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The relative contributions of histamine and prostanoids to bronchoconstriction provoked by isocapnic hyperventilation in asthma

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The relative contributions of histamine and prostanoids to bronchoconstriction provoked by isocapnic hyperventilation in asthma. J.P. Finnerty, A. Harvey, S.T. Holgate.
ABSTRACT: It has been proposed that exercise provokes bronchoconstriction in asthma by inducing mast cell degranulation, and that this occurs secondary to the hyperpnoea of exercise causing hypertonicity of the airway lining fluid. We investigated the contribution of the mast cell products, histamine and prostaglandins, to the bronchoconstriction induced by isocapnic hyperventilation (ISH) using single doses of terfenadine, a specific histamine H_1-receptor antagonist, and flurbiprofen, a potent cyclooxygenase inhibitor. We also investigated the effect of flurbioprofen in single dose on bronchial histamine reactivity.

Eleven asthmatics took part in a two phase, double-blind, randomized study. In phase 1, subjects attended on three occasions and received either terfenadine 180 mg, flurbiprofen 150 mg, or placebo, prior to 6 min of ISH. The mean maximum percentage fall in forced expiratory volume in one second (FEV_1) induced by ISH was 31.5(±3.2)% following placebo, 29.7(±4.4)% following flurbiprofen (NS), and reduced to 16.6(±3.7)% following terfenadine ($p<0.01$). In phase 2, subjects received bronchial challenge with histamine following either flurbiprofen 150 mg or placebo. No significant change in bronchial reactivity following flurbiprofen was seen.

We conclude that as administered in this study, flurbiprofen has no effect on baseline bronchial reactivity to histamine. The inhibitory effect of terfenadine indicates that histamine, probably from airway mast cells, makes an important contribution to bronchoconstriction induced by isocapnic hyperventilation, whereas prostaglandin release has no significant role.
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Exercise is a common and troublesome cause of provoked asthma. It is widely believed that it is the hyperpnoea of exercise, rather than exercise *per se*, that provides the stimulus for exercise-induced bronchoconstriction in asthmatic subjects [1], for when isocapnic hyperventilation (ISH) is matched to produce the same ventilation as that achieved during an exercise task, a similar degree of bronchoconstriction is induced [2, 3]. While the mechanism of exercise-induced asthma (EIA) is not established, there is evidence that it may be mediated by mast cell activation, possibly secondary to hypertonicity of the airway lining fluid, consequent upon conditioning of the inspired air [4]. Elevated plasma histamine levels have been found in association with EIA [5–7], although the cellular provenance of this mediator has not been established with certainty. Prostaglandin D_2 (PGD_2), a newly-generated mast cell product, may contribute to both EIA and ISH-induced bronchoconstriction, since increased concentrations of this eicosanoid have been reported following nasal challenge with cold air in man [8], and high airflow challenge of canine airways [9].

As a pharmacologic approach to measure the contrbution of histamine and prostanoids to different forms of provoked bronchoconstriction in asthma, we have previously reported the use of selective histamine H_1-receptor antagonism and inhibition of cyclooxygenase. In the present study, we have applied this approach to ISH-induced asthma by observing the effects of terfen-adine, a selective H_1-receptor antagonist and flurbiprofen, a potent cyclooxygenase inhibitor, on the airways response to ISH in asthma. Because of the possiblity that some of the bronchoconstrictor effect of released histamine might occur as a consequence of prostanoid release [10], we have also assessed the effect of flurbiprofen on the airways response to inhaled histamine.

Methods

Subjects

Eleven asthmatic subjects (7 male) with a mean age of 28.7 yrs (range 18–48 yrs) took part in the study. None had a history of dyspepsia or aspirin intolerance. All were nonsmokers and all were atopic, as judged by at least one positive wheal >3 mm in diameter on skin prick testing to *Dermatophagoides pteronyssinus*, mixed grass pollens, and cat dander (Bencard, Middlesex, UK). Their mean forced expiratory flow in one second (FEV_1) was 104% of their predicted values [11]. All took inhaled β_2-agonists as required, and two were taking regular inhaled corticosteroids (table 1). All medication was withheld for 6 h prior to each study visit, and subjects were asked to abstain from caffeine-containing drinks. The study was approved by the Southampton University and Hospitals Ethical Subcommittee and written informed consent was obtained from each subject.

Table 1. – Characteristics of subjects studied

Subject no.	Age yrs	Sex	FEV_1 (% pred)	Medication	Target ventilation $l \cdot min^{-1}$	% of IBMC
1	20	F	76	S as needed	23.3	31
2	20	M	88	T as needed C 2 mg *q.i.d*	31.7	28
3	29	M	122	S as needed	55.0	29
4	19	F	94	T as needed	36.5	32
5	43	M	121	S as needed	36.0	24
6	18	M	98	S as needed B 100 μg *b.i.d.*	40.0	24
7	24	F	137	S as needed	30.0	19
8	23	F	114	S as needed	34.0	25
9	48	M	72	S as needed	48.0	53
10	29	M	126	S as needed B 100 μg *q.i.d.*	50.0	26
11	43	M	93	S as needed	35.0	27

S: salbutamol aerosol inhaler; T: terbutaline aerosol inhaler; B: beclomethasone dipropionate aerosol inhaler; C: sodium cromoglycate inhaler; IBMC: Indirect maximum breathing capacity; FEV_1: forced expiratory volume in one second.

Histamine bronchial provocation

Histamine provocation was performed using the five breath technique modified from that of CHAI *et al.* [12]. The lowest concentration of histamine monophosphate (Sigma, Poole, Dorset, UK) used was 0.03 $mg \cdot ml^{-1}$ (0.1 $\mu mol \cdot l^{-1}$) and doubling concentrations were administered up to a maximum of 16 $mg \cdot ml^{-1}$ (52 $\mu mol \cdot l^{-1}$), using an initial volume in the nebulizer of 3 ml. Prior to challenge, a baseline FEV_1 was obtained, being the highest of three technically satisfactory recordings using a dry wedge spirometer (Vitalograph, Buckingham, UK). Subjects were then asked to take five breaths of aerosol from functional residual capacity to total lung capacity *via* a mouthpiece from an Inspiron nebulizer (C.R. Bard International, Sunderland, UK), from which normal saline was nebulized using compressed air at a flow rate of 8 $l \cdot min^{-1}$. Under these conditions particles of a mass median diameter of 4.7 μm are generated [13]. Two measurements of FEV_1 were performed at 1 and 3 min, the higher value being recorded at each time-point. If the minimum post-saline FEV_1 was within 10% of the baseline, then the histamine challenge was undertaken. Increasing doubling concentrations of histamine were administered at 5 min intervals, and paired FEV_1 measurements made at 1 and 3 min with the higher reading at each time-point being recorded. The test continued until a 20% fall in FEV_1 from the post-saline value had occurred. From a plot of the percentage fall in FEV_1 against the natural logarithm of the cumulative concentration of histamine administered, the concentration of histamine which provoked a 20% fall in FEV_1 (PD_{20}) was derived by linear interpolation.

Isocapnic hyperventilation challenge

The method used was adapted from that of PHILIPPS *et al.* [3]. The challenge was conducted with subjects seated and wearing a noseclip. Subjects inspired dry air containing 5% CO_2 at room temperature and atmospheric pressure for 6 min from a prefilled Douglas bag *via* a mouthpiece connected to a two-way valve, and expired into ambient air. Type K thermocouples (Tempcon Instrumentation Ltd, Chichester, UK) with time constants in air of 0.6 s were placed in the expiratory and inspiratory ports of the valve and used to record inspiratory and expiratory air temperatures breath-by-breath. The thermocouples were connected to voltage conversion circuitry and the output connected to an analogue to digital converter on a BBC microcomputer, which was programmed to use the mean inspiratory and the peak expiratory temperature recordings in subsequent calculations. The volume of inspired air was measured using a Parkinson Cowan gas meter (PK Morgan Ltd) and also input to the microcomputer.

During each challenge, the inspired volume was measured breath-by-breath, and displayed as a line on the BBC microcomputer monitor. The desired ventilation rate was represented on the screen by a horizontal line, and the subject was instructed to ventilate at a rate which kept as close as possible to this line. With practice, all subjects were able to hyperventilate at the desired rate. The highest of three readings of FEV_1 performed immediately prior to ISH challenge was taken as the baseline value, and after the challenge single measurements of FEV_1 were made at 1, 3, 5, 10, 15, 20, 25 and 30 min.

Respiratory heat exchange during the isocapnic hyperventilation test was calculated by the microcomputer using an adaptation of the formula of DEAL *et al.* [1], which assumes 100% saturation of expired air:

$$RHE = V(HC(T_I - T_E) + HV(WC_I - WC_E))$$

where RHE is respiratory heat exchange (kJ); V is total volume respired (*l*); HC is heat capacity of air (0.00127 $kJ \cdot l^{-1} \cdot °C$); T_I is inspired air temperature and T_E is expired air temperature (°C); HV is heat of vapourization of water (0.0024 $kJ \cdot mg^{-1}$); WC_I is water content of inspired air and WC_E is water content of expired air ($mg \cdot l^{-1}$). The calculated water loss during ISH was also recorded.

Study protocol

All subjects had a history of exercise-induced asthma and, where known, the initial ISH ventilatory task was the same as had occurred spontaneously during a previous exercise challenge. Otherwise, an initial ISH ventilation of 150 *l* over 6 min was performed. Each subject then underwent at least two further ISH challenges, with the ventilation rate being altered in successive tests if required to achieve a >25% fall in FEV_1 postchallenge. Once an appropriate ventilation rate had been established for each subject, this ventilation rate was repeated for all subsequent ISH challenges. The final target minute ventilation is given in table 1, and expressed as a percentage of indirect maximum breathing capacity (IMBC). IMBC was calculated using the following formula:

$$40 \times (0.92\ FEV_1 - 0.07)\ [14]$$

The study was randomized, double-blind and placebo-controlled, and was conducted in two phases. In the first phase, each subject performed three ISH challenges, each at the same time of day, with intervals of 7–15 days between test days. Three hours prior to each challenge, they receive either terfenadine 180 mg orally or matched placebo tablets. Two hours prior to challenge, they receive either flurbiprofen 150 mg orally or matched placebo tablets. Thus, on the three study days, they received either terfenadine and placebo; flurbiprofen and placebo; or double placebo. ISH challenge was performed as described, with the prescribed ventilation being constant for each subject. The bronchoconstrictor response was assessed over 30 min following ISH challenge.

In the second phase, subjects attended the laboratory on two occasions at least seven days apart. Both visits were at the same time of day. Each subject took either flurbiprofen 150 mg or matched placebo tablets 2 h prior to a histamine dose-response challenge being performed. Terfenadine was obtained from Merrel Dow Laboratories, flurbiprofen from Boots Co. PLC, and matched placebos were prepared in the hospital pharmacy.

Data analysis

The airways response to ISH challenge in phase 1 was assessed both as the maximum percentage fall in FEV_1 from the prechallenge baseline value, and as the area under the curve (AUC) of the percentage fall in FEV_1 from the prechallenge baseline value against time over 30 min calculated by trapezoidal integration. Percentage inhibition of the maximum bronchoconstrictor response after each drug treatment was calculated for each subject as follows:

$$\frac{100 \times [(\text{max\% fall after placebo}) - (\text{max\% fall after active drug})]}{\text{Max\% fall after placebo}}$$

Student's paired t-tests were used to compare baseline FEV_1 values and bronchoconstrictor responses to ISH on each of the study days. Since an increase in prechallenge FEV_1 was observed following terfenadine, multiple linear regression analysis was performed on the data from the placebo and terfenadine days, using the percentage bronchoconstriction as the dependent variable and the subjects, drugs used and prechallenge FEV_1 as the independent variables. Repeatability of the bronchoconstriction following ISH challenge was assessed by comparing the maximum percentage falls in FEV_1 following the final practice ISH challenges with those that occurred in the study period on the placebo days, using the method described by BLAND and ALTMAN [15]. Repeatability was expressed both as the coefficient of repeatability and as the coefficient of variation derived from analysis of variance (ANOVA) [16].

In phase 2, a Student's paired t-test was used to compare the PC_{20} values for histamine, after logarithmic transformation of the data.

Results

Phase 1

After oral placebo, the baseline FEV_1 prior to ISH challenge had a mean(±SEM) of 3.17±0.29 *l*. Following flurbiprofen, the mean baseline FEV_1 value was 3.76±0.32 *l* (NS), whilst following terfenadine, the mean baseline FEV_1 was 4.26±0.27 *l* which was significantly greater than on either of the other two study days ($p<0.01$) (table 2).

The volume respired during each ISH challenge, the estimated respiratory heat exchange (RHE) and water loss did not differ significantly between study days (table 3). The coefficient of variation for each was 1.1% for volume respired, 10.4% for RHE and 10.4% for water loss. The coefficient of repeatability of the maximum percentage fall in FEV_1 occurring after ISH challenge was 23.8% (CI 12.7–34.9%). From ANOVA, the coefficient of variation of the maximum percentage fall in FEV_1 was 22.6%. For each individual over the three tests performed, the range (SD) for the inspired air temperature was 2.7 (1.3)°C, while for each individual over the three tests performed, the range (SD) for the mean expired temperature was 2.0 (1.4)°C).

Table 2. – Baseline FEV_1 values prior to each isocapnic hyperventilation challenge, and protection afforded by terfenadine and flurbiprofen expressed as a percentage of the placebo values, for each subject

Subject no.	Drug treatment				
	Placebo	Terfenadine		Flurbiprofen	
	FEV_1 *l*	FEV_1 *l*	%protection	FEV_1 *l*	%protection
1	1.75	3.00	70	1.90	13
2	3.50	4.50	48	3.50	-8
3	4.75	5.15	-23	4.55	-136
4	3.20	3.55	55	3.10	66
5	4.10	4.30	62	4.35	-44
6	4.60	5.05	-15	4.85	-30
7	4.30	4.60	97	4.20	85
8	3.80	4.45	70	4.00	20
9	2.50	2.70	-1	2.40	-14
10	4.75	5.50	59	5.40	47
11	3.55	4.05	62	3.10	-29
Mean	3.71	4.26	44	3.76	-3
(SEM)	(0.29)	(0.27)	(12)	(0.32)	(18)

FEV_1: Forced expiratory volume in one second.

Table 3. – Mean values for volume respired (BTPS), and calculated respiratory heat exchange (RHE) and water loss during each hyperventilation challenge

	Volume *l*	RHE kJ	Water loss ml	Mean T_I °C	Mean T_E °C
Placebo					
Mean	258.5	21.5	7.76	21.1	29.5
(SEM)	(19.3)	(2.0)	(0.68)	(0.4)	(0.5)
Flurbiprofen					
Mean	258.6	19.8	7.27	21.1	29.0
(SEM)	(18.8)	(1.6)	(0.58)	(1.0)	(0.5)
Terfenadine					
Mean	257.9	21.5	7.76	21.5	29.9
(SEM)	(19.2)	(1.7)	(0.64)	(0.6)	(0.4)

BTPS: body temperature, atmospheric pressure, saturated.; T_I, T_E: temperature of inspired and expired air respectively.

The mean maximum percentage fall in FEV_1 provoked by ISH after pretreatment with placebo was 31.5±3.2% and the mean AUC of percentage fall in FEV_1 over 30 min was 635±93 % min (table 4). Following flurbiprofen the mean maximum percentage fall in FEV_1 provoked by ISH was 29.7±4.4% and the mean AUC was 528±96 % min, neither of which were significantly different from the response after placebo. The AUC over 30 min could not be calculated for subject no. 9 on the flurbiprofen day as he received inhaled salbutamol following slow recovery from the challenge. Terfenadine had a marked inhibitory effect on the bronchoconstrictor response to ISH, with a reduction in the mean maximum percentage fall in FEV_1 from baseline to 16.6±3.7% ($p<0.01$) and a reduction in the mean AUC to 324±102 % min ($p<0.01$). The inhibition of the maximum percentage fall by terfenadine was 43.7±11.7%. Using multiple linear regression analysis as described earlier, there was significant negative correlation between the bronchodilation achieved by terfenadine and the bronchoconstriction induced by ISH, but even taking this confounding factor into account, the inhibitory effect of terfenadine remained significant ($p=0.022$).

Table 4. – The effects of terfenadine, flurbiprofen and placebo on the maximum percentage fall in FEV_1 and AUC 0–30 min after ISH-challenge

Subject no.	Placebo		Terfenadine		Flurbiprofen	
	FEV_1 % fall	AUC % min	FEV_1 % fall	AUC % min	FEV_1 % fall	AUC % min
1	48.6	898	15.0	349	42.1	578
2	34.3	605	17.8	277	27.1	453
3	12.6	247	15.5	267	29.7	508
4	28.1	334	12.7	81	9.7	199
5	18.3	370	7.0	146	26.4	658
6	29.3	654	33.7	849	38.1	768
7	31.4	524	1.1	13	4.8	98
8	40.8	1129	12.4	232	32.5	699
9	44.0	1159	44.4	1083	50.0	*
10	26.3	440	10.9	80	13.9	222
11	32.4	621	12.4	189	41.9	1098
Mean	31.5	635	16.6	324	29.7	528
(SEM)	(3.2)	(93)	(3.7)	(102)	(4.4)	(96)

*: this subject received inhaled salbutamol during the recovery period, so an AUC over 30 min could not be calculated. FEV_1: Forced expiratory volume in one second; AUC: area under curve; ISH: isocapnic hyperventilation.

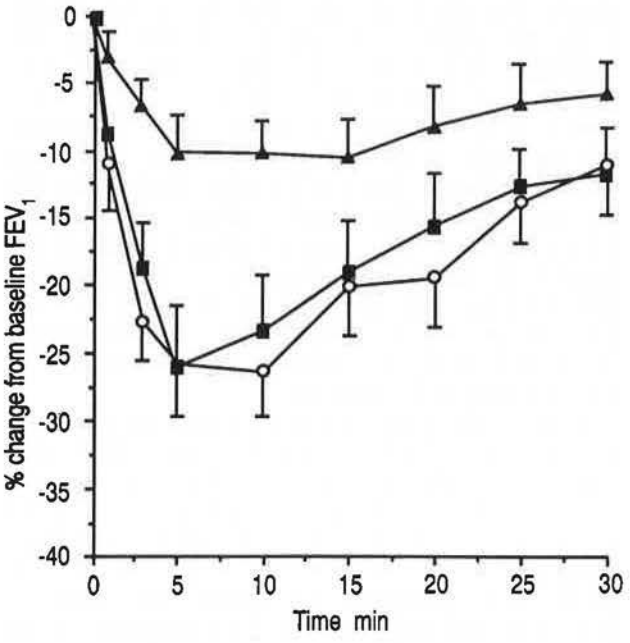

Fig. 1. – The effect of placebo (open circles), flurbiprofen (closed squares) and terfenadine (closed triangles) on the mean percentage fall in FEV_1 from baseline following 6 min of isocapnic hyperventilation at room temperature (n=11)

The time course of the mean percentage fall in FEV_1 following ISH after each drug treatment is shown in figure 1. Following placebo the maximum mean

percentage fall in FEV_1 of 26.5±3.2% occurred at 10 min after challenge and diminished to 11.4±3.3% at 30 min. Following flurbiprofen the maximum mean percentage fall in FEV_1 of 26.2±4.7% occurred at 5 min postchallenge and did not differ significantly from placebo at any time-point measured. However, with terfenadine pretreatment, the maximum mean percentage fall in FEV_1 of 10.6±2.9% occurred later, at 15 min postchallenge, and the mean percentage fall was significantly reduced at 3 and 10 min at the 1% level of significance, and at all time-points up to and including 20 min at the 5% level of significance.

Phase 2

The mean baseline FEV_1 prior to histamine bronchial challenge was 3.68±0.26 *l* following placebo and 3.71±0.29 *l* following flurbiprofen (NS). Following placebo pretreatment, the geometric mean (±geometric SEM) PC_{20} histamine was 1.19 (0.88–1.61) $mg \cdot ml^{-1}$ (table 5) and was not altered significantly by pretreatment with flurbiprofen (PC_{20} 1.03 (0.69–1.53) $mg \cdot ml^{-1}$.

Table 5. – PC_{20} histamine after treatment with placebo or flurbiprofen 150 mg

Subject no.	PC_{20} histamine $mg \cdot ml^{-1}$ Placebo	flurbiprofen
1	0.52	0.65
2	0.77	0.38
3	6.64	1.78
4	0.72	0.65
5	0.63	0.76
6	0.60	0.12
7	1.80	12.09
8	0.43	0.42
9	4.30	2.56
10	4.50	5.76
11	0.65	0.68
Geometric mean (±SEM)	1.19 (0.88–1.61)	1.03 (0.69–1.53)

PC_{20}: concentration of histamine provoking a 20% fall in forced expiratory volume in one second.

Discussion

This study has demonstrated that blockade of histamine H_1-receptors by terfenadine in asthmatic subjects significantly inhibits the bronchoconstrictor response to isocapnic hyperventilation challenge at room temperature, thus implicating the release of histamine in the airway response to this stimulus. In this dose used, flurbiprofen had no significant effect on airway responsiveness to histamine and also had no effect on the bronchoconstrictor response to ISH. We interpret this as indicating that prostanoid generation has little role to play in the observed airway response to ISH.

Exercise-induced asthma (EIA) is believed to be induced principally by the physical stimulus of conditioning a large volume of air over a short space of time, which can be shown to lead to a loss of water from the airways [17]. This is believed to induce transient hypertonicity of the airway lining fluid, which in turn induces degranulation of airway mast cells [4, 18]. The observation that ISH can mimic the bronchoconstrictor response to exercise [2, 3] has provided a useful tool to investigate mechanisms. In the present study the ISH stimulus applied to the airways on each of the study days was quantified in terms of calculated respiratory heat and water loss. Since these indices are considered to be the principal physical stimuli inducing bronchoconstriction following ISH and exercise challenge [1, 4], the very small variation observed in these measures over the three drug study days provides evidence for the repeatability of the ISH challenge. Our mean value for water loss of respired air of 30 $mg \cdot l^{-1}$ (calculated from table 3) is closely similar to the value of 28.9 $mg \cdot l^{-1}$ found by SMITH and ANDERSON [4], although our mean calculated expiratory temperature of 29.5°C is slightly less than their mean figure of 31.2°C [4], and probably represents an underestimate. However, our average figure for the temperature of expired air does not take into account the size of the breath measured. Our technique of measuring RHE breath-by-breath means that a small volume of rapidly expired breath, where perhaps the thermocouple's response time did not permit accurate measurement of the maximum expired temperature, would have less weight in the final estimated RHE than a larger volume breath, where a more accurate measurement of expired air temperature would be expected. Further evidence on repeatability of the challenge is provided by comparing the airway response achieved with the same ISH challenge on the final prestudy practice and the placebo day. This showed a coefficient of variation of 22.6% which is almost identical to the coefficient of variation of 21% in maximum percentage fall in peak expiratory flow previously shown for exercise testing in asthma repeated at one week intervals [16].

Evidence cited to support a role for mast cell products in EIA is the finding of elevated plasma levels of histamine [5] and neutrophilchemotactic factor [6, 7] in association with EIA, and the inhibitory effect of sodium cromoglycate on both exercise- and ISH-induced bronchoconstriction [19, 20]. By stimulating the physical stimulus of EIA, ISH should induce bronchoconstriction in asthmatics by an identical mechanism. However, following ISH most investigators have failed to demonstrate any increase in concentrations of plasma histamine [5, 21, 22]. Failure to find mediators in the peripheral circulation may be due to the absence of a major haemodynamic component to ISH, which with exercise might flush mediators released in the lung into the circulation. However, even following exercise several investigators have failed to demonstrate any difference between normal and asthmatic subjects in plasma histamine concentrations [23–25].

Another approach to the problem is to antagonize the effects of histamine pharmacologically, using the specific and potent histamine H_1-receptor antagonist, terfenadine. In the dose administered in this study, terfenadine has been shown to produce a 35 fold protection of the airways against the bronchoconstrictor action of inhaled histamine [26], and is without significant anticholinergic activity in the airways [27]. The bronchodilator effect of terfenadine observed in this study has been observed previously [27]. Although it is impossible to completely discount the confounding effect of bronchodilatation on the protection observed against ISH-induced bronchoconstriction, when multiple linear regression was performed to take baseline airway calibre into account, the protective effect of terfenadine remained significant.

We and others have shown that prior administration of terfenadine inhibits the bronchoconstrictor response to exercise [28, 29]. A study by WIEBICKE *et al.* [30], looking at young asthmatics aged 13–25 yrs, showed a protective effect against EIA of terfenadine in a dose of 120 mg, but failed to show a protective effect against isocapnic hyperventilation of cold air in the same subjects. Our results with isocapnic hyperventilation at ambient temperature clearly conflict with this finding, since we have demonstrated a 43.7% inhibition of the bronchoconstrictor response using terfenadine. Although the apparent inhibitory effect of terfenadine varied between subjects (table 4), the variance of the results on the terfenadine day was similar to that observed on the placebo day. Furthermore, the variance observed with terfenadine in the present study could as easily be accounted for by the variability in the degree of histamine antagonism afforded by terfenadine [26] as by any heterogeneity in the contribution of histamine to the bronchoconstrictor response. It is likely that ambient temperature and freezing air challenges do not stimulate the airways by the same mechanism, especially since normal subjects respond to frigid air by bronchoconstricting [31]. Thus, while O'BYRNE *et al.* [32] demonstrated a small protective effect of chlorpheniramine against cold air challenge, the same study demonstrated a significant anticholinergic activity of the drug, and atropine was shown to exert a similar protective effect.

Our study confirms that ISH at ambient temperature induces bronchoconstriction, with a time-course similar to that observed with exercise, reaching a maximum approximately 10 min after challenge [33, 34], and that it is significantly inhibited by terfenadine at every time-point measured over the first 20 min, implicating histamine as an important mediator of the response. Our findings are consistent with those of BADIER *et al.* [35], who demonstrated a protective effect of 120 mg of terfenadine against hyperventilation challenge using ambient temperature air, with an increase in the mean hyperventilation stimulus required to increase specific airways resistance by 100% from 44 to 64 $l \cdot min^{-1}$. The extent of the inhibition produced by terfenadine against the airway effects of ISH (43.7±11.7%) is greater than the inhibition we have previously observed, where bronchoconstriction was induced by exercise [29], but is similar to that produced against hypertonic saline (56.4±10.2%) [36]. These data support the view that ISH and hypertonic saline challenge share a common dependence on mast cell histamine release in provoking bronchoconstriction, whereas this is less obvious with exercise.

Evidence suggesting a possible role for prostanoids in hyperventilation-induced bronchoconstriction derives from studies using high airflow as the stimulus. TOGIAS *et al.* [8] found elevated levels of prostaglandin D_2 (PGD_2) in nasal washings following cold air challenge in subjects with rhinorrhoea. In anaesthetized dogs, FREED *et al.* [9] showed an increase in peripheral airways resistance and an increase in levels of PGD_2 in lavage fluid after high flow dry air challenge. At first sight, these observations appear inconsistent with the idea that hypertonic mast cell degranulation accounts for airflow-induced bronchoconstriction, since mast cells respond to hypertonic challenge *in vitro* by releasing histamine but not prostanoids [37]. However, in asthma direct challenge of the bronchi with hypertonic saline has been shown to increase the concentrations of histamine, PGD_2, $PGF_{2\alpha}$, and thromboxane B_2 in bronchoalveolar lavage [38]. It remains true that as for histamine the demonstration of release of a potential mediator of bronchoconstriction following bronchial provocation does not permit evaluation of its importance in the ensuing bronchoconstrictor response.

To investigate the contribution of newly generated prostanoids to ISH-provoked bronchoconstriction, we used flurbiprofen, a potent cyclooxygenase inhibitor. Flurbiprofen has been shown to be 2,000 times more potent than aspirin and 10 times more potent than indomethacin as an inhibitor of guinea-pig lung microsomal, having an median inhibitory concentration (IC_{50}) of 10^{-7} [39]. When administered for three days prior to challenge, flurbiprofen has been shown to inhibit the immediate bronchoconstrictor response to allergen challenge in asthmatics [10]. However, when cyclooxygenase inhibitors are administered over several days, a reduction in bronchial sensitivity to histamine has been observed, making the specificity of the observed reduction in bronchoconstriction to subsequent allergen challenge difficult to interpret [10, 40]. Phase 2 of this study was, therefore, undertaken to assess any nonspecific effect that a single dose of 150 mg of flurbiprofen might have on bronchial reactivity in our subjects. When given in single dose prior to challenge, flurbiprofen significantly inhibits bronchoconstriction induced by inhaled adenosine 5'-monophosphate (AMP) [41] and bradykinin [42], but in a dose of 100 mg it is without effect on histamine reactivity [41]. Similarly, in the present study we have demonstrated that when used in a single dose of 150 mg, flurbiprofen has no significant effect on bronchial histamine reactivity. Thus, the failure of flurbiprofen to inhibit the bronchoconstriction induced by ISH indicates that prostanoid generation plays little or no role in the events leading to

bronchoconstriction. This is consistent with our previous work with hypertonic saline challenge in asthma, where flurbiprofen had no significant inhibitory effect on the bronchoconstrictor action of hypertonic saline when administered as a single challenge, although a small inhibitory effect on the more protracted dose-response to hypertonic saline was noted [36]. In contrast, flurbiprofen in the same dose as used in the present study causes a 31% inhibition of exercise-induced bronchoconstriction [29], supporting the view that bronchoconstriction provoked by exercise and ISH are not identical.

In conclusion, the potent inhibitory effect of terfenadine against ISH-induced asthma suggests that histamine is an important mediator of the response and is similar in this respect to asthma provoked by hypertonic saline. The absence of inhibition of ISH-induced asthma by a potent cyclooxygenase inhibitor makes a role for prostanoids in the response unlikely. In the dose used in this study, flurbiprofen significantly inhibited the bronchoconstrictor response to exercise and AMP, demonstrating its capacity to inhibit airway events. Our data support the view that activation of airway mast cells accounts for a considerable proportion of the bronchoconstrictor response to ISH and inhalation of hypertonic saline, but this mechanism is less important in EIA.

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