Thorax 1986;**41**:753–758

Effect of verapamil and sodium cromoglycate on leukotriene D_4 induced bronchoconstriction in patients with asthma

J A ROBERTS, I W RODGER, N C THOMSON

From the Department of Respiratory Medicine, Western Infirmary, Glasgow

ABSTRACT Leukotriene D_4 (LTD_4) may be an important mediator in asthma. The effect of verapamil and sodium cromoglycate on LTD_4 induced bronchoconstriction has been examined in seven patients with asthma. The bronchoconstrictor response to increasing concentrations of inhaled LTD_4 (0·0032–50 μg/ml) was assessed by measuring changes in FEV_1, specific airways conductance, and flow rate at 30% of vital capacity ($\dot{V}_{30(p)}$). Results were expressed as the provocation concentration (PC) producing a 10% fall in FEV_1 ($PC_{10}FEV_1$), a 35% fall in specific airways conductance (PC_{35}sGaw), and a 30% fall in flow at 30% of vital capacity ($PC_{30}\ \dot{V}_{30(p)}$). Neither verapamil nor cromoglycate inhibited LTD_4 induced bronchoconstriction in asthmatic subjects. These results suggest that in asthmatic patients LTD_4 induced bronchoconstriction is not mediated via verapamil or cromoglycate sensitive mechanisms.

The leukotrienes (LTs), including LTD_4, may be important mediators in asthma. LTD_4 is released both in vitro and in vivo after allergen challenge,[1–3] and is a potent bronchoconstrictor.[4 5] The mechanism of LTD_4 induced bronchospasm in asthma has not, however, been established. The calcium channel blocker verapamil partially inhibits the bronchoconstrictor response to LTD_4 in vivo but not in vitro in non-asthmatic human bronchi,[6] and only at very high concentrations in isolated trachea from the guinea pig.[7] These results suggest that in guinea pigs and non-asthmatic human subjects LTD_4 induced bronchoconstriction occurs as a result both of a direct effect on airway smooth muscle that is insensitive to the inhibitory action of verapamil and of an indirect effect via a verapamil sensitive mechanism. Similarly, in guinea pigs sodium cromoglycate partially inhibits the contractile response to LTD_4 in vivo but not in vitro.[7] Thus in this species cromoglycate appears capable of inhibiting an indirectly mediated bronchoconstrictor response of LTD_4.

In this study we examined the effect of pretreatment with verapamil and sodium cromoglycate on LTD_4 induced bronchoconstriction to determine whether in asthma LTD_4 induced airway narrowing depends on a mechanism sensitive to verapamil or to sodium cromoglycate or on both types of mechanism.

Address for reprint requests: Dr J A Roberts, Department of Respiratory Medicine, Western Infirmary, Glasgow G11 6NT.

Accepted 12 May 1986

Methods

PATIENTS

We studied seven patients with asthma (table 1), four of whom were women. Their ages ranged from 22 to 49 years. All were atopic and were non-smokers. All were taking inhaled β_2 adrenoceptor agonists by pressurised aerosol, three were taking inhaled corticosteroids regularly, and two were taking sodium cromoglycate. All β_2 agonists were discontinued 12 hours before testing and sodium cromoglycate 24 hours before testing. Inhaled corticosteroids were continued. All subjects gave informed consent and the experimental protocol was approved by the Western Infirmary ethical committee.

IN VIVO MEASUREMENTS

Airways resistance (Raw) and thoracic gas volume (TGV) were measured in a constant volume body plethysmograph (Fenyves and Gut), a computerised data collection and analysis system[8] based on the method of DuBois *et al*[9] being used. The results were expressed as specific airways conductance (sGaw) (= 1/Raw × TGV). The mean of eight measurements was taken for the sGaw value. The maximum ex-

Thorax: first published as 10.1136/thx.41.10.753 on 1 October 1986. Downloaded from http://thorax.bmj.com/ on April 10, 2024 by guest. Protected by copyright.

Table 1 *Characteristics of the patients*

Patient No	Age (y)	Sex	FEV_1 (l)	FEV_1 (% pred)	Atopy	Methacholine $PC_{20}FEV_1$ (mg/ml)	Current treatment*
1	28	M	2·96	86	+	2·6	S, B
2	49	F	3·43	139	+	1·3	S
3	25	F	2·59	83	+	0·95	S, B
4	42	M	3·00	86	+	0·97	S, B
5	22	F	3·49	103	+	0·23	S, SCG
6	24	M	4·57	113	+	0·90	S
7	24	F	4·21	121	+	0·81	S, SCG

*S—salbutamol inhaler; B—beclomethasone dipropionate inhaler; SCG—sodium cromoglycate.

piratory flow at 70% of expired vital capacity, obtained from a partial flow-volume ($\dot{V}_{30(p)}$) curve, and the forced expiratory volume in one second (FEV_1) were measured automatically (Collingwood Measurement). The flow-volume curves were obtained as follows[7]: after a period of normal tidal breathing, the subject expired maximally from end tidal inspiratory volume to residual volume (RV) to obtain the partial expiratory flow-volume (PEFV) curve. When RV was reached the subject inspired to total lung capacity (TLC) and expired maximally to RV. From this manoeuvre FEV_1 was calculated. Body plethysmographic measurements preceded flow-volume recordings. Aerosols were generated with a Wright nebuliser by air of 50 lb/in^2 (345 kPa) at a flow rate of 8 l min^{-1} to achieve an output of 0·15 ml min^{-1}.

DOSE-RESPONSE CURVES

The study was performed in two parts, which were carried out sequentially two months apart. In the first each patient (Nos 1–6) received either verapamil (2·5 mg/ml) or normal saline in a randomised double blind manner on three separate days (two saline). After baseline measurements of sGaw (mean of eight readings) and $\dot{V}_{30(p)}$ and FEV_1 (mean of five readings), the solutions were inhaled for five minutes. After 10 minutes lung function tests were repeated and each subject then inhaled increasing concentrations of leukotriene (0·0032–50 μg/ml). Each concentration was inhaled for two minutes and inhalations were repeated every 15 minutes. Results were expressed as the provocation concentration (PC) producing a 35% fall in sGaw (PC_{35}sGaw), a 30% fall in $\dot{V}_{30(p)}$ ($PC_{30}\dot{V}_{30(p)}$), and a 10% fall in FEV_1 ($PC_{10}FEV_1$).

In the second part of the study patients (Nos 1–4, 6, 7) inhaled either sodium cromoglycate (10 mg/ml) or placebo for five minutes in a randomised double blind manner. Measurements were taken before and 10 minutes after each inhalation. A dose-response curve for LTD_4 was then constructed as described above. On a separate day a dose-response curve for methacholine was obtained in a single blind manner according to the protocol described by Hargreave *et al.*[10] The average PC value for the two postsaline LTD_4 dose-response curves was used for the comparison with the results obtained after inhalation of verapamil. Results were compared by means of analysis of variance and Student's *t* test.

Results

The $PC_{20}FEV_1$ for methacholine ranged from 0·17 to 0·64 mg/ml, confirming that these patients had bronchial responsiveness values in the asthmatic range.[10 11]

Inhalation of verapamil did not alter baseline FEV_1, sGaw, or $\dot{V}_{30(p)}$ (table 2). All patients developed appreciable bronchoconstriction as assessed by $PC_{10}FEV_1$, PC_{35}sGaw and $PC_{30}\dot{V}_{30(p)}$. Pretreatment with verapamil did not modify this response (fig 1). The geometric mean $PC_{10}FEV_1$ was 0·35 μg/ml after verapamil compared with 0·47 μg/ml after the control (NS). PC_{35}sGaw was 0·69 μg/ml after control and 0·37 μg/ml after verapamil (NS). Mean $PC_{30}\dot{V}_{30(p)}$ was 0·41 μg/ml after the control and 0·31 μg/ml after verapamil (NS). Sample dose-response curves for

Table 2 *Effect of verapamil on baseline airway function* (means with standard errors in parentheses)*

	FEV_1 (l)		sGaw (s^{-1} kPa^{-1})		$\dot{V}_{30}(p)$ ($l\,s^{-1}$)	
	Baseline	After treatment	Baseline	After treatment	Baseline	After treatment
Control	2·59 (0·36)	2·60 (0·36)	1·13 (0·16)	1·03 (0·13)	1·2 (0·2)	1·36 (0·22)
Verapamil	2·38 (0·51)	2·37 (0·54)	1·39 (0·49)	1·07 (0·28)	1·05 (0·3)	1·02 (0·63)

*No significant differences in baseline and post-treatment values of FEV_1, specific airways conductance, and partial flow-volume curve ($\dot{V}_{30}$(p)) within or between treatments.
Conversion: SI to traditional units—sGaw: 1 s^{-1} kPa^{-1} = 1 s^{-1} mm H_2O^{-1}.

Thorax: first published as 10.1136/thx.41.10.753 on 1 October 1986. Downloaded from http://thorax.bmj.com/ on April 10, 2024 by guest. Protected by copyright.

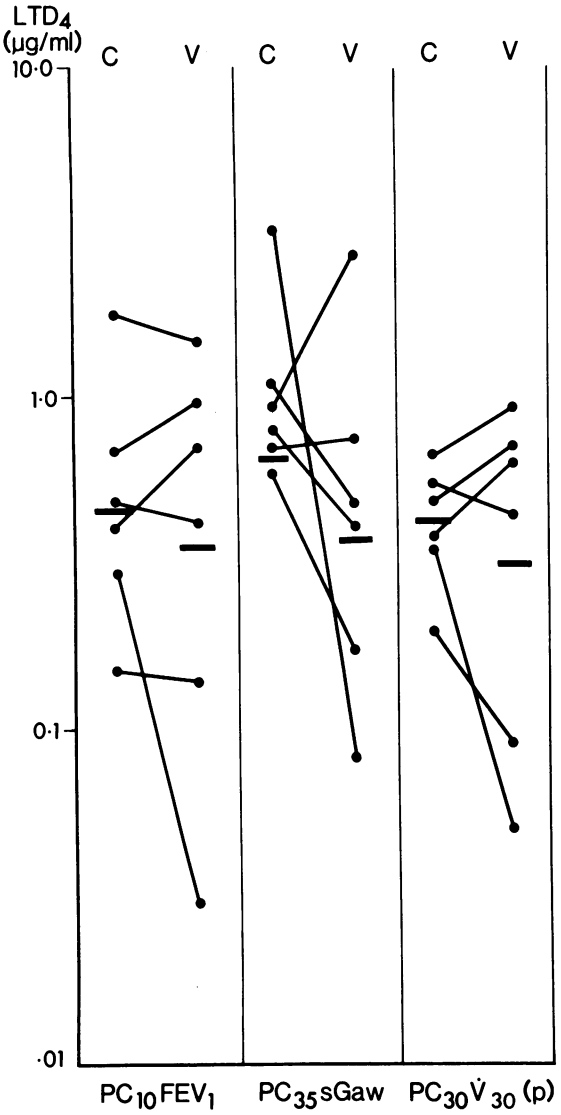

Fig 1 *Effect of pretreatment with verapamil (2·5 mg/ml) or control (phosphate buffered saline) on airway responsiveness to leukotriene D_4 (LTD_4). Results are expressed as the provocation concentration (PC) producing a 10% decrease in FEV_1 ($PC_{10}FEV_1$), a 35% decrease in specific airways conductance (sGaw—PC_{35}sGaw), and a 30% fall in the partial flow-volume curve ($\dot{V}_{30(p)}$—$PC_{30}\dot{V}_{30(p)}$). Mean values of $PC_{10}FEV_1$, PC_{35}sGaw, and $PC_{30}\dot{V}_{30(p)}$ are shown as horizontal bars.*

sGaw against log concentration LTD_4 (two after placebo and one after verapamil) are shown in figure 2.

Sodium cromoglycate did not significantly alter baseline FEV_1 sGaw or $\dot{V}_{30(p)}$ (table 3) and did not alter responsiveness to LTD_4 (fig 3). The geometric mean PC_{10} FEV_1 was 0·22 μg/ml after control and 0·24 μg/ml after sodium cromoglycate (NS). PC_{35}sGaw was 0·21 μg/ml after control and 0·19 μg/ml after sodium cromoglycate. $PC_{30}\dot{V}_{30(p)}$ was 0·21 μg/ml after control and 0·19 μg/ml after sodium cromoglycate. Sample dose-response curves for sGaw against log concentration LTD_4 (after placebo and cromoglycate) are shown (fig 4). All patients developed chest tightness but none coughed after LTD_4.

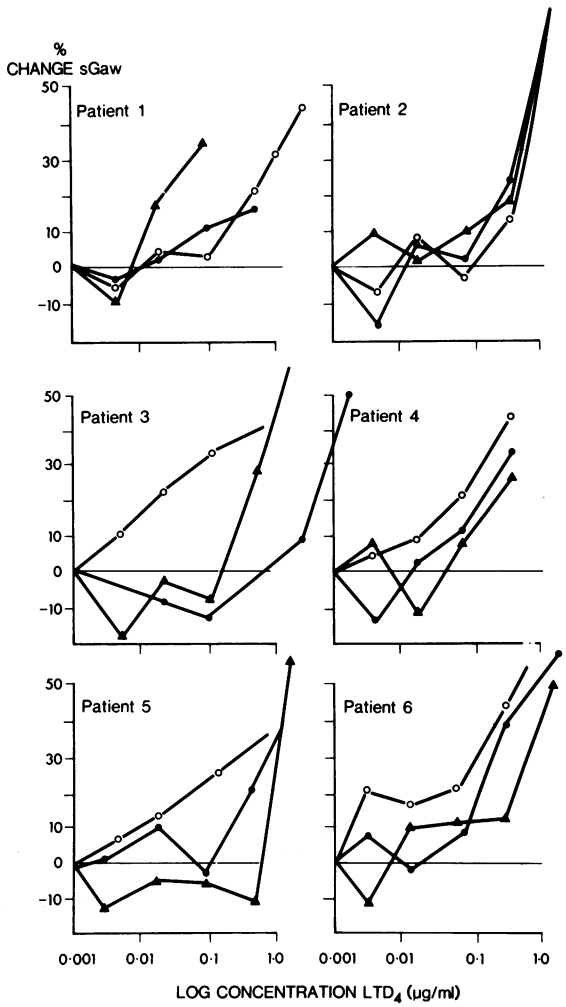

Fig 2 *Graph of percentage change in specific airways conductance (sGaw) against log concentration of inhaled leukotriene D_4 (LTD_4) after placebo 1 (○——○), placebo 2 (●——●), and verapamil (▲——▲).*

Thorax: first published as 10.1136/thx.41.10.753 on 1 October 1986. Downloaded from http://thorax.bmj.com/ on April 10, 2024 by guest. Protected by copyright.

Table 3 *Effect of sodium cromoglycate on baseline airway function* (means with standard errors in parentheses)*

	FEV_1 (l)		sGaw (s^{-1} kPa^{-1})		$\dot{V}_{30(p)}$ ($l\,s^{-1}$)	
	Baseline	*After treatment*	*Baseline*	*After treatment*	*Baseline*	*After treatment*
Control	2·35 (0·2)	2·46 (0·21)	1·20 (0·9)	1·14 (0·9)	1·00 (0·43)	1·07 (0·2)
Sodium cromoglycate	2·42 (0·18)	2·51 (0·21)	1·2 (0·8)	1·17 (0·9)	0·99 (0·25)	1·09 (0·29)

*No significant differences in baseline and after treatment values of FEV_1, specific airways conductance (sGaw), and partial flow-volume curve ($\dot{V}_{30(p)}$) within or between treatments.
Conversion: SI to traditional units—sGaw: $1\,s^{-1}\,kPa^{-1} = 1\,s^{-1}\,mm\,H_2O^{-1}$.

Discussion

These results show that neither the calcium channel blocker verapamil nor sodium cromoglycate modifies LTD_4 induced bronchoconstriction in patients with asthma. In contrast to the finding with verapamil, we recently showed that in non-asthmatic subjects[6] the calcium channel blocker significantly reduced the constrictor response to LTD_4.[6]

Why should verapamil have a protective effect against LTD_4 induced bronchoconstriction in normal subjects but not in asthmatic patients? It has been suggested that there may be heterogeneity of LTD_4 receptors, and that drugs vary in their ability to block the response to LTD_4 according to the different affinities of their LTD_4 receptors.[1,2] In support of this hypothesis, the calcium channel blocker diltiazem inhibits the contraction of guinea pig lung parenchymal strips in response to high dose LTD_4 whereas the

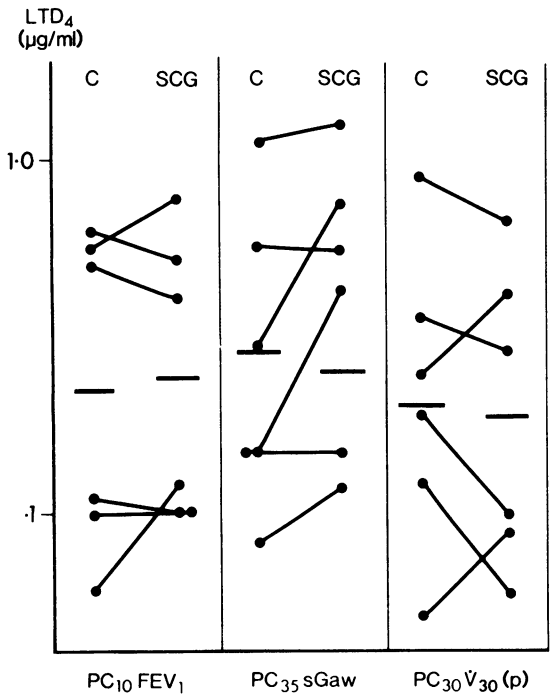

Fig 3 *Effect of pretreatment with sodium cromoglycate (10 mg/ml) or control (phosphate buffered saline) on airway responsiveness to leukotriene D_4 (LTD_4). Results are expressed in a similar manner to those in figure 1. Sodium cromoglycate did not significantly reduce the response to LTD_4.*

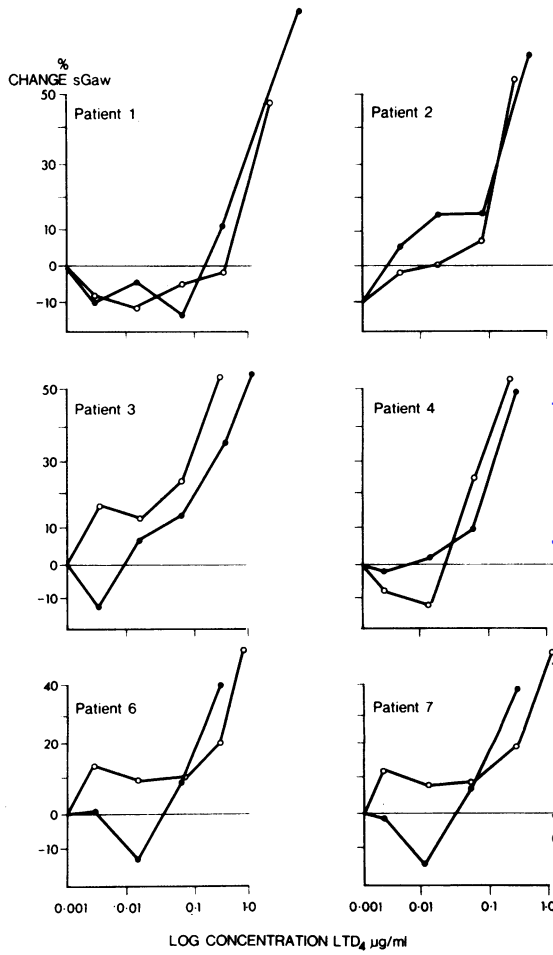

Fig 4 *Graph of percentage change in specific airways conductance (sGaw) against log concentration of inhaled leukotriene (LTD_4): results after placebo (○——○) and sodium cromoglycate (●——●).*

Thorax: first published as 10.1136/thx.41.10.753 on 1 October 1986. Downloaded from http://thorax.bmj.com/ on April 10, 2024 by guest. Protected by copyright.

SRS-A antagonist FPL55712 inhibits only the low dose part of LTD_4 induced contraction.[12] Since the non-asthmatic subjects inhaled higher concentrations of LTD_4 than the asthmatic patients,[6] this may have meant that the low affinity LTD_4 receptors sensitive to calcium channel blockers were stimulated only in the normal subjects. An argument against this mechanism is that verapamil does not significantly inhibit the contractile response of in vitro preparations of human bronchi to different doses of LTD_4, although a slight non-significant reduction of response to the highest concentration of LTD_4 was observed.[6]

An alternative explanation of these findings is that LTD_4 induced bronchoconstriction in asthmatic patients may be due to a direct effect on airway smooth muscle, whereas in non-asthmatic subjects airway narrowing occurs because of a combination of direct action via bronchial smooth muscle receptors and indirect verapamil sensitive mechanisms. This indirect mechanism may have a higher threshold before LTD_4 produces an effect. Possible indirect mechanisms by which LTD_4 might cause bronchoconstriction include reflex vagal bronchoconstriction[7 13] and release of secondary mediators.[14]

A third possibility is that the action of verapamil is dependent on an intact respiratory epithelium. Recently Raeburn *et al*[15] have shown that verapamil inhibits induction of airway smooth muscle contraction by LTD_4 in the rabbit only when the airway mucosa is intact. Since patients with even mild asthma have damaged mucosa,[16] the absence of an inhibitory effect of verapamil on LTD_4 induced bronchoconstriction could be due to the lack of an intact mucosa in those with asthma.

If LTD_4 is confirmed as an important mediator in asthma, it could be predicted from our findings that verapamil would not be an effective drug in asthma. The results of most studies using calcium channel blocking drugs in asthma would support this suggestion. Neither verapamil nor nifedipine significantly alter resting bronchomotor tone and they produce little or no protection against bronchoconstriction induced by allergen.[17 18] They are moderately effective in inhibiting exercise induced asthma.[19 20]

Sodium cromoglycate inhibits mast cell degranulation, but may have other modes of action.[21] Its duration of action at concentrations used in our protocol is two to four hours.[22] In guinea pigs in vivo sodium cromoglycate partially inhibits LTD_4 induced bronchoconstriction.[7] In patients with aspirin induced asthma the response to aspirin challenge can be blocked by sodium cromoglycate.[23] The pathogenesis of aspirin induced asthma is unknown but increased production of lipoxygenase products such as LTD_4 may play a part.[24] Possibly sodium cromoglycate acts as a leukotriene antagonist. Our results show that sodium cromoglycate is not a specific inhibitor of LTD_4 in patients with asthma. This finding confirms and extends the work of Holroyde *et al*,[5] who showed that in normal subjects sodium cromoglycate had no effect on airway narrowing induced by LTD_4

In summary, we have shown that neither verapamil nor sodium cromoglycate inhibits LTD_4 induced bronchospasm in patients with asthma. This contrasts with the action of verapamil in non-asthmatic subjects.

References

1 Orange RP, Austen KF. Slow reacting substance of anaphylaxis. *Adv Immunol* 1969;**10**:105–44.

2 Dahlen SE, Hansson G, Heqvist P, Bjork T, Granstrom E, Dahlen B. Allergen challenge of lung tissue from asthmatics elicits bronchial contraction that correlates with the release of leukotriene C_4, D_4 and E_4. *Proc Natl Acad Sci USA* 1983;**80**:1712–6.

3 Creticos PS, Peters SP, Adkinson NF, *et al*. Peptide leukotriene release after antigen challenge in patients sensitive to ragweed. *N Engl J Med* 1984;**310**:1626–30.

4 Dahlen SE, Hedqvist P, Hammerstrom S, Samuelsson B. Leukotrienes are potent constrictors of human bronchi. *Nature* 1980;**299**:484–6.

5 Holroyde MC, Altounyan REC, Cole M, Dixon M, Elliot EV. Bronchoconstriction produced in man by leukotriene C and D. *Lancet* 1981;ii:17–8.

6 Roberts JA, Giembycz MA, Raeburn D, Rodger IW, Thomson NC. In vitro and in vivo effect of verapamil on human airway responsiveness to leukotriene D_4. *Thorax* 1986;**41**:12–6.

7 Advenier C, Cerrina J, Durroux P, Floch A, Pradel J, Renier A. Sodium cromoglycate, verapamil and nicardipine antagonism to leukotriene D_4 bronchoconstriction. *Br J Pharmacol* 1983;**78**:301–6.

8 Roberts JA, Pugh JR, Thomson NC. A new adaptable computerised system for the measurement of specific airways conductance. *Br J Dis Chest* (in press).

9 Dubois AB, Botelho SY, Comroe JH. A new method for measuring airway resistance in man using a body plethysmograph: values in normal subjects and patients with respiratory disease. *J Clin Invest* 1956; **35**:327–35.

10 Hargreave FE, Ryan G, Thomson NC, *et al*. Bronchial responsiveness to histamine and methocholine in asthma: measurement and clinical significance. *J Allergy Clin Immunol* 1981;**63**:347–55.

11 Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977;**7**:235–43.

12 Lewis RA, Austen KF. The biologically active leukotrienes. Biosynthesis, metabolism, receptors, functions and pharmacology. *J Clin Invest* 1983;**73**:889–97.

13 Hirscham CA, Davnell M, Brugeman T, Peters J. Airway constriction effects of leukotriene D_4 in dogs with

Thorax: first published as 10.1136/thx.41.10.753 on 1 October 1986. Downloaded from http://thorax.bmj.com/ on April 10, 2024 by guest. Protected by copyright.

hyper-reactive airways. *Prostaglandins* 1983;**25**: 481–90.
14 Weichman BM, Muccitelli RM, Osborn RR, Holden DA, Gleason JG, Wasserman MA. In vitro and in vivo mechanisms of leukotriene mediated bronchoconstriction in the guinea pig. *J Pharmacol Exp Ther* 1982;**222**:202–8.
15 Raeburn D, Hay DWP, Robinson VA, Farmer SG, Fleming WW, Fedan JS. The effect of verapamil is reduced in isolated airway smooth muscle preparations lacking the epithelium. *Life Science* 1986; **38**:809–16.
16 Laitinen LA, Heino M, Laitinen A, Kava T, Haatelin T. Damage of the airway epithelium and bronchial reactivity in patients with asthma. *Am Rev Respir Dis* 1985;**131**:599–606.
17 Patel KR, Al Shama MR, Kerr JW. The effect of inhaled verapamil on allergen induced bronchoconstriction. *Clin Allergy* 1983;**13**:119–22.
18 Henderson AF, Dunlop LS, Costello JF. Effect of nifedipine on antigen-induced bronchoconstriction. *Am Rev Respir Dis* 1983;**127**:549–53.
19 Cerrina J, Denjean A, Alexandre G, Lockhart A, Durroux P. Inhibition of exercise induced asthma by a calcium antagonist, nifedipine. *Am Rev Respir Dis* 1981;**123**:156–60.
20 Patel KR. Calcium antagonists in exercise-induced asthma. *Br Med J* 1981;**282**:932–3.
21 Stokes TC, Morley J. Prospects for an oral intal. *Br J Dis Chest* 1981;**75**:1–14.
22 Patel KR, Tullett WM, Neale MG, Wall RT. Dose duration effect of sodium cromoglycate aerosol in exercise asthma [abstract]. *Thorax* 1985;**40**:706.
23 Martelli NA, Usandivaras G. Inhibition of aspirin-induced bronchoconstriction by sodium cromoglycate inhalation. *Thorax* 1977;**32**:684–90.
24 Szczeklik A, Gryglewski RJ, Czerniawska-Mysik G. Relationship of inhibition of prostaglandin biosynthesis by analgesia to asthma attacks in aspirin-sensitive patients. *Br Med J* 1975;i:67–9.

Thorax: first published as 10.1136/thx.41.10.753 on 1 October 1986. Downloaded from http://thorax.bmj.com/ on April 10, 2024 by guest. Protected by copyright.