

Airways hyper-responsiveness to bradykinin and methacholine: effects of inhaled fluticasone

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Summary

Background Although inhaled corticosteroids are the most effective anti-inflammatory agents available for the treatment of asthma, they have, at best, only modest effects on airways responsiveness to methacholine. Thus, hyper-responsiveness to methacholine is a relatively insensitive monitor of the effectiveness of glucocorticoids in asthmatic subjects.

Objective The study aimed to determine if airways hyper-responsiveness to bradykinin provides a more sensitive index of glucocorticoid responsiveness in asthmatic subjects than does hyper-responsiveness to methacholine.

Methods A double-blind, placebo-controlled, parallel group study comparing the effects of inhaled fluticasone (220 µg twice daily) on responsiveness to the two stimuli in asthmatic subjects who had never previously received corticosteroid therapy. Drug ($n = 13$) or placebo ($n = 12$) were administered for 16 weeks. Responsiveness to bradykinin and methacholine was determined at baseline and at 4 week intervals.

Results Placebo did not alter responsiveness to either stimulus compared to baseline. Fluticasone treatment significantly reduced responsiveness to bradykinin ($P < 0.001$ by Friedman ANOVA) and methacholine ($P = 0.02$), but changes in responsiveness to bradykinin were significantly greater than those in methacholine responsiveness ($P = 0.002$). Bradykinin responsiveness was decreased at all treatment times compared to baseline, while methacholine responsiveness was not decreased until 8 weeks of therapy. When data were analyzed as changes from baseline ($\Delta\text{Log PD}_{20}$), $\Delta\text{Log PD}_{20}$ for methacholine was not different at any time-point between the two treatment groups. By contrast, $\Delta\text{Log PD}_{20}$ for bradykinin was significantly greater in patients receiving fluticasone compared to those on placebo at all but the 16-week treatment time. Ten of 13 subjects receiving fluticasone failed, on at least one post-treatment visit, to show a 20% fall in forced expiratory volume (FEV_1), even at the highest dose of bradykinin.

Conclusions Airways responsiveness to bradykinin is more profoundly, and more rapidly, reduced by inhaled glucocorticoids than is responsiveness to methacholine. Airways hyper-responsiveness to bradykinin provides a convenient and sensitive monitor of glucocorticoid responsiveness in asthma.

Keywords asthma, bradykinin, corticosteroids, hyper-responsiveness, methacholine

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Introduction

Bradykinin and lysylbradykinin are potent pro-inflammatory peptides that can be generated during inflammatory events *in vivo*. Increased levels of kinins have been detected in bronchoalveolar lavage fluids of asthmatic subjects responding to allergen challenge [1, 2], as well as from asthmatics with active disease [3]. The generation of kinins during asthmatic reactions, together with the observation that bradykinin is a potent inducer of bronchoconstriction, cough and retrosternal discomfort when inhaled by asthmatic subjects [4, 5], has led to the suggestion that kinins may contribute to the pathogenesis of

asthma [6, 7]. Further support for this concept was provided by the demonstration that 4-week administration of an inhaled bradykinin antagonist significantly improved pulmonary function in moderately severe asthmatics [8].

In contrast to methacholine, bradykinin is, at best, a very weak spasmogen of isolated human airway smooth muscle [5, 9–11], suggesting that bronchoconstriction *in vivo* occurs via indirect mechanisms. The ability of ipratropium bromide to inhibit bradykinin-induced airflow limitation in asthmatic subjects indicates a role for cholinergic reflexes [5]. The ability of bradykinin to induce reflex-mediated bronchoconstriction, however, seems to be restricted to inflamed airways. Bradykinin does not induce bronchoconstriction in normal subjects. Moreover, airway responsiveness of asthmatic subjects to bradykinin correlates with the degree of airway eosinophilic inflammation [12, 13], and enhancement of airway inflammation by allergen provocation markedly increases airway responsiveness to

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bradykinin [14]. Subjects with active allergic inflammation also show nasal hyper-reactivity to bradykinin [15, 16]. This hyper-reactivity is also mediated via stimulation of neuronal reflexes that are not observed in normal, non-inflamed airways [15].

Inhaled corticosteroids are considered to be among the most effective anti-inflammatory agents for the treatment of asthma. Numerous studies have demonstrated that they alleviate symptoms and reduce many aspects of the airway inflammation that is characteristic of this disease [17–20]. Despite these potent effects on symptoms and inflammation, however, inhaled corticosteroids usually have, at best, modest effects on airways responsiveness to methacholine [18, 19, 21–23]. Moreover, several studies have observed that airways responsiveness to methacholine is not related to inflammatory status in asthmatic subjects [24–27].

We hypothesized that, if hyper-responsiveness to bradykinin is, indeed, more dependent on the inflammatory status of the airways than methacholine responsiveness, then responsiveness to bradykinin may provide a more sensitive monitor of the effectiveness of anti-inflammatory medications. To test this hypothesis, we performed a 16-week, double-blind, placebo-controlled, parallel group study comparing the effects of inhaled fluticasone (220 µg twice daily) on airways responsiveness to the two stimuli in asthmatic subjects who had never previously received corticosteroid therapy. Our data clearly demonstrate that inhaled corticosteroids inhibit airways responsiveness to bradykinin more rapidly and effectively than responsiveness to methacholine, and support the concept that bradykinin is a more sensitive stimulus with which to monitor the effectiveness of anti-inflammatory medications in asthmatic subjects.

Methods

Subjects

Twenty-five otherwise healthy subjects with mild asthma were enrolled in the study. To qualify for the study, all subjects had to have a baseline forced expiratory volume (FEV₁) of > 60% of the predicted value [28]. In addition, the cumulative provocative dose of methacholine or bradykinin, calculated in breath units (1 breath unit = 1 breath of a 1-mg/mL solution) and log transformed (Log PD₂₀) required to reduce FEV₁ by more than 20% compared to diluent control had to be ≤ 1.74 and ≤ 1.35 log units, respectively. These translate to provocative concentrations (PC₂₀) of ≤ 7.5 mg/mL and ≤ 3 mg/mL, respectively. Forty-seven subjects were screened; 25 satisfied the criteria. All subjects denied symptoms of an upper respiratory infection during the 4 weeks prior to enrolment. No medications other than intermittent inhaled β-adrenergic agonists were used by the subjects and these were withheld for at least 8 h before each study visit. The study was approved by the Institutional Review Board of the Johns Hopkins Bayview Medical Center and all 47 subjects gave written informed consent.

Study design and protocol

The study used a randomized double-blind, placebo-controlled, parallel group design. Thirteen subjects received inhaled fluticasone (220 µg twice daily) and 12 received placebo for 16 weeks. Pulmonary function and airways responsiveness to

methacholine and bradykinin were measured at baseline and at 4-week intervals throughout the study using previously published methods [14]. At each visit, baseline FEV₁ was assessed and the highest of three consecutive measurements was recorded. After this, 1 mL of sterile phosphate-buffered saline was placed in a DeVilbiss Model 646 nebulizer (DeVilbiss, Somerset, PA, USA). Subjects then activated a Rosenthal–French model D-2 A dosimeter connected to compressed air at a pressure of 20 lb/in², thereby allowing the dose to be synchronized with the subject's inspiration and delivered over 0.6 s. Subjects were instructed to take five slow breaths from functional residual capacity to total lung capacity, holding each breath for 3 s. After a 3-min period, pulmonary function testing was repeated, with the highest FEV₁ value serving as the baseline for the upcoming provocation. Increasing doses of methacholine or bradykinin were given until FEV₁ values were reduced from baseline by 20% or until the maximum dose of agonist was reached. The maximum dose of agonists administered were 2.26 Log units for methacholine (equivalent to a concentration of 25 mg/mL); and 1.86 Log units for bradykinin (10 mg/mL). Subjects were permitted to leave the laboratory when FEV₁ returned to within 10% of the initial value. On each occasion, responsiveness to methacholine was measured first, followed 24 h later by measurement of reactivity to bradykinin. For each agonist, the Log PD₂₀ was calculated by interpolation of the dose–response curve.

Data analysis

For data analysis, airways responsiveness was expressed as Log PD₂₀. Changes from baseline were expressed as ΔLog PD₂₀. For statistical purposes, in the event that a subject did not show a 20% fall in FEV₁ at the highest concentration of agonist used, a Log PD₂₀ value was calculated in a standard manner designed to provide the most conservative assessment of the change in airways responsiveness. Thus, if FEV₁ had fallen by less than 10% at the top dose given, a fall of 20% FEV₁ was assigned for the next hypothetical dose of methacholine (2.74 Log units; equivalent to a PC₂₀ of 75 mg/mL) or bradykinin (2.35 Log units; equivalent to a PC₂₀ 30 mg/mL). If FEV₁ had already fallen by more than 10% at the top dose of agonist then a fall of 20% FEV₁ was assigned to that top dose (2.26 Log units or a PC₂₀ 25 mg/mL and 1.86 Log units or a PC₂₀ of 10 mg/mL, respectively).

Given that, as described above, values of Log PD₂₀ had to be assigned in cases where a 20% fall in FEV₁ was not achieved, data were not normally distributed. Therefore, non-parametric statistical methods were used to compare all responsiveness data. For initial assessments of changes with time or treatment, Friedman's two-way ANOVA was used. Subsequent *post-hoc* analysis used Wilcoxon matched-pairs signed ranks test for paired comparisons and Mann–Whitney *U*-tests for non-paired analysis. Spearman rank correlation analysis was used to examine the relationship between maximal fluticasone-induced changes in airways responsiveness to bradykinin and methacholine, as measured by ΔLog PD₂₀. Values of FEV₁ were normally distributed, permitting the use of parametric statistics. The effects of inhaled fluticasone and placebo on FEV₁ were compared therefore by two-way ANOVA, with time and treatment as dependent variables. For all statistical analyses, significance was assumed for values of *P* < 0.05.

Results

Demographic information, as well as baseline pulmonary function and airways responsiveness data for all participants are shown in Table 1. The two groups of subjects receiving fluticasone or placebo were of comparable mean age and were not significantly different in terms of measures of baseline pulmonary function. In addition, at baseline, the two groups did not differ in terms of airways responsiveness to either bradykinin ($P = 0.48$ by Mann–Whitney U -test) or methacholine ($P = 0.83$).

Patients receiving inhaled fluticasone showed an improvement in FEV₁, while placebo treatment was without effect. Mean changes from baseline at 4, 8, 12 and 16 weeks of treatment were 0.11, 0.10, 0.20 and 0.19 L on fluticasone, and 0, −0.06, −0.01 and −0.01 L on placebo. Lung function was significantly increased ($P = 0.01$ by two-way ANOVA) with fluticasone compared to placebo treatment.

To assess the effects of placebo and active treatment on airways responsiveness to methacholine and bradykinin, we first performed Friedman's two-way ANOVA on the baseline and all four time-points at which evaluation of responsiveness was performed (4, 8, 12 and 16 weeks) using pooled data from both treatments (placebo, active). For both methacholine and bradykinin, these analyses rejected the null hypotheses that the repeated measures data were derived from the same sample (for methacholine, $P = 0.006$; for bradykinin, $P = 0.0004$).

Next we separately used the same test on the placebo and active treatment groups. For methacholine (Fig. 1), only the active treatment group showed a significant difference among the five time-points (fluticasone, $P = 0.02$; placebo, $P = 0.34$). *Post-hoc* analysis showed that methacholine responsiveness did not differ from baseline after 4 weeks of treatment ($P = 0.22$), but was significantly different from baseline at weeks 8, 12 and 16 ($P = 0.02$, $P = 0.006$ and $P = 0.009$, respectively). The maximum change observed in mean Log PD₂₀ was 0.5 Log units (between baseline and week 12).

For bradykinin (Fig. 2), analysis of both the active and the placebo group yielded results indicating differences among the five time-points (placebo, $P = 0.02$; fluticasone, $P = 0.0004$). *Post-hoc* analyses were performed on both datasets. In the placebo group, when data from weeks 4, 8, 12 and 16 were compared to baseline, no significant differences were detected ($P = 0.75$, $P = 0.67$, $P = 0.08$, and $P = 0.29$, respectively).

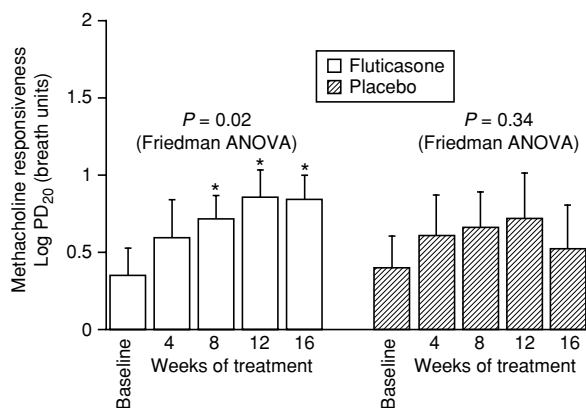


Fig. 1. Methacholine responsiveness (Log PD₂₀) at baseline and at 4-week intervals during treatment with fluticasone propionate (220 µg twice daily) or placebo. Asterisks indicate significant differences from respective baseline.

The only significant difference obtained was in the comparison between data from weeks 4 and 12 ($P = 0.002$). By contrast, in the fluticasone treatment group, airways responsiveness at all four treatment times were significantly different from baseline ($P = -0.006$, $P = 0.002$, $P = 0.002$ and $P = 0.03$ for weeks 4, 8,

Table 1. Subject characteristics

Subject no.	Gender	Age	FEV ₁ (L)	% predicted	Log PD ₂₀ bradykinin (breath units)	Log PD ₂₀ methacholine (breath units)
Fluticasone						
1	Male	30	3.6	93	1.18	0.04
2	Male	28	2.6	62	0.46	0.47
3	Female	45	2.6	91	0.34	−0.34
4	Female	40	2.3	81	0.86	−0.06
5	Female	56	2.0	76	−0.17	1.65
6	Female	23	3.1	94	1.07	1.25
7	Female	24	2.8	93	−1.45	−0.38
8	Female	40	2.2	68	1.03	0.74
9	Female	59	2.0	87	0.35	0.21
10	Female	27	2.8	101	0.10	0.24
11	Male	23	3.7	82	1.15	0.62
12	Male	52	2.5	68	−1.22	−0.03
13	Female	25	2.1	73	−0.30	0.46
Mean		36.3	2.6	82	0.3	0.4
SD		13.2	0.6	12	0.9	0.6
Placebo						
1	Male	35	3.4	77	0.44	0.21
2	Female	26	3.0	88	1.08	1.67
3	Female	41	2.4	91	1.01	0.42
4	Female	48	2.8	81	1.25	1.38
5	Female	45	2.0	73	0.35	0.20
6	Female	18	3.7	104	−0.88	−0.12
7	Female	28	3.6	103	0.47	−0.15
8	Female	18	2.7	72	0.62	−0.37
9	Female	27	3.3	92	1.06	1.19
10	Female	30	2.9	89	1.17	0.74
11	Male	23	3.8	82	0.10	−0.13
12	Female	34	2.2	75	−2.16	−0.40
Mean		31.1	3.0	86	0.4	0.4
SD		9.8	0.6	11	1.0	0.7

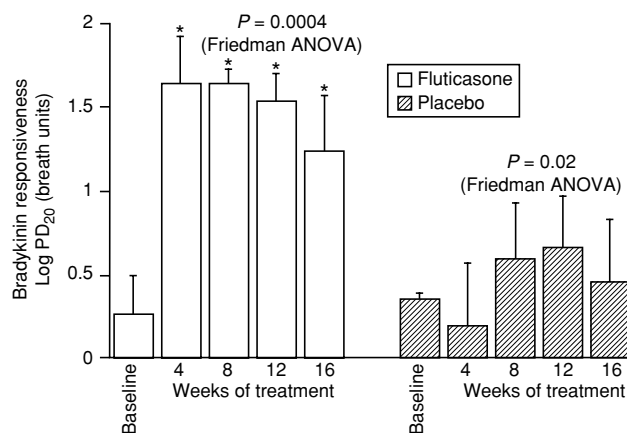


Fig. 2. Bradykinin responsiveness (Log PD₂₀) at baseline and at 4-week intervals during treatment with fluticasone propionate (220 µg twice daily) or placebo. Asterisks indicate significant differences from respective baseline.

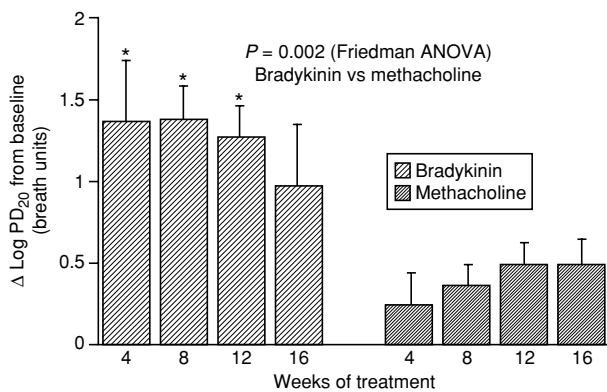


Fig. 3. Comparison of changes from baseline in responsiveness ($\Delta\text{Log PD}_{20}$) to bradykinin and methacholine observed at 4-week intervals during treatment with fluticasone propionate ($220\text{ }\mu\text{g}$ twice daily). Asterisks indicate significant differences between effects of fluticasone on bradykinin and methacholine responsiveness.

12 and 16, respectively). By contrast to the modest effects of fluticasone on methacholine responsiveness, mean Log PD_{20} to bradykinin increased by an apparent 1.3–1.5 Log units (approximately five doubling-doses) at various post-treatment times. Given, however, that 10 of 13 subjects failed, on at least one post-treatment visit, to show a 20% fall in FEV_1 at the highest dose of bradykinin administered, our conservative approach to assigning a Log PD_{20} value to these subjects means that the changes in bradykinin responsiveness noted above are clearly underestimates of the actual effect. Indeed, it is of interest to note that, of the four subjects with the greatest baseline responsiveness to bradykinin in the fluticasone group, treatment reduced the Log PD_{20} by over 3 Log units in three subjects, and by over 2 Log units in the fourth. Moreover, 11 of the 13 subjects showed a more than 1 Log reduction in bradykinin PD_{20} at some time-point during treatment. By contrast, only two subjects had responsiveness to methacholine that was reduced by more than 1 Log at any time during treatment (max 1.35 Log units).

Data for airways responsiveness to methacholine and bradykinin also were expressed as change from baseline ($\Delta\text{Log PD}_{20}$) and, for each treatment time-point, a Mann–Whitney *U*-test was used to compare effects of placebo and fluticasone. For methacholine, no differences between fluticasone and placebo were detected, at any time-point ($P=0.48$, $P=0.23$, $P=0.23$ and $P=0.18$ for the 4, 8, 12, and 16-week time-points, respectively). For bradykinin, statistically significant differences were obtained in all but the 16-week time-point ($P=0.002$, $P=0.002$, $P=0.002$ and $P=0.09$, for the 4, 8, 12 and 16-week time-points, respectively).

When data from all four treatment time-points (4, 8, 12, and 16 weeks) were used to compare the fluticasone-induced changes in airways responsiveness to bradykinin and methacholine using Friedman's two-way ANOVA, a significant ($P=0.002$) difference was detected (Fig. 3). *Post-hoc* analysis was performed with the Wilcoxon test. At three of the four treatment time-points, the effect of fluticasone on the bradykinin Log PD_{20} was statistically greater than its effect on the methacholine Log PD_{20} ($P=0.002$, $P=0.005$, $P=0.004$ and $P=0.16$, for weeks 4, 8, 12 and 16, respectively).

Finally, to assess the relationship between the fluticasone-induced changes in airways responsiveness to methacholine

and to bradykinin ($\Delta\text{Log PD}_{20}$), Spearman rank correlation coefficients were calculated. The results at 4, 8, 12 and 16 weeks of treatment, respectively, are as follows: $r_s=0.58$, $P=0.04$; $r_s=0.18$, $P=0.55$; $r_s=0.43$, $P=0.14$; $r_s=0.47$, $P=0.10$. Moreover, when the maximum fluticasone-induced improvements in airways responsiveness to methacholine and bradykinin were compared, the r_s was -0.005 ($P=0.98$).

Discussion

Because the underlying inflammatory nature of asthma is now well recognized, guidelines for the management of this disease now emphasize the aggressive use of anti-inflammatory medications in the treatment of all persistent asthma, from mild to severe [29]. Inhaled corticosteroids are among the most effective anti-inflammatory medications currently available for this purpose [30]. Fluticasone propionate is among the most potent anti-inflammatory glucocorticoids currently available [31]. The dose of fluticasone used in our current study ($220\text{ }\mu\text{g}$ twice daily) was selected to be in the moderate range of those used clinically, to provide adequate anti-inflammatory efficacy without any marked systemic absorption. Although we did not monitor any specific inflammatory parameters in our current study, it has been demonstrated previously that 6-week treatment of asthmatic subjects with fluticasone in this dose range reduces several inflammatory parameters, including numbers of eosinophils and mast cells in bronchial biopsies, as well as adhesion molecule expression [19].

In the current study, subjects receiving fluticasone showed an improvement in FEV_1 , confirming the efficacy of this dose of drug. Moreover, the changes observed in methacholine responsiveness in our present study (0.3–0.5 Log) are of a similar magnitude to those observed by Olivieri and co-workers [19]. The results of the current study clearly demonstrate, however, that airways responsiveness of asthmatic subjects to bradykinin is profoundly inhibited by this dose of inhaled fluticasone compared with the effects on methacholine. Moreover, inhibition of hyper-responsiveness to bradykinin is observed sooner in the treatment regimen. Although it is feasible that this is simply an issue of sensitivity and that a comparable magnitude of effect on methacholine responsiveness may eventually be observed at higher doses of fluticasone, this seems unlikely, for two reasons. First, in a prior study in which asthmatics were treated with $2000\text{ }\mu\text{g}$ daily of fluticasone propionate for 3 months, the reported reduction in methacholine responsiveness (1.8 doubling doses) was comparable to that observed in our current study [32]. Second, we found no significant correlation between the maximal shifts in methacholine and bradykinin responsiveness, implying that the underlying basis of hyper-responsiveness to these two compounds may differ.

Our results differ somewhat from those of two earlier studies examining the effects of inhaled corticosteroids on hyper-responsiveness to bradykinin. A study using $400\text{ }\mu\text{g}$ daily of beclomethasone in children failed to find any effect of corticosteroids on PD_{20} to either methacholine or bradykinin [33]. These children were recruited, however, only on the basis of recurrent wheezing and the majority of subjects showed no hyper-responsiveness to either bradykinin (73% non-responsive) or methacholine (58% non-responsive) at study entry. Thus, it would be difficult to show an effect of

beclomethasone in these subjects. In the other study, 3-week treatment of adult asthmatics with budesonide (1.2 mg daily) did reduce hyper-responsiveness to bradykinin but only to a comparable degree to that observed for methacholine [34]. This may reflect the shorter duration of glucocorticoid treatment, or may be due to technical differences. Fuller and colleagues assessed hyper-responsiveness by measurement of specific airways conductance, rather than FEV₁. Data also may have been affected by the fact that bradykinin was prepared in solution containing ethanol, and that responsiveness to bradykinin was assessed immediately after methacholine responsiveness testing, rather than 1 day later, as in the current study [34].

Airways hyper-responsiveness to a wide variety of stimuli is a characteristic feature of asthma, but the mechanisms underlying this phenomenon are still not fully understood. Methacholine and histamine, the most commonly used stimuli for monitoring hyper-responsiveness, are both direct smooth muscle spasmogens. Although several studies have found a relationship between the degree of airway inflammation and hyper-responsiveness to methacholine or histamine in asthmatic subjects [35–37], others have failed to find such a relationship [24–27, 38, 39]. Moreover, exacerbation of inflammation by allergen provocation [14, 40, 41], or reduction of inflammation using corticosteroids [18, 19, 21–23], tend to have, at best, modest effects on hyper-responsiveness to these agents. Thus, hyper-responsiveness to the direct spasmogens, methacholine and histamine, seems to be a relatively stable index that may reflect some long-term underlying structural or functional change in the airways.

In contrast to methacholine and histamine, there are a variety of stimuli that are believed to cause airflow limitation by indirect mechanisms. These include not only physical stimuli (e.g. exercise, cold, dry air or non-isotonic aerosols), but also a range of chemical agonists [e.g. adenosine 5' monophosphate (AMP), prostaglandins, sodium metabisulfite]. The mechanisms by which many of these chemical stimuli cause airflow limitation are not established, but may vary with the stimulus. Mast cell activation has been implicated in the actions of AMP [42]. By contrast, bradykinin does not activate human mast cells [43], and its effects on airflow limitation are not blocked by administration of antihistamines or cyclooxygenase inhibitors [5, 44]. Rather, bradykinin probably functions via stimulation of neural reflexes. Given that airways hyper-responsiveness to one bronchoconstrictive agent often correlates poorly with responsiveness to another agent, there has been some debate on which stimuli may provide useful assessment of disease activity. Given that airways responsiveness to AMP correlates more closely with levels of airway eosinophilia [45], and is affected more robustly by inhaled corticosteroids [46], than airway responsiveness to methacholine, it has been suggested that provocation with AMP may provide a more discriminatory test for airway inflammation in asthma than provocation with direct spasmogens, such as methacholine and histamine [47].

We suggest that, in a similar fashion, bradykinin also provides a useful and appropriate stimulus with which to monitor the effects of anti-inflammatory medications in asthma. This peptide is generated naturally in the asthmatic airways in response to allergen provocation, and is present in increased amounts in the airways of subjects with active asthma. We have previously shown that bradykinin provocation testing in asthmatic subjects is reliable, with the reproducibility being at

least comparable to that reported for methacholine testing [14]. Moreover, in contrast to several other stimuli, bradykinin does not induce bronchoconstriction in normal subjects, even at high doses. In asthmatic subjects, airways responsiveness to bradykinin has been shown to correlate more closely with eosinophilic inflammation than does responsiveness to methacholine [12, 13], and we have previously shown that increasing inflammation by allergen provocation exacerbates responsiveness to bradykinin more than that to methacholine [14]. Our current data showing profound inhibition of bradykinin responsiveness upon treatment with fluticasone propionate, provide further strong support for the use of bradykinin as a stimulus to monitor the effectiveness of anti-inflammatory medications in asthma.

In summary, therefore, the current study clearly demonstrates that administration of inhaled fluticasone to asthmatic subjects more rapidly and profoundly inhibits airways responsiveness to bradykinin compared to that to methacholine. We suggest that bradykinin responsiveness may prove a useful index for monitoring airway responses to anti-inflammatory medications.

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