, it only resulted in inhibition of 13% of the overall response suggesting that histamine release contributes only a minor component to the airways response (Miszkiet *et al.*, 1987).

In addition to the release of granule-derived mediators, both immunological and non-immunological stimulation of human lung mast cells (Agius *et al.*, 1985; Holgate *et al.*, 1984) results in the release of newly generated oxidative products of arachidonic acid. In particular, mast cells generate the potent bronchoconstrictor mediators prostaglandin (PG) D₂ (Holgate *et al.*, 1984) and leukotriene (LT) C₄ (Bisgaard *et al.*, 1987b). Since these lipid mediators are considered to contribute to airflow limitation in asthma it is also possible that with mast cell activation they also contribute to the adverse effect of inhaled BC.

When inhaled by asthmatic subjects, BC is irritant. Thus an alternative or additional possibility is that this substance stimulates reflex narrowing of the airways through a cholinergic-mediated mechanism. Activation of both the rapidly adapting irritant receptors and nerve endings of non-myelinated C-fibres present in the airway epithelium could result in bronchoconstriction through stimulation of peripheral and central reflex pathways (Widdicombe, 1986).

produced by BC by observing the effects of ipratropium bromide and sodium cromoglycate on the response in both dose-response and time course experiments in patients with asthma.

Subjects

Nine atopic mild asthmatic subjects (five male, four female) with a mean age of 31 years (range 19–62) participated in the study (Table 1). All patients had stable asthma controlled with an inhaled β_2 -adrenoreceptor agonist alone or with inhaled corticosteroids. No subject was receiving oral corticosteroids, theophylline, antihistamines, sodium cromoglycate or ketotifen for routine treatment. All inhaled therapy was omitted for at least 6 h before bronchial challenge testing. The study was approved by the Southampton University and Hospitals Ethics Committee and written informed consent was obtained from each subject.

Bronchial provocation

Airway calibre was measured before and during the inhalation challenges as the forced expiratory volume in 1 s (FEV₁) using a dry wedge spirometer (Vitalograph, Buckingham, U.K.). At

Table 1 Patient characteristics

Patient	Sex	Age (years)	FEV ₁ (l)	FEV ₁ (% predicted)	Baseline treatment†
1	M	62	1.41	38	S, B
2	M	19	3.37	81	S, B
3	M	24	4.65	97	S
4	M	20	4.75	105	S, B
5	M	29	3.84	84	S
6	F	23	3.52	105	S, B
7	F	59	1.37	58	S, B
8	F	22	3.80	105	S, B
9	F	21	3.45	102	S
Mean	5M	31.0	3.35	86.1	
± s.e. mean	4F	± 10.3	± 0.41	± 8.0	

† S: Salbutamol; B: Beclomethasone dipropionate.

Furthermore mediators released from activated mast cells may cause bronchoconstriction via cholinergic mediated mechanisms in addition to direct contractile effects on bronchial smooth muscle (Beasley *et al.*, 1987b).

In this study we have investigated the potential role of reflex and mast cell-dependent mechanisms in the pathogenesis of bronchoconstriction

each time point the higher of two consecutive measurements was used for analysis.

Benzalkonium chloride solutions (Boehringer Ingelheim, Bracknell, U.K.) were made up freshly on each challenge day in 0.9% saline to produce a range of doubling concentrations of 0.4–50 mg ml⁻¹. The BC was administered as an aerosol from a starting volume of 3 ml using an

Inspiron nebuliser (C.R. Bard International, Sunderland, U.K.) driven by compressed air at 8 l min^{-1} . Under these conditions the nebuliser output was 1 ml min^{-1} and the mass median particle diameter was 4.7μ (Chai *et al.*, 1975). Subjects were instructed to inhale 5 breaths of the nebuliser solution from a tidal volume to full inspiratory capacity through a mouthpiece.

Study design

The study was carried out in four phases. During the first phase patients attended the laboratory at the same time of day on two separate occasions to participate in a concentration-response study with inhaled BC. After 10 min rest, two initial baseline measurements of FEV_1 were made, and then either 0.9% saline (placebo) or preservative-free isotonic ipratropium bromide (0.25 mg ml^{-1} , total dose 1 mg) (Boehringer Ingelheim, Bracknell, U.K.) was administered double-blind and in random order. The subjects inhaled the nebulised solutions during tidal breathing from a starting volume of 4 ml to dryness. The concentration-response study was then undertaken 45 min later. After two measurements of FEV_1 (post-treatment baseline), BC was administered in a series of doubling concentrations and paired measurements of FEV_1 were made at 1 and 2 min after inhalation of each dose. The inhalations and measurements were continued until the FEV_1 had fallen by more than 20% of the post-treatment baseline value. The percentage fall in FEV_1 was plotted against the agonist concentration on a logarithmic scale and the concentration of BC causing a 20% fall in FEV_1 (PC_{20}) derived by linear interpolation.

During the second phase of the study, the subjects attended the laboratory at the same time of day on two separate occasions to undertake time course studies. After 10 min rest, two initial baseline measurements of FEV_1 were made and the subjects then inhaled 4 ml of either 0.9% saline (placebo) or 1 mg preservative-free isotonic ipratropium bromide. After 45 min, post-treatment baseline FEV_1 recordings were made and followed by inhalation of the concentration of BC previously shown to produce a greater than 20% fall in FEV_1 . Paired measurements of FEV_1 were then made at regular intervals up to 45 min post BC.

In the final two phases of the study, the effect of pretreatment with sodium cromoglycate or saline on the concentration-response and time-course of BC-induced bronchoconstriction was investigated. An identical protocol was used as in phase 1 and 2 of the study, except that the 4 ml solutions of sodium cromoglycate (10 mg ml^{-1}

total dose 40 mg) (Fisons Pharmaceuticals, Loughborough, U.K.) and 0.9% saline (placebo) were administered by nebulisation 15 min before inhalation challenge with BC.

Data analysis

The initial and post-treatment baseline FEV_1 values were compared within and between study days by Student's *t*-test for paired data. Of the paired measurements of FEV_1 recorded at each time point only the higher value was used for analysis. The values of FEV_1 recorded at each agonist concentration or time point were expressed as a percentage of the post-treatment baseline FEV_1 .

In the concentration-response studies, when the FEV_1 was recorded 1 and 3 min after inhalation the lower value was used for comparison with the post-treatment baseline FEV_1 . Analysis of variance was used to assess whether the concentration-response curves for each treatment group differed significantly from parallel. After logarithmic transformation, the PC_{20} BC values obtained after inhalation of saline were compared with those after ipratropium bromide or sodium cromoglycate using Student's *t*-test for paired data and geometric mean PC_{20} values calculated for each treatment group. The protective effect of ipratropium bromide or sodium cromoglycate treatment against the airway effects of BC were expressed as the concentration ratios i.e. PC_{20} BC after ipratropium bromide and sodium cromoglycate divided by that after saline. The repeatability of the measurement of the PC_{20} values obtained for BC after saline, was determined using the method described by Altman & Bland (1983). Following logarithmic transformation of the values, so that repeatability could be expressed as a percentage of the magnitude of the measurement, rather than an absolute value, the standard deviation of the differences between paired measurements was calculated.

For the time course studies the mean percentage fall in FEV_1 at each time point after saline were compared with those after ipratropium bromide and sodium cromoglycate treatment were compared as the overall bronchoconstrictor response to BC between 0 and 45 min post-challenge calculated by trapezoid integration as the area under FEV_1 response time course curve (AUC).

Results

Patient 5 was withdrawn from the study due to paradoxical bronchoconstriction following inhal-

Table 2 Provocation concentration of benzalkonium chloride required to reduce FEV₁ by 20% after treatment with saline, ipratropium bromide and sodium cromoglycate.

Subject	Saline control	Ipratropium bromide	Saline control	Sodium cromoglycate
1	11.50*	9.80	12.50	15.40
2	1.43	10.70	1.12	7.35
3	3.78	7.45	1.98	6.70
4	19.50	52.50	15.60	14.80
5	—†	—†	1.02	2.19
6	9.80	11.70	7.25	14.80
7	0.48	1.80	0.37	0.96
8	8.20	6.10	2.98	18.30
9	5.00	2.82	—‡	—
10	8.20	4.95	—‡	—
Geometric mean	4.99	7.48	2.77	7.10
	NS		<i>P</i> < 0.05	

* PC₂₀ value mg ml⁻¹; † Patient experienced paradoxical bronchoconstriction with ipratropium bromide;

‡ Patients unable to take part in this limb of the study.

ation of ipratropium bromide. Patient 9 did not attend the laboratory for the final 3 phases of the study.

There was no significant difference in baseline FEV₁ values on any of the treatment days. Pre-treatment with saline did not significantly affect the baseline FEV₁ after 45 min (2.5 ± 1.7%, NS). Administration of 1 mg ipratropium bromide increased the mean baseline FEV₁ by 13.3 ± 3.1% (*P* < 0.001) after 15 min.

After pretreatment with saline or ipratropium bromide the slopes of the benzalkonium chloride concentration-response curves did not significantly depart from parallel. Benzalkonium chloride caused concentration related falls in FEV₁ in all subjects. The geometric mean PC₂₀ value was 4.99 mg ml⁻¹ (range 0.48–19.5 mg ml⁻¹) after saline and 7.48 mg ml⁻¹ (range 1.8–

52.5 mg ml⁻¹) after ipratropium bromide which were not significantly different (Table 2). The mean concentration ratio for the benzalkonium chloride PC₂₀ after administration of ipratropium bromide and placebo was 2.2 ± 0.8.

Following logarithmic transformation, the standard deviation of the differences between paired measurements of the PC₂₀ for benzalkonium chloride (after saline) was 0.15.

After saline inhalation of BC caused rapid bronchoconstriction, with a mean maximum fall in FEV₁ of 23.3 ± 2.9% after 5 min. The FEV₁ remained significantly below baseline for the 45 min during which measurements were made. Ipratropium bromide significantly inhibited the bronchoconstrictor response to BC for the first 30 min of the time course period (Figure 1). The mean fall in FEV₁ after 5 min was 13.2 ± 5.4% which was significantly lower than that achieved after inhaled saline (*P* < 0.02). The mean area under the FEV₁-time course curve after saline and ipratropium bromide pretreatment were 653 ± 127 and 291 ± 150 respectively, representing 56% inhibition of the overall bronchoconstrictor response (*P* < 0.02) (Table 3).

There was no significant change in FEV₁ 15 min after inhalation of either sodium cromoglycate (40 mg) or saline (placebo). The slopes of the BC concentration-response curves following the sodium cromoglycate or saline pretreatment did not significantly depart from parallel. Sodium cromoglycate significantly displaced the concentration-response curve for BC to the right in all subjects, resulting in a geometric mean PC₂₀ of 7.10 mg ml⁻¹ (range 0.96–18.3 mg ml⁻¹) after sodium cromoglycate, compared with 2.77 (range 0.37–15.6 mg ml⁻¹) after saline (*P*

Figure 1 The effect of pretreatment with inhaled saline (●) and ipratropium bromide 1 mg (◆) on airway calibre following inhalation of benzalkonium chloride by 9 asthmatic subjects. Each point represents the mean FEV₁ expressed as percent of post-treatment baseline and each bar the SEM.

Table 3 Effect of inhaled saline, ipratropium bromide and sodium cromoglycate on the AUC between 0–45 for percentage fall in FEV₁ following inhalation of the PC₂₀ benzalkonium chloride.

Subject	Saline control	Ipratropium bromide	Saline control	Sodium cromoglycate
1	376*	-96	1566	389
2	603	-222	454	334
3	319	153	540	162
4	568	368	592	427
5	-†	-	1816	-73
6	1453	1023	1510	-131
7	710	-14	898	919
8	430	697	662	-237
9	-‡	-	-‡	-
10	770	416	-	-
Mean $\pm$ s.e. mean	653 $\pm$ 127	291 $\pm$ 150	1009 $\pm$ 191	224 $\pm$ 133
	$P < 0.02$		$P < 0.01$	

* AUC, arbitrary units; † Patient experienced paradoxical bronchoconstriction with ipratropium bromide;

‡ Patient unable to take part in these limbs of the study.

Figure 2 The effect of pretreatment with inhaled saline (●), and sodium cromoglycate 40 mg (▲) on airway calibre following inhalation of benzalkonium chloride by eight asthmatic subjects. Each point represents the mean FEV₁ expressed as percent of post-treatment baseline and each bar the s.e. mean.

< 0.05) (Table 2). The mean concentration ratio for BC after administration of sodium cromoglycate was 3.1 ± 0.8 .

Pretreatment with sodium cromoglycate markedly attenuated the airway response to benzalkonium chloride throughout the 45 min time course period (Figure 2). The maximum fall in FEV₁ was $10.7 \pm 3.8\%$ at 5 min, compared with $29.1 \pm 5.8\%$ after saline ($P < 0.01$). The mean area under the time course FEV₁ response curves for BC was 1009 ± 191 and 224 ± 133 for saline placebo and sodium cromoglycate respectively. This represents a 78% inhibition by sodium cromoglycate of the overall bronchoconstrictor response due to BC ($P < 0.001$). (Table 3).

Discussion

In this study we have demonstrated that in asthmatic subjects, bronchoconstriction provoked

by BC is inhibited by pretreatment with both ipratropium bromide and sodium cromoglycate. In association with our previous findings (Miszkiel *et al.*, 1987), this suggests that the airways response due to BC is mediated both by the bronchoconstrictor effects of mediators released from mast cells within the airways, and also by the stimulation of central and/or local neural reflex pathways.

The initial experiments were undertaken to determine the contribution of cholinergic-mediated pathways to bronchoconstriction provoked by inhaled BC. To achieve effective cholinergic blockade the muscarinic receptor antagonist ipratropium bromide was administered in a dose of 1 mg, 45 min prior to BC challenge. The peak anticholinergic effect of this muscarinic antagonist on the airways occurs after this time (Allen & Campbell, 1986), and we have previously demonstrated that this dose results in > 100 -fold shift to the right of the methacholine concentration-FEV₁ response curve (Mann *et al.*, 1985; Beasley *et al.*, 1987b). To eliminate the change in baseline airway calibre caused by ipratropium bromide when examining its effect on BC-induced bronchoconstriction, we expressed bronchoconstriction as the percentage change in FEV₁ from the post-drug baseline value (Chung *et al.*, 1982). We have recently used this approach in a group of asthmatic patients to demonstrate that ipratropium bromide in the same dose used in the present study produced a 69% increase in mean baseline specific airways conductance and a 196-fold shift in the methacholine dose-response curve but had no significant effect in displacing the bronchoconstriction dose-response curve to inhaled adenosine 5'-monophosphate indicating that a change in baseline airway calibre *per se* is unlikely to influence the position of a broncho-

constrictor dose-response curve (Mann *et al.*, 1985). However this will only apply to a competitive antagonist such as ipratropium bromide not to a functional antagonist such as a β_2 -adrenoceptor agonist.

Over the 45 min period of observation, ipratropium bromide inhibited BC-induced bronchoconstriction by over 50%, with the maximum inhibitory effect occurring during the first 15 min after challenge. With other exogenous stimuli considered to stimulate cholinergic bronchoconstriction, for example sulphur dioxide (Nadel *et al.*, 1965; Snashall & Baldwin, 1982) and distilled water (Fuller & Collier, 1983), the degree of protection afforded by muscarinic blockade is of a similar degree. The inability of ipratropium bromide to displace significantly the concentration-response curves is unlikely to be due to poor repeatability of our measurements, for repeat benzalkonium PC₂₀ values were within one doubling of the initial values, representing a degree of repeatability superior to that reported previously (Britton *et al.*, 1986). It is more likely to relate to the time points at which FEV₁ were made after successive inhalation of BC. Although the maximum bronchoconstriction due to BC was observed after 5 min, and the differences between ipratropium bromide and saline greatest after 10 min, measurements of FEV₁ were made 1 and 3 min after successive inhalations.

These results suggest that a major component of the bronchoconstriction induced by BC results from vagal reflex mechanisms. This response is likely to result from the stimulation of rapidly adapting irritant receptors of myelinated nerves and the nerve endings of non-myelinated C fibres (Widdicombe, 1986) by a direct action of BC, following the release of mediators from mast cells (Beasley *et al.*, 1987b), or a combination of both mechanisms. Alternatively, benzalkonium chloride could directly stimulate cholinergic ganglia in the airways as has been suggested with certain mediators (Omini *et al.*, 1986).

Sodium cromoglycate was subsequently used to assess further the contribution of mast cell activation and neural reflex mechanisms to bronchoconstriction provoked by BC. Sodium cromoglycate inhibits the release of both preformed and newly generated mediators from mast cells when activated by either immunological and non-immunological mechanisms (Bernstein 1985). In addition this drug also inhibits bronchoconstriction provoked by agents

which activate local and central neural reflexes (Richards, 1982). When inhaled in equivalent doses to that used in this study, sodium cromoglycate is at least as effective as atropine at inhibiting bronchoconstriction provoked by inhaled sulphur dioxide (Snashall & Baldwin, 1981) and distilled water (Fuller & Collier, 1983), stimuli which are considered to activate vagal reflex pathways. There is also some circumstantial evidence that sodium cromoglycate may inhibit the local axon reflex which results in the release of bronchoconstrictor sensory neuropeptides such as substance P, neurokinin A and calcitonin gene-related peptide (Barnes, 1986), a mechanism which is unaffected by muscarinic blockade. In support of this mechanism, sodium cromoglycate inhibits bronchial C-fibre reflex activity in laboratory animals (Richards, 1982; Dixon *et al.*, 1980) and also attenuates bradykinin-induced bronchoconstriction, which is thought to occur following the release of substance P from C-fibre endings (Fuller *et al.*, 1987).

Although sodium cromoglycate did not affect baseline airway calibre, it markedly attenuated the bronchoconstrictor response to benzalkonium chloride throughout the 45 min time course period. Sodium cromoglycate inhibited also 80% of the overall bronchoconstrictor response, which contrasts with the 13% and 55% inhibition associated with H₁-histamine (Miszkief *et al.*, 1987) and muscarinic receptor blockade respectively. Furthermore sodium cromoglycate displaced the concentration response curves of benzalkonium chloride approximately three fold to the right.

In conclusion, this present study suggests that benzalkonium chloride provokes bronchoconstriction in asthmatic subjects due to a combination of mast cell activation and stimulation of peripheral and central cholinergic neural pathways. These studies provide yet another example of how a bronchoconstrictor agonist can produce its airway effects by more than one mechanism and may provide a useful experimental model for studying the relationship between mast cell activation and stimulation of neural pathway in asthma.

We are grateful to Dr A. Eyre-Brook of Boehringer Ingelheim Ltd for supplying the benzalkonium chloride, to Mrs S. Foulkes for preparation of the manuscript, and to the Wellcome Trust and the Medical Research Council of New Zealand for financial support.

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(Received 15 September 1987,
accepted 9 May 1988)