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Protective effect of drugs on histamine-induced asthma

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Cockcroft, D. W., Killian, D. N., Mellon, J. J. A., and Hargreave, F. E. (1977). *Thorax*, **32**, 429–437. **Protective effect of drugs on histamine-induced asthma.** Controlled standardised histamine inhalation tests were carried out in 21 asthmatics to determine the degree of non-specific bronchial hyperreactivity with and without prior treatment with several anti-asthmatic drugs. A significant protective effect was produced by inhaled salbutamol, 200 μg, ingested salbutamol, 4 mg, inhaled Sch1000, 40 μg, inhaled atropine sulphate, 290 μg, and ingested choline theophyllinate (200 or 400 mg) producing serum theophylline levels over 10 mg/l. Inhaled salbutamol was consistently the most effective and was significantly better than the other drugs. The protective effect between the other four was not significantly different. Drug side-effects occurred only with the ingested drugs. No significant protection was detected after ingested choline theophyllinate producing serum theophylline levels of less than 10 mg/l, inhaled sodium cromoglycate, 20 mg given once or six-hourly for one week, or ingested ascorbic acid, 1 gram.

In most persons with asthma there is a non-specific increase in the reactivity of the bronchial smooth muscle (Orehek and Gayrard, 1976). This increased reactivity can be demonstrated in the laboratory by inhalation of histamine (Curry, 1946; Makino, 1966; Laitinen, 1974). Histamine, in low concentrations, is thought to act chiefly as an irritant (Sellick and Widdicombe, 1971), although it may also exert a direct effect on bronchial smooth muscle (Altounyan, 1971). Naturally occurring irritants such as dust (Sellick and Widdicombe, 1971), smoke (Simonsson *et al.*, 1967), and cold air (Wells *et al.*, 1960) are important triggers of asthma (*Lancet*, 1975). The avoidance of them and protection against their effects by drugs are important aspects of treatment.

In this study we have examined and compared the protective effect of recommended therapeutic doses of a number of drugs on histamine-induced bronchoconstriction, including salbutamol, atropine sulphate, Sch1000, sodium cromoglycate, choline theophyllinate, and ascorbic acid.

Methods

Twenty-one patients with asthma (Ciba Foundation Guest Symposium, 1959) of varying severity were selected from the Chest and Allergy Clinic at St. Joseph's Hospital, Hamilton (Table 1). All had airways obstruction reversible by bronchodilators and/or corticosteroids. At the time of study the forced expired volume in one second (FEV_1) was between 57 and 97% (mean 84%± 11 SD) of the patient's previous best values. All had been noted to have a raised total blood eosinophil count of over 400/mm^3 and/or an increase in sputum eosinophils. Thirteen of the patients were considered atopic, having evidence from history and skin tests of allergic factors. The skin tests were carried out by the modified prick technique using 16 common allergen extracts. There were no other complicating respiratory diseases. Informed consent was obtained. All of the patients understood that they were to be given a series of histamine inhalation tests and certain medicines currently in use for the treatment of asthma, but they did not know the nature of the medicines.

The standard histamine inhalation test was performed in the following way. FEV_1 was measured in triplicate with a Collins' 14-litre water spirometer. Five millilitres of the test solution was

Table 1 *Patient data*

No.	Age	Sex	Atopy	Treatment		FEV_1 (litres)	Threshold HAP concentration (mg/ml)	Drugs investigated								
				Regular bronchodilators	Others			Si	So	SCH	AS	CT > 10	CT < 10	SCG	SCG_7	AA
1	28	M	+			3·5	4	+	+	+	+		+	+		+
2	35	F	+			2·4	4							+	+	
3	40	M				3·7	2	+		+	+			+		
4	21	F	+			2·6	2						+			
5	36	F		+	B400	2·8	2					+				
6	31	F		+	P15	1·6	1	+	+					+		
7	29	F	+			2·6	1	+								+
8	27	F	+	+	B400	3·1	0·5	+		+	+		+			+
9	49	F				2·4	0·5							+	+	
10	35	F		+	P10	2·6	0·5	+	+	+	+		+	+		+
11	46	M		+	P10	2·5	0·5	+	+		+			+		
12	50	F	+	+	B100	1·7	0·25					+	+			
13	19	F	+	+	P10	2·2	0·25	+	+		+			+		
14	51	F	+	+	SCG	1·7	0·25	+		+	+					
15	22	M	+	+	B400	2·3	0·25	+	+			+	+			
16	28	M	+	+		2·8	0·25						+			
17	26	F	+	+		3·2	0·125							+	+	
18	29	M	+			2·0	0·125	+	+	+		+	+			
19	23	M	+	+		3·1	0·125	+	+				+			
20	46	F		+	P25	1·2	0·063	+	+	+	+	+		+		
21	58	F		+	B200	1·0	0·031					+				

Abbreviations: P = prednisone (mg/day), B = beclomethasone (μg/day), SCG = sodium cromoglycate, Si = inhaled salbutamol, So = oral salbutamol, SCH = Sch1000, AS = atropine sulphate, CT > 10 = choline theophyllinate with serum theophylline levels > 10 mg/l, CT < 10 = choline theophyllinate with serum theophylline levels < 10 mg/l, SCG_7 = sodium cromoglycate for one week, AA = ascorbic acid.

placed in a Wright nebuliser and an aerosol was generated with an oxygen flow of 7 litres per minute (particle size—1·3 μ mass median diameter; nebuliser output 0·13 ml/min). A nose clip was applied and the subject inhaled the aerosol via a face mask by quiet tidal breathing for 2 minutes. FEV_1 was repeated 30 and 90 seconds after completion of the inhalation. The first solution inhaled was phosphate buffered saline as a control. This was followed, at 5-minute intervals, by inhalation of successively doubled concentrations of histamine acid phosphate (HAP) (0·03, 0·06, 0·125, 0·25, 0·5, 1, 2, 4, and 8 mg/ml) until a fall in FEV_1 of 20% was produced or until the maximum concentration of 8 mg/ml was reached. FEV_1 was then also repeated at 3 and 5 minutes. The percentage fall in FEV_1 was calculated from the lowest post-saline FEV_1 (FEV_S) and the lowest post-HAP FEV_1 (FEV_H) by the formula:

$$100 \times (FEV_S - FEV_H)/FEV_S$$

The lowest concentration of HAP which produced a greater than 20% fall in FEV_1 was termed the threshold concentration. When the test is performed in this way the step up in dose at 5-minute intervals does not produce a cumulative effect or tolerance, so that if the test is repeated using only the threshold concentration the response is the same (Cockcroft *et al.*, in preparation).

Patients were studied on three or more days at the same time of day within a two-week period. On the first day, a standard histamine inhalation test was performed as outlined above. On subsequent day(s) patients were pretreated with a test drug (Table 1). After an interval, chosen to coincide with the time of maximum effect of the test drug (Table 2), the histamine inhalation test was repeated, omitting the saline inhalation and starting with the threshold HAP concentration identified on the first day. On the final day the test was repeated with no test drug, to check that the response was similar to that of the first day. On the second and all subsequent days the fall in FEV_1 was calculated from the lowest initial and lowest post-HAP values.

Table 2 *Drugs investigated*

Drug	Dose	Route of administration	Interval before test (min)
Salbutamol	200 μg	Inhaled[1]	15
Salbutamol	4 mg	Oral	60
Sch1000	40 μg	Inhaled[1]	60
Atropine sulphate	290 μg	Inhaled[2]	60
Choline theophyllinate	200–400 mg	Oral	180
Sodium cromoglycate	20 mg	Inhaled[3]	15
	20 mg qid 7 days	Inhaled[3]	15
Ascorbic acid	1 g	Oral	60

[1]Two puffs from a meterised canister.
[2]Atropine sulphate 0·2% nebulised in Wright nebuliser by oxygen flow 7 l/min and inhaled for 1 minute by tidal breathing.
[3]Spinhaler.

Throughout the study an attempt was made to control factors known, or thought, to influence the level of non-specific bronchial reactivity. None of the patients smoked, none had evidence of recent respiratory infection, and none was exposed to relevant allergens. In all patients the initial FEV_1 was within 20% of that on the first day; in 18 of the 21 it was within 10%. The use of other anti-asthmatic drugs was carefully controlled. Salbutamol and sodium cromoglycate were withheld for 6 hours and choline theophyllinate for 24 hours. Corticosteroids were continued in the same dose.

Serum theophylline levels were measured by a modified method of Schack and Waxler (1949) on days when the effect of choline theophyllinate was under study. On these days the blood sample was obtained immediately before histamine challenge.

In an analysis of the results, bronchial reactivity was expressed as the HAP provocation concentration required to produce a 20% FEV_1 fall (PC_{20}). The PC_{20} was calculated from the histamine dose-response curve or, in subjects in whom a single HAP concentration produced more than a 20% FEV_1 fall, from the formula:

$$PC_{20}=20\%\times\text{HAP concentration}/\%\text{ fall in } FEV_1$$

The PC_{20} in normal subjects, using the same method of histamine inhalation, is usually >8 mg/ml (Cockcroft *et al.*, in preparation). Reproducibility of the first and final control tests was assessed by calculation of the coefficient of variation:

$$\frac{100\times\text{SD of difference between two tests}}{\sqrt{2}\times\text{mean value of test result}}$$

Protection against histamine-induced asthma was indicated by a rise in PC_{20} above that observed on either control day. Geometric means and SD for PC_{20} were calculated using logarithmic transformation. They were compared for significance using the paired or unpaired Student's *t* test where appropriate. Linear regression analysis was done by the method of least squares. Differences were considered significant if $P<0.05$.

Results

The first and final (control) histamine inhalation tests showed, for the same inhaled HAP concentration, a difference in the percentage FEV_1 fall of 0 to 18, mean 5·5%, coefficient of variation 15%.

Significant bronchodilatation was seen after salbutamol, Sch1000, atropine sulphate, and choline theophyllinate, producing serum theophylline levels over 10 mg/l (Table 3). Inhaled salbutamol was the most effective and in paired samples was superior to ingested salbutamol (n=9, $P<0.05$) and to atropine sulphate (n=8, $P<0.05$) but not to Sch1000 (n=7, $P<0.20$). No significant bronchodilatation was seen after sodium cromoglycate, ascorbic acid, or choline theophyllinate producing serum theophylline levels less than 10 mg/l.

Significant protection against histamine-induced asthma was observed after inhaled and ingested salbutamol, Sch1000, atropine sulphate, and choline theophyllinate with serum theophylline levels over 10 mg/l (Fig. 1; Table 3). No protection was seen after sodium cromoglycate, ascorbic acid, or choline theophyllinate with serum theophylline levels under 10 mg/l.

Inhaled salbutamol was the most effective drug in protecting against histamine-induced asthma in all 13 subjects receiving it. This is illustrated by comparative dose-response curves from a repre-

Table 3 *Comparative drug protection in histamine-induced asthma*

Drug	*No. of patients tested*	*Bronchodilatation*			*Improvement in bronchial reactivity*				
		FEV_1			*Patients improved*		*Histamine provocation concentration (PC_{20})*		
		Mean % increase	*SD*	*P*	*No.*	*%*	*Control PC_{20} geometric mean (mg/ml)*	*Treated PC_{20} geometric mean (mg/ml)*	*Significance of difference*
Si	13	13·0	8·5	<0·001	13	100	0·33	3·99	P<0·001
So	9	6·7	7·3	<0·05	5	56	0·21	0·44	P<0·02
SCH	7	10·7	7·6	<0·02	5	71	0·28	0·78	P<0·05
AS	8	6·0	4·9	<0·02	6	75	0·28	0·65	P<0·01
CT<10	6	11·3	7·6	<0·05	4	67	0·12	0·26	P<0·05
CT>10	9	2·9	4·3	>0·10	2	22	0·30	0·39	P>0·1
SCG	10	0	3·6	>0·20	2	20	0·46	0·51	P>0·2
SCG_7	3	0	—	>0·20	0	0	0·42	0·44	P>0·2
AA	4	2·0	2·5	>0·20	0	0	0·73	0·73	P>0·2

Abbreviations as in Table 1.

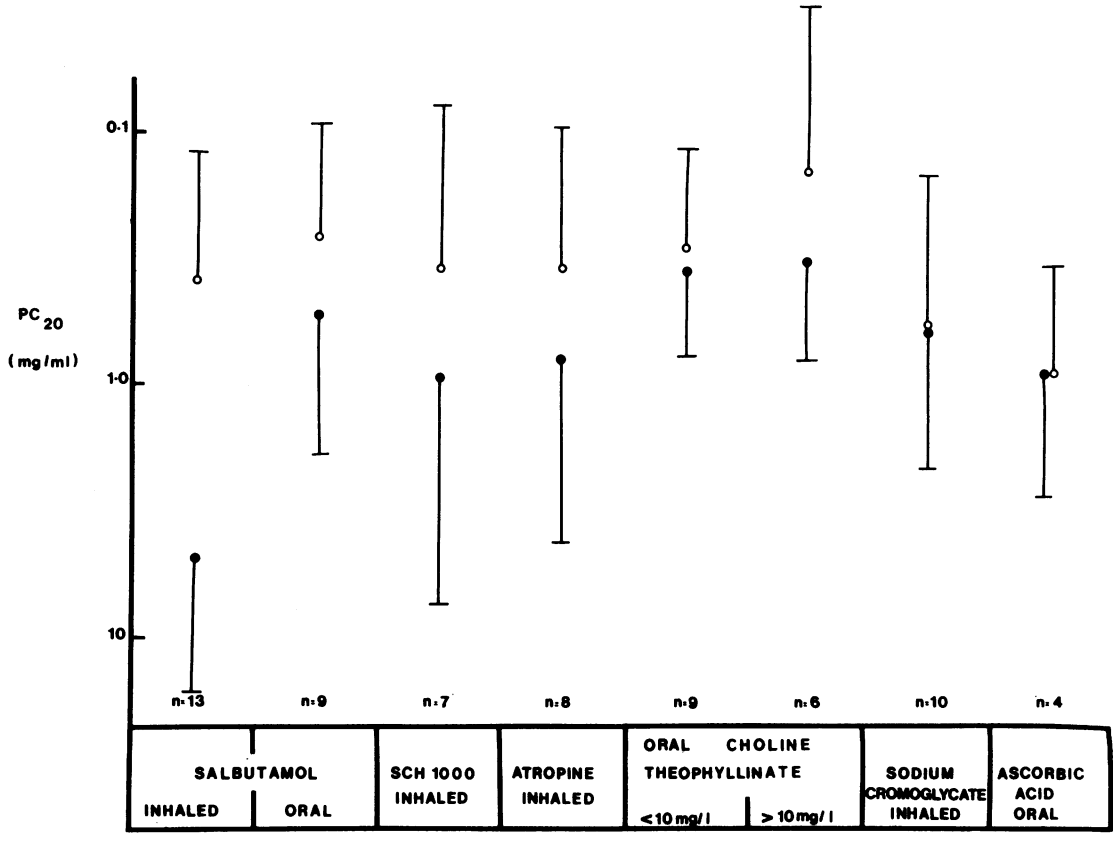

Fig. 1 *Geometric mean PC_{20} (mg/ml). Open circles represent control values, closed circles represent values after administration of noted drugs, and vertical bars represent one geometric standard deviation.*

sentative sample of subjects in Figures 2 and 3. After the inhalation of salbutamol, the geometric mean PC_{20} increased 12-fold over that in the two control tests. This compares with the 2- to 3-fold increases seen for the other four effective drugs (Table 3). A comparison of protective effect using paired samples showed that inhaled salbutamol provided significantly greater protection against histamine-induced asthma than did ingested salbutamol ($n=9$, $P<0.01$), atropine sulphate ($n=8$, $P<0.01$) or Sch1000 ($n=7$, $P<0.05$). In an unpaired comparison, inhaled salbutamol provided significantly greater protection than choline theophyllinate with serum theophylline levels >10 mg/l ($n_1+n_2=19$, $P<0.001$). There was no significant difference in the effect of ingested salbutamol, atropine, Sch1000, or choline theophyllinate.

The relationship of the degree of protection against histamine-induced asthma and the degree of bronchodilatation produced by the test drug was examined. Six of 18 patients who received bronchodilators had no bronchodilatation, yet they demonstrated a similar degree of protection against histamine-induced asthma. There was no correlation, for any drug, between the degree of bronchodilatation (% improvement in FEV_1) and the degree of protection (log change in PC_{20}) ($0<r<0.2$, $P>0.1$).

Drug-induced side-effects were noted by seven patients. These included tremulousness after 4 mg ingested salbutamol in two of nine patients (22%), and anorexia, nausea, and/or headache after 400 mg oral choline theophyllinate (two with serum theophylline under 10 mg/l) in five of 12 patients (42%).

Serum theophylline levels over 10 mg/l (mean 13.3 ± 3.4 SD) were achieved in four subjects after 400 mg choline theophyllinate and in two after 200 mg. Levels of under 10 mg/l (mean

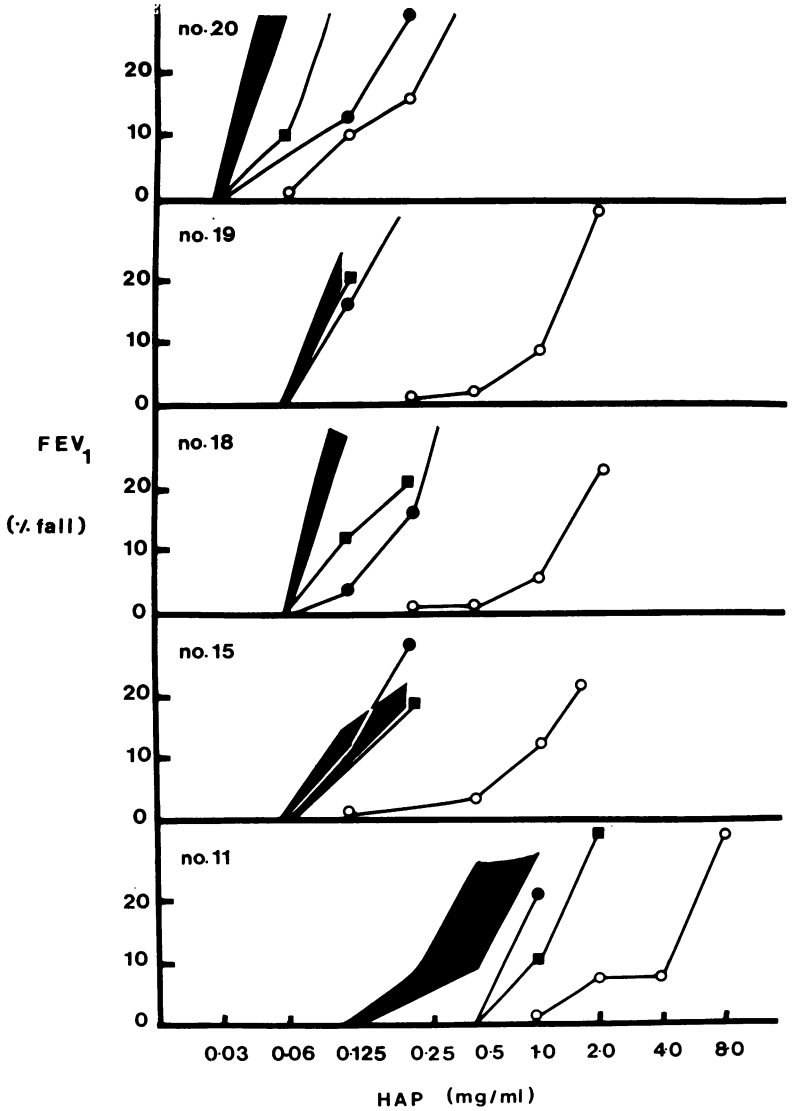

Fig. 2 *Comparative protective effect of inhaled and ingested salbutamol and ingested choline theophyllinate in five representative subjects. Shaded area—control range; open circles—inhaled salbutamol; closed circles—ingested salbutamol; squares—choline theophyllinate (serum theophylline levels; patient 20, 17 mg/l; patient 19, 5 mg/l; patient 18, 12·6 mg/l; patient 15, 6 mg/l; and patient 11, not determined).*

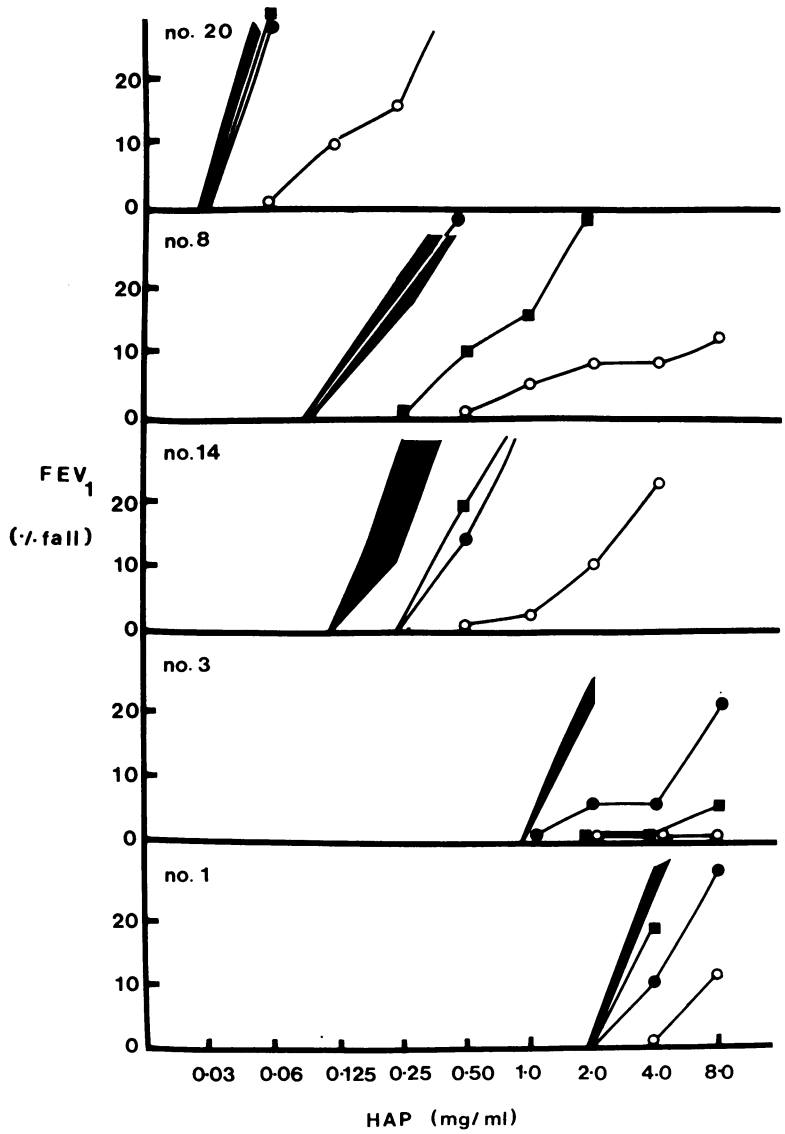

Fig. 3 *Comparative protective effect of inhaled salbutamol, Sch1000, and atropine sulphate in five representative subjects. Shaded area—control range; open circles—salbutamol; closed circles—atropine sulphate; squares—Sch1000.*

6·8±1·6 SD) were seen after 400 mg choline theophyllinate in four subjects and after 200 mg in five.

Discussion

The present study has examined the protective effect of a number of drugs on histamine-induced asthma. Protection was provided by salbutamol, Sch1000, atropine sulphate, and choline theophyllinate producing serum theophylline levels above 10 mg/l. Inhaled salbutamol was the most effective; there was no difference between the protective effect of the others, including ingested salbutamol. No protection was provided by choline theophyllinate giving serum theophylline levels less than 10 mg/l, sodium cromoglycate, and ascorbic acid.

Each drug was used in the recommended therapeutic dose which probably produces a maximum or near maximum effect, and was tested at a time regarded as optimal. No attempt was made to examine the effect of the drugs in different doses, except with choline theophyllinate, or at different times. It would have been preferable to include placebos for the inhaled and ingested drugs and to have tested each drug in each patient. However, there was, in most instances, administration of a drug which had no effect which could be taken to represent a placebo, and the study was single-blind. The patient was unaware of the nature of the drug and the dose of histamine administered. The histamine inhalation test, as carried out in this study, was reproducible in each patient over the test period as shown by comparison between first and final tests.

One factor contributing to the protective effect of the different drugs on histamine-induced asthma is their bronchodilating effect. It is well known that as airway calibre increases the response to inhaled histamine decreases (Benson, 1975). In the present study, the drug producing the greatest bronchodilatation also produced the greatest protection. However, there is also clearly another factor independent of the degree of bronchodilatation. Thus Sch1000 produced as good bronchodilatation as inhaled salbutamol but gave less protection. Six subjects had no bronchodilatation following bronchodilator but had equal protection to those exhibiting bronchodilatation. Within the groups of patients tested with each drug no significant relationship was observed between the degree of bronchodilatation and the degree of protection. Altounyan (1974) has also observed that maximum bronchodilatation produced by atropine sulphate and maximum protection against carbacol-induced asthma do not coincide; maximum bronchodilatation occurs at one hour and maximum protection immediately.

Inhaled histamine is thought to act chiefly by stimulating irritant receptors (Sellick and Widdicombe, 1971), and in this study it was used as a model of irritant-induced asthma. In previous studies of histamine on irritant-induced asthma protection has been observed with isoprenaline (Dubois and Dautrebande, 1958; Wells *et al.*, 1960; Harnett and Spector, 1976), atropine (Altounyan, 1964; Nadel *et al.*, 1965), and Sch1000 (Harnett and Spector, 1976) but not with salbutamol or theophylline. The clinical use of isoprenaline is no longer recommended because of its beta-one cardiac stimulating effects and the short duration of action. The present study identifies inhaled salbutamol, or presumably similar selective beta-two stimulants like inhaled orciprenaline and terbutaline, as the most effective drugs in protecting against histamine-induced, and possibly irritant-induced asthma. Ingested theophylline is not recommended because it is effective only when it produces serum levels of greater than 10 mg/l and these are associated with a high incidence of side-effects.

Inhaled irritants act through irritant receptors (Fillenz and Woods, 1970; Mills *et al.*, 1970) in central airways and a reflex arc in vagal parasympathetic fibres (Simonsson *et al.*, 1967). Their effects are blocked by anticholinergics. In the present study the failure of histamine-induced asthma to be blocked most effectively by anticholinergics may mean that histamine is exerting its effect, at least partly, through other mechanisms (Altounyan, 1971). In one other study Harnett and Spector (1976) report that Sch1000 protects more effectively than isoprenaline against histamine-induced asthma. Altounyan (1964), however, found isoprenaline better than atropine.

The parasympathetic reflex mechanism involved in irritant-induced asthma also participates in allergen-induced asthma (AIA) and may play a role in exercise-induced asthma (EIA). In AIA the level of non-specific reactivity measured by histamine inhalation influences the response to allergen (Killian *et al.*, 1976); AIA is triggered more easily as the level of hyperreactivity increases. In EIA hyperventilation stimulates irritant receptors (Simonsson *et al.*, 1967). One would therefore expect drugs effective in irritant-induced asthma to be also effective in AIA and EIA, and this has been observed with beta-adrenergic stimulants (Booij-Nord *et al.*, 1972;

Kershnar *et al.*, 1976; Godfrey and König, 1976) and anticholinergics (Itkin and Anand, 1970; Kersten, 1974; Godfrey and König, 1976; Kershnar *et al.*, 1976). Godfrey and König (1976) found that salbutamol was also the most effective drug in EIA. He noted that oral salbutamol was as effective as inhaled salbutamol but only two cases of the former were examined. However, recently Anderson *et al.* (1976) have shown inhaled salbutamol to be superior to oral salbutamol in preventing EIA and suggested that the mechanism of this was independent of the bronchodilating effect. The effect of atropine varies in both EIA (Godfrey and König, 1976) and AIA (Itkin and Anand, 1970; Rosenthal *et al.*, 1974) and this probably reflects different degrees of involvement of the parasympathetic reflex in different patients.

The lack of effect of sodium cromoglycate on histamine-induced asthma has been recorded by others (Cox, 1967; Ryo *et al.*, 1971; Townley *et al.*, 1973). This contrasts with its effectiveness in EIA (McNeill *et al.*, 1966; Kershnar *et al.*, 1976; Kiechel *et al.*, 1976) and AIA (Pepys *et al.*, 1968). Its mode of action in EIA is unknown. Patel *et al.* (1976) have recently suggested that its effect is related to the phosphodiesterase inhibiting action identified by Lavin *et al.* (1976). If this action is significant, it should produce bronchodilatation and protection against irritant-induced asthma. Sodium cromoglycate is effective in AIA by preventing release of mediators from mast cells (Cox, 1967). Allergen exposure may secondarily increase non-specific bronchial reactivity to inhaled histamine, and Altounyan (1970) has shown that when sodium cromoglycate is used regularly in relation to allergen exposure it may prevent or reverse this increase.

We were unable to show any protective effect of ascorbic acid. However, Zuskin *et al.* (1973) have demonstrated a significant protective effect of ascorbic acid against histamine inhalations in normal individuals using more sensitive maximal expiratory flow volume curves. The protection they observed, however, was very small in comparison with the degree of protection produced by various medications in the present study.

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