Inhibition of methoxamine-induced bronchoconstriction by ipratropium bromide and disodium cromoglycate in asthmatic subjects

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1 We compared the effects of pretreatment with saline, ipratropium bromide, and disodium cromoglycate (DSCG) on bronchoconstriction induced by methoxamine—an α -adrenoceptor agonist, in asthmatic subjects.

2 All 12 patients bronchoconstricted in response to methoxamine after saline. The PD₂₀ (the dose of methoxamine causing a 20% fall in forced expiratory volume in 1 s [FEV₁]) ranged from 0.3–18 μ mol.

3 Ipratropium bromide (200 μ g administered by aerosol) significantly inhibited ($P < 0.05$) the response to methoxamine in all patients without producing significant changes in the mean baseline lung function. The mean PD₂₀ for methoxamine after saline was 6.8 μ mol and 95% confidence limits (CL) were 3.6, 12.7 μ mol. The mean PD₂₀ for methoxamine after ipratropium bromide was 35.4 (95% CL 28.8, 43.6) μ mol.

4 DSCG also produced significant ($P < 0.05$) shifts to the right in the methoxamine dose response curves, but did not affect resting airway calibre as measured by the FEV₁. The mean PD₂₀ for methoxamine increased from 3.3 μ mol (95% CL 1.1, 10.0 μ mol) after saline to 25.1 μ mol (95% CL 14.1, 44.6) after DSCG pretreatment.

5 These findings suggest that α -adrenoceptors in the airways of asthmatic subjects may be located at sites other than smooth muscle—possibly on mast cells but more likely on nerve endings and/or parasympathetic ganglia.

Keywords α -adrenoceptors asthma disodium cromoglycate ipratropium bromide methoxamine

Introduction

We have previously reported that methoxamine, an α -adrenoceptor agonist, produces bronchoconstriction in asthmatic subjects (Black *et al.*, 1982). Since there have been reports of methoxamine possessing β -adrenoceptor antagonist properties (Karim, 1965) it was possible that methoxamine-induced bronchoconstriction was the result of β -adrenoceptor blockade and not α -adrenoceptor stimulation. Recently we investigated the effect of prazosin, an α_1 -adrenoceptor antagonist, on broncho-

constrictor responses to methoxamine. Prazosin significantly antagonized the effects of methoxamine, providing evidence that the responses were in fact mediated via α -adrenoceptors (Black *et al.*, 1984).

It is still not known however, where these α -adrenoceptors are situated. *In vitro* evidence for their existence on human bronchial muscle is scanty (Guirgis & McNeill, 1969; Mathe *et al.*, 1971; Simonsson *et al.*, 1972; Kneussl & Richardson, 1978). Kaliner *et al.* (1972) have

postulated that α -adrenoceptors are present on mast cells and that stimulation of these will result in mediator release. Although similar observations have been made in murine neoplastic mast cells (Moroni *et al.*, 1977) no studies have been published which corroborate this finding in human lung. It is possible that the α -adrenoceptors detected in our *in vivo* studies in asthmatics may lie on the bronchial smooth muscle itself, or elsewhere in the airways—on mast cells, or on nerve endings or ganglia. In order to shed light on the mechanism of α -adrenoceptor induced bronchoconstriction, the present study investigates the effect of disodium cromoglycate (DSCG) and an anticholinergic agent ipratropium bromide on responses to methoxamine in asthmatic subjects.

Methods

We studied 12 asthmatic subjects with documented bronchial hyperresponsiveness to histamine. The mean histamine PD₂₀ for the group was 0.1 μ mol (95% CL 0.04, 0.3 μ mol). All patients were atopic. The study protocol was approved by the Ethics Review Committee of Sydney University, and all patients agreed to withhold medications for 4–6 h prior to the challenge procedures. Details of the patients and their maintenance therapy are shown in Table 1.

Table 1 Details of patients studied

Patient	Sex	Age (years)	Treatment
1	M	23	S, B
2	F	55	S, B
3	F	32	S, B
4	F	21	S, B
5	F	24	S, B
6	M	24	S, B
7	M	32	S, D
8	M	31	S
9	M	22	S, B, T
10	M	24	S
11	F	29	S, B, D
12	M	26	S

S salbutamol, B beclomethasone, D disodium cromoglycate, T theophylline

All subjects visited the laboratory on two to three occasions at the same time of day within a 2 week period. On the first occasion a methoxamine challenge was carried out as has been previously described (Black *et al.*, 1982, 1984) following placebo pretreatment of 0.9% w/v saline. Briefly, resting forced expiratory volume in 1 s (FEV₁) and forced vital capacity (FVC) were

measured using a dry vitalograph spirometer and following this, 2 ml of 0.9% w/v saline were administered over approximately 10 min using a Hudson's nebulizer with a flow rate of 6–7 l/min. The FEV₁ and FVC were again recorded and methoxamine challenge commenced. The methoxamine was administered from a DeVilbiss # 646 nebulizer with a 3 s delivery time controlled by a Nebulization-Dosimeter (Rosenthal-French, Baltimore USA) as has been described previously (Black *et al.*, 1982). Patients received one, three, or five inhalations of solutions of 1 mg/ml and 10 mg/ml. The challenge procedure was stopped when a 20% fall in the FEV₁ from the post pre-treatment values had been achieved or a total of 42 μ mol (10.4 mg) methoxamine had been delivered. On the second challenge day the pretreatment consisted of 10 inhalations of ipratropium bromide (200 μ g) administered from a metered dose inhaler. Immediately after the FEV₁ had again been recorded, the methoxamine challenge was commenced. On the third day 2 ml of DSCG (20 mg) was administered via a Hudson's nebulizer before the methoxamine challenge. Some patients were studied with either ipratropium bromide or DSCG, whereas others participated in both studies.

All falls in FEV₁ were expressed as a percentage of the post pretreatment value. Graphs were constructed of the relationship between log cumulative dose of methoxamine and percentage fall in FEV₁. From these the dose of methoxamine producing a 20% fall in FEV₁ (PD₂₀) was estimated by linear interpolation of two adjacent data points. The mean PD₂₀ for the group for methoxamine after saline was compared firstly with that after ipratropium bromide and secondly with that after DSCG. Student's *t*-tests were performed on log-transformed data. If the distribution of the two samples was shown to be different using a variance ratio (*F*) test, the modified *t*-test described by Cochran & Cox (1957) was used. Results were considered significant when *P* < 0.05.

Results

All 12 patients experienced a $\geq$ 20% fall in FEV₁ in response to methoxamine after saline. The PD₂₀ ranged from 0.3 to 18 μ mol. Pretreatment with ipratropium bromide markedly inhibited the bronchoconstrictor response to methoxamine in all patients (*n* = 9) tested, in five of whom no PD₂₀ to methoxamine could be obtained i.e. a 20% fall in FEV₁ to 42 μ mol methoxamine did not occur after ipratropium

bromide. In those patients in whom a dose-response curve to methoxamine could be obtained in the presence of ipratropium bromide, the methoxamine dose response curves were shifted to the right in a parallel fashion (Figure 1). The mean PD_{20} (95% CL) for methoxamine after saline was 6.8 (3.6, 12.7) μmol and after ipratropium bromide was 35.4 (28.8, 43.6) μmol . These values were significantly different ($P < 0.05$). Except in one patient, (no. 1) ipratropium bromide did not cause an immediate change in baseline lung function. There was no significant difference ($P > 0.05$) between the FEV_1 expressed as a percentage of predicted values on the two challenge days, $82 \pm 12\%$ (mean $\pm$ s.d.) for day 1 and $86 \pm 11\%$ for day 2.

Figure 1 Percentage fall in forced expiratory volume in 1 s (FEV_1) to methoxamine in the absence (—) and presence (----) of ipratropium bromide in three patients (● ▲ ■).

Again in the nine patients in whom the effects of DSCG on methoxamine responses were examined, (day 3), all patients demonstrated a significant increase in the PD_{20} to methoxamine. Thus the PD_{20} for the group was significantly greater in the presence of DSCG, 25.1 μmol (95% CL 14.1, 44.6) than in its absence, 3.3 μmol (95% CL 1.1, 10.0). The mean FEV_1 percent predicted was $89 \pm 11\%$ for day 1 compared with $91 \pm 15\%$ for day 3, and these values were not significantly different ($P > 0.05$).

Results from individual patients are shown in Table 2 and the dose-response curves to methoxamine after saline, ipratropium bromide and DSCG in a single patient are shown in Figure 2.

Discussion

This study has shown that bronchoconstriction induced by an α -adrenoceptor agonist, methoxamine, can be significantly inhibited by both ipratropium bromide and DSCG. These findings have several important implications for the location of α -adrenoceptors within the respiratory tract.

It is difficult to demonstrate α -adrenoceptor-mediated contraction in human bronchial smooth muscle *in vitro*. This may reflect the fact that specimens of human bronchus studied *in vitro* did not originate from asthmatic subjects. Those patients who come to thoracotomy do so usually for resection of carcinoma of the lung. It is true that some of these may exhibit some degree of bronchial hyperresponsiveness (Vincenc *et al.*, 1983; Armour *et al.*, 1984) but they do not have clinical asthma. It may be that lung tissue from asthmatic subjects responds differently *in vitro* to α -adrenoceptor stimulation. There is indeed some evidence for this from the relatively small number of *post mortem* lung specimens from patients with and without respiratory disease studied by Kneussl & Richardson (1978). In addition, our *in vivo* studies have shown that bronchial hyperresponsiveness *per se* is not necessarily associated with a bronchoconstrictor response to methoxamine (Black *et al.*, 1982) which seems to require the presence of clinical asthma.

The inhibition of methoxamine responses by ipratropium is interesting in view of the study of Snashall *et al.* (1978). These authors pretreated their asthmatic subjects with atropine methonitrate before methoxamine challenge. After observing what tended to be rather small changes in airway function in response to methoxamine, they concluded that bronchoconstriction was not the result of stimulation of vagal reflex pathways. It is difficult to assess the dose of atropine administered and whether complete vagal blockade would have been achieved. In addition, the response to methoxamine was not observed in the absence of atropine, in which case bronchoconstrictor responses may well have been greater. The dose of ipratropium bromide used in the present study is sufficient to block a standard methacholine challenge (Woolcock *et al.*, 1984) although we did not test its effect against methacholine in the present study. Our findings with ipratropium lead us to suspect, unlike Snashall *et al.* (1978) that responses to methoxamine may be mediated by cholinergic mechanisms. Methoxamine may stimulate vagal reflex arcs. Alternatively, although there is evidence to suggest that

Table 2 Effect of ipratropium bromide (IB) and disodium cromoglycate (DSCG) on the dose of methoxamine (PD₂₀) producing a 20% fall in forced expiratory volume in 1 s (FEV₁). Values for FEV₁ expressed as a percentage of predicted values on each day are shown, together with the actual FEV₁ immediately following the three pretreatments.

Patient	Methoxamine after saline			Methoxamine after IB			Methoxamine after DSCG		
	FEV ₁ %Pred	(l)	PD ₂₀ (μmol)	FEV ₁ %Pred	(l)	PD ₂₀ (μmol)	FEV ₁ %Pred	(l)	PD ₂₀ (μmol)
1	81	3.2	13.5	84	4.2	> 42	—	—	—
2	82	1.8	11.0	86	1.9	> 42	—	—	—
3	61	1.8	2.9	71	2.3	32	—	—	—
4	92	3.0	0.3	—	—	—	118	3.8	20
5	91	2.9	4.4	—	—	—	83	2.7	5.4
6	107	4.5	0.54	—	—	—	105	3.9	18.0
7	102	4.3	18.0	105	4.2	> 42	102	4.1	> 42
8	79	3.7	2.0	81	3.7	> 42	76	3.6	> 42
9	75	3.6	6.0	71	3.2	30	73	3.3	17
10	79	3.3	3.8	88	4.2	38	82	4.1	> 42
11	95	2.9	14.0	92	3.1	> 42	85	2.8	> 42
12	80	3.8	6.0	98	4.4	18.5	98	4.2	38

sympathetic nerve fibres do not terminate on human bronchial smooth muscle (Richardson & Beland, 1976) α -adrenoceptors may be situated on parasympathetic ganglia. Stimulation of these receptors may result in increased cholinergic traffic and a bronchoconstrictor response which can be blocked by an anticholinergic agent such as ipratropium. Skoogh & Svedmyr (1984) have demonstrated the presence on parasympathetic preganglionic nerve endings, of β_2 -adrenoceptors, stimulation of which inhibits transmission through parasympathetic ganglia. It is not difficult to imagine that α -adrenoceptors may also modulate ganglionic transmission. It is interesting that DSCG inhibited methoxamine-induced bronchoconstriction. This may indicate that α -adrenoceptors are present on mast cells and that their stimulation results in release of bronchoconstricting mediators. However the time course of methoxamine-induced bronchoconstriction resembles that of histamine and methacholine, which presumably act directly on airway smooth muscle rather than that induced by antigen which is slower and results from mediator release. Additional mechanisms of action for DSCG *viz* an effect on vagal reflex arcs or a direct effect on bronchial smooth muscle have been suggested. This is based on reports that DSCG can inhibit bronchospasm resulting from a wide spectrum of stimuli such as exercise (Schoeffel *et al.*, 1983) cold air (Breslin *et al.*, 1980) SO₂ (Harries *et al.*, 1981) and hypotonic nebulized solutions in man (Fuller & Collier, 1984) and that following stimulation of reflex arcs in dogs (Dixon *et al.*, 1980). Mast cell-derived mediators are not likely to be involved in all these forms of bronchoconstriction. It is

unlikely that the effects of DSCG in the present study represented a direct effect on airway smooth muscle, since Patel (1984) has reported that DSCG in similar doses does not affect histamine or methacholine-induced bronchoconstriction in asthmatic subjects.

In this study it was not possible to administer the placebo and active pretreatments in exactly the same manner. Saline and DSCG were both given by nebulizer whereas ipratropium was

Figure 2 Percentage fall in forced expiratory volume in 1 s (FEV₁) to methoxamine after saline (●), disodium cromoglycate (○) and ipratropium bromide (□) in patient no. 9.

administered via metered dose inhaler. We did not use ipratropium nebulizer solution as it contains metabisulphite to which a number of asthmatics are sensitive (Baker *et al.*, 1981). It is not likely that this different mode of administration would have greatly influenced our results, since antagonism of methoxamine responses by ipratropium bromide was observed consistently in all patients.

It is unlikely that the effects of ipratropium and DSCG could be attributed to differences in starting airway calibre on the three challenge days (Tattersfield, 1981). There was no significant difference between the mean values for FEV₁ after the three pretreatments. Although ipratropium bromide does cause bronchodilatation, this is slow in onset. We administered methoxamine immediately after ipratropium so that, although cholinergic blockade was achieved,

except in one patient, no bronchodilatation resulted. Ipratropium bromide-induced bronchodilatation may have occurred during the course of the methoxamine dose response curve. However the shift in the curves was a parallel one which makes this unlikely. Moreover others have shown (Anderson *et al.*, 1983) that there is only a poor relationship between FEV₁ and PD₂₀ in asthmatic subjects. In conclusion, our results have shown that α -adrenoceptor mediated bronchoconstriction can be significantly inhibited by both ipratropium bromide and DSCG and provide evidence for the location of airway α -adrenoceptors at sites other than bronchial smooth muscle.

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References

- Anderson, S. D., Schoeffel, R. E. & Finney, M. (1983). Evaluation of ultrasonically nebulised solutions for provocation testing in patients with asthma. *Thorax*, **38**, 284–291.
- Armour, C. L., Lazar, N. M., Schellenberg, R. R., Chan, N., Hogg, J. C. & Pare, P. D. (1984). A comparison of *in vivo* and *in vitro* human airway reactivity to histamine. *Am. Rev. resp. Dis.*, **129**, 907–910.
- Baker, G. J., Collett, P. & Allen, D. H. (1981). Bronchospasm induced by metabisulphite containing foods and drugs. *Med. J. Aust.*, **ii**, 614–617.
- Black, J. L., Salome, C. M., Yan, K. & Shaw, J. (1982). Comparison between airways response to an α -adrenoceptor agonist and histamine in asthmatic and non-asthmatic subjects. *Br. J. clin. Pharmacol.*, **14**, 464–466.
- Black, J. L., Salome, C., Yan, K. & Shaw, J. (1984). The action of prazosin and propylene glycol on methoxamine-induced bronchoconstriction in asthmatic subjects. *Br. J. clin. Pharmacol.*, **18**, 349–353.
- Breslin, F. J., McFadden, E. R. Jr. & Ingram, R. H. Jr. (1980). The effects of cromolyn sodium on the airway response to hyperpnea and cold air in asthma. *Am. Rev. resp. Dis.*, **122**, 11–15.
- Cochran, W. G. & Cox, A. M. (1957). *Experimental designs*, 2nd edition. New York: Wiley.
- Dixon, M., Jackson, D. M. & Richards, I. M. (1980). The actions of sodium cromoglycate on 'C' fibre endings in the dog lung. *Br. J. Pharmacol.*, **70**, 11–13.
- Fuller, R. W. & Collier, J. G. (1984). Sodium cromoglycate and atropine block the fall in FEV₁ but not the cough induced by hypotonic mist. *Thorax*, **39**, 766–770.
- Guirgis, H. M. & McNeill, R. S. (1969). The nature of the adrenergic receptors in isolated human bronchi. *Thorax*, **24**, 613–615.
- Harries, M. G., Parkes, P. E. G. & Lessof, M. H. (1981). Role of bronchial irritant receptors in asthma. *Lancet*, **i**, 5–7.
- Kaliner, M., Orange, R. P. & Austen, K. F. (1972). Immunological release of histamine and slow reacting substance of anaphylaxis from human lung. *J. exp. Med.*, **136**, 556–567.
- Karim, S. M. M. (1965). Sympathetic beta-receptor blocking action of methoxamine. *Br. J. Pharmacol.*, **24**, 365–374.
- Kneussl, M. P. & Richardson, J. B. (1978). Alpha-adrenergic receptors in human and canine tracheal and bronchial smooth muscle. *J. appl. Physiol.*, **45**, 307–311.
- Mathe, A. A., Astrom, A. & Persson, N.-A. (1971). Some bronchoconstricting and bronchodilating responses of human isolated bronchi: evidence for the existence of α -adrenoceptors. *J. Pharm. Pharmacol.*, **23**, 905–910.
- Moroni, F., Fantozzi, R., Masini, E. & Mannaioni, P. F. (1977). The modulation of histamine release by alpha-adrenoceptors: evidence in murine neoplastic mast cells. *Agents Actions*, **7**, 57–61.
- Patel, K. R. (1984). Sodium cromoglycate in histamine and methacholine reactivity in asthma. *Clin. Allergy*, **14**, 143–145.
- Richardson, J. & Beland, J. (1976). Nonadrenergic inhibitory nervous system in human airways. *J. appl. Physiol.*, **41**, 764–771.
- Schoeffel, R. E., Anderson, S. D. & Lindsay, D. A. (1983). Sodium cromoglycate as a pressurised aerosol (Vicrom) in exercise-induced asthma. *Aust. NZ. J. Med.*, **13**, 157–161.
- Simonsson, B. G., Svedmyr, N., Skoogh, B.-E., Andersson, R. & Bergh, N. P. (1972). In vivo and in vitro studies on alpha-receptors in human airways. Potentiation with bacterial endotoxin. *Scand. J. resp. Dis.*, **53**, 227–236.

- Skoogh, B.-E. & Svedmyr, N. (1984). β_2 adrenoceptor stimulation inhibits ganglionic transmission in ferret trachea. *Am. Rev. resp. Dis.*, **129**, 232.
- Snashall, P. D., Boother, F. A. & Sterling, G. M. (1978). The effect of α -adrenoreceptor stimulation on the airways of normal and asthmatic man. *Clin. Sci. mol. Med.*, **54**, 283–289.
- Tattersfield, A. E. (1981). Measurement of bronchial reactivity: a question of interpretation. *Thorax*, **36**, 561–565.
- Vincenc, K. S., Black, J. L., Yan, K., Armour, C. ♡, Donnelly, P. & Woolcock, A. J. (1983). Comparison of *in vivo* and *in vitro* responses to histamine in human airways. *Am. Rev. resp. Dis.*, **128**, 875–879.
- Woolcock, A. J., Salome, C. M. & Yan, K. (1984). The shape of the dose-response curve to histamine in asthmatic and normal subjects. *Am. Rev. resp. Dis.*, **130**, 71–75.

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