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Effects of intranasal salmeterol and fluticasone given alone and in combination in persistent allergic rhinitis

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ABSTRACT

Background: β_2 -Agonists have previously been shown to be effective inhibitors of mediator release from airway mucosal mast cells.

Objective: To evaluate the effects of intranasal salmeterol and fluticasone propionate alone and in combination on the response to nasal adenosine monophosphate (AMP) challenge to assess mast cell activation.

Methods: Twenty-three patients with persistent allergic rhinitis completed a randomized, double-blind, placebo-controlled, 4-way crossover trial. They received once daily treatment with placebo, salmeterol, 50 μ g, fluticasone propionate, 500 μ g, or fluticasone propionate and salmeterol combination, 500/50 μ g, delivered via an antistatic spacer with nasal adapter for 1 week each, with trough measurements being made 12 hours after the first and last dose. The primary outcome was the maximum percentage decrease in peak nasal inspiratory flow after nasal AMP challenge.

Results: For the primary outcome there was significant protection after single and long-term dosing with fluticasone alone and fluticasone-salmeterol combination, whereas salmeterol alone only afforded protection after the first dose. Fluticasone-salmeterol combination and fluticasone but not salmeterol conferred significant chronic dosing effects on secondary outcomes of nasal symptoms and disease-specific quality of life. There was no potentiation of the response to fluticasone by salmeterol on any outcomes when given in combination.

Conclusion: Chronic dosing with fluticasone but not salmeterol confers anti-inflammatory activity against nasal AMP challenge, but there was no potentiation of fluticasone when given in combination with salmeterol. Thus, salmeterol may not be an effective treatment for use in allergic rhinitis.

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Introduction

Nasal mucosal inflammation and associated upper airway hyperresponsiveness (AHR) are integral features of allergic rhinitis (AR). The IgE-mediated inflammation, which is central to AR and related conditions, triggers the signaling cascade, which leads to the generation of the inflammatory mediators and cytokines, which are responsible for the symptoms, nasal airway obstruction, and AHR. In particular, the recruitment and expression of mast cells, eosinophils, and basophils are considered critical to disease expression.¹ One method for assessing mast cell-mediated AHR involved the use of nasal adenosine monophosphate (AMP) challenge.

Intranasal corticosteroids form the cornerstone of anti-inflammatory therapy in AR, with antihistamines being used as add-on

therapy in persistent cases.² Both intranasal corticosteroids and antihistamines have been shown to attenuate nasal AHR in response to AMP challenge in persistent AR, although not in an additive fashion.^{3–7} β_2 -Adrenoreceptors, although traditionally associated with bronchial smooth muscle, have been identified on several inflammatory cells, including eosinophils, neutrophils, lymphocytes, macrophages, and mast cells.⁸ β_2 -Adrenoceptor agonists have been shown to be effective inhibitors of mediator release from mast cells in asthma.^{9–14} Russo et al¹⁵ showed that intranasal salbutamol is effective in attenuating AMP-induced nasal response in patients with AR. This effect is presumably due to the stimulation of β_2 -adrenoreceptors present on nasal mucosal mast cells, thus inhibiting degranulation. However, the effects of the long-acting β_2 -agonist (LABA) salmeterol on nasal AMP challenge have not previously been reported, especially when given in combination with the corticosteroid fluticasone propionate. In one study a single dose of intranasal salmeterol produced protection against nasal allergen-induced responses but did not confer any synergistic effects to fluticasone.¹⁶ Regular exposure to LABAs in asthmatic patients results in adaptive β_2 -adrenoceptor downregulation and associated subsensitivity of response, even when given in conjunction with an

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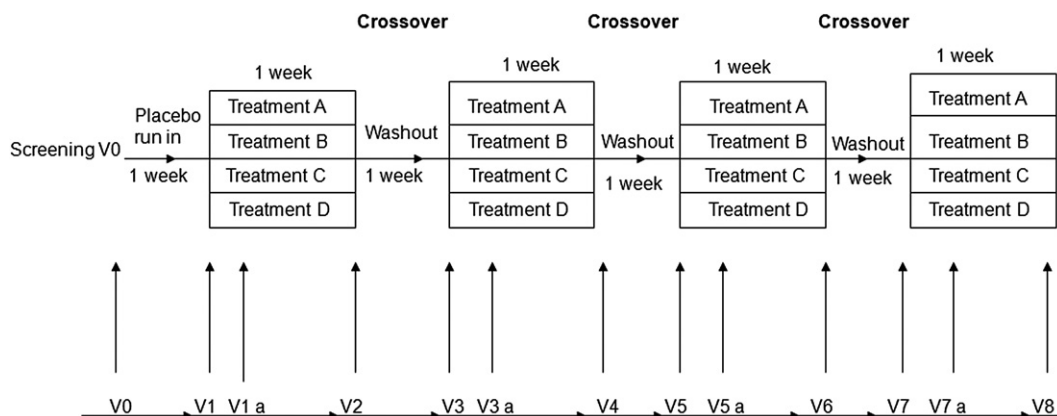


Fig. 1. Study flowchart. Nasal adenosine monophosphate challenge was performed at all visits. Visits 1a, 3a, 5a, and 7a all took place 12 hours after the first dose of randomized treatments, whereas visits 2, 4, 6, and 8 took place 12 hours after the last dose after 1 week. Randomized treatments A, B, C, and D were placebo; fluticasone, 500 µg; salmeterol, 50 µg; or fluticasone-salmeterol combination, 500/50 µg (all given once daily at nighttime). Baseline measurements were made after run-in and washout period at visits 1, 3, 5, and 7.

inhaled corticosteroid, as evidenced by loss of protection against direct or indirect AHR.¹⁷

In vitro and ex vivo data have suggested that salmeterol may exhibit ligand-independent activation of glucocorticoid receptors, which might result in potentiation of fluticasone by salmeterol when given in combination. Although no such evidence of synergy was reported with fluticasone-salmeterol combination in the nose with allergen challenge,¹⁶ no data are available on AMP challenge in the nose. The purpose of the present study was to compare for the first time, to our knowledge, the single and long-term dosing effects of intranasal fluticasone alone, salmeterol alone, and fluticasone-salmeterol combination on nasal AMP challenge in persistent AR.

Methods

Study participants

This study was approved by Tayside Committee on Medical Research Ethics (EudraCT 2005-005523-34). We included nonasthmatic patients between 18 and 65 years of age with at least 1 positive skin prick test result and a history of persistent AR to perennial or seasonal allergens.² Patients who were sensitized to seasonal allergens were studied while symptomatic in season. At baseline they were required to demonstrate a 20% or greater decrease in peak nasal inspiratory flow (PNIF) after nasal AMP challenge. All patients were required to show a willingness and ability to comply with the protocol, and all participants gave written informed consent.

Study design

A randomized, double-blind, placebo-controlled, 4-way crossover design (Fig 1) was used to compare the effects of intranasal salmeterol, intranasal fluticasone, and intranasal fluticasone-salmeterol combination. Participants discontinued use of their nasal corticosteroid, leukotriene receptor antagonist, and antihistamine 1 week before starting their randomized treatment during the run-in phase and washout periods. Intranasal sodium cromoglycate spray 4% (Rhinocrom Aqueous; Sanofi-Aventis, Surrey, United Kingdom) was provided for rescue use throughout the study but was withdrawn for at least 24 hours (ie, 4 half-lives) before challenge. Nasal AMP challenges were performed at baseline, after run-in and each washout, and 12 hours after the first and last evening dose of the study medication. A Zerostat Antistatic 280-mL spacer (Cipla, Mumbai, India) was used¹⁸ to administer the randomized treatments having been adapted with a nasal olive for intranasal use instead of the conventional mouthpiece (Fig 2).

The randomized treatments were all given once daily at night-time as follows: placebo hydrofluoroalkane, 2 puffs (Cipla, Mumbai, India); salmeterol-hydrofluoroalkane, 2 puffs of 25 µg (Serevent Evohaler; Allen and Hanburys, Middlesex, United Kingdom); fluticasone-salmeterol hydrofluoroalkane, 2 puffs of 250/25 µg (Seretide Evohaler; Allen and Hanburys); and fluticasone-hydrofluoroalkane, 2 puffs of 250 µg (Flixotide Evohaler; Allen and Hanburys). The randomization process was double-blind and computer generated from www.randomization.com. Trial drug randomization was performed under the direct supervision of the clinical trial pharmacist at Ninewells University Hospital. Active fluticasone, salmeterol, and fluticasone-salmeterol combination were procured from GlaxoSmithKline (Allen and Hanburys) by the clinical trial pharmacy and matched placebo was provided by Cipla. Patients were provided randomized, labeled treatment packs that would contain a single inhaler, which could be either active or placebo at each study treatment visit. Blinding of patients and investigators was ensured by using identical colored actuators for each treatment and labeling of active or placebo canisters with trial-specific labels; thus, all the inhalers appeared identical in each treatment pack. Measurements were all made at 12 hours after the first and last dose of each randomized treatment.

Measurements

At initial screening, nasal endoscopy was performed using a rigid, 2.7-mm, 30° Hopkins rod lens telescope to exclude significant nasal polyp disease (greater than grade 1), concha bullosa, and septal deviation (>50%). A Niox Flex nitric oxide analyzer (Aerocrine AB, Solna, Sweden) was used to measure nasal nitric oxide at a flow rate of 50 mL/s as previously described. The standard aspi-



Fig. 2. Zerostat polycarbonate antistatic spacer adapted with nasal olive for intranasal delivery.

Table 1
PNIF in response to nasal adenosine monophosphate challenge

Variable	Mean (95% CI)		
	Δ Fluticasone propionate	Δ Fluticasone-salmeterol combination	Δ Salmeterol
Change in maximum percentage decrease in PNIF from pooled placebo (first dose)	8.7 (2.0-15.4) ($P < .05$)	8.6 (2.2-14.9) ($P < .05$)	6.6 (0.7-12.5) ($P < .05$)
Change in maximum percentage decrease in PNIF from pooled placebo (1 week)	10.2 (3.0-17.5) ($P < .01$)	9.4 (4.1-14.7) ($P < .01$)	-1.5 (-8.4 to 5.4) ($P = .91$)

Abbreviations: CI, confidence interval; NS, nonsignificant; PNIF, peak nasal inspiratory flow.

ration technique recommended by the American Thoracic Society/European Respiratory Society guidelines was used with a unilateral, nasal olive, breath-holding, and velum closure at a flow rate of 50 mL/s.¹⁹

For nasal AMP challenge, PNIF was measured before and every 5 minutes for 60 minutes after 4 squirts of 0.1 mL per actuation in each nostril of 400 mg/mL of AMP (ie, 320-mg total dose) was delivered to the nose. The maximum decrease in PNIF was measured as previously described²⁰⁻²² using an In-check Nasal Inspiratory Flow Meter (Clement Clarke International Ltd, Harlow, United Kingdom). In this regard it has been shown that measuring PNIF response is more sensitive than measuring nasal airway resistance and is also more reproducible.²¹⁻²³ Moreover, PNIF correlates well vs symptoms in response to treatment in AR patients and has been shown to have a minimal clinically important difference of 5 L/min.²¹⁻²⁴ At screening, patients were instructed in the correct technique with particular attention to horizontal positioning of the meter, a closed mouth with maximal peak nasal inspiratory effort, and an adequate mask seal around the mouth and nose. Nasal impulse oscillometry measurements for nasal airway resistance at 5 Hz were performed using a Jaeger MasterScreen impulse oscillometry system (Erich Jaeger, Hoechst, Germany). The total nasal symptom 4 (TNS4) scores were recorded at all visits, with a total score of 12 for the sum of the 4 separate domains for nasal blockage, run, itch, and sneeze, each rated from 0 to 3. The Juniper Mini Rhinoconjunctivitis Quality of Life Questionnaire (Mini-RQLQ)²⁵ was completed by the participant immediately before and a week after each randomized treatment. The global RQLQ is the mean of the 5 individual domains, with individual domains scored from 0 to 6. Serum eosinophilic cationic protein was measured using an enzyme-linked immunoassay technique (UniCAP; Sweden Diagnostics UK Ltd, Milton Keynes, United Kingdom) with an intra-assay coefficient of variation of 3.3%.

Statistical analysis

Data analysis was performed using SPSS statistical software for Windows, version 17 (SPSS Inc, Chicago, Illinois). The study was powered (>80%) on a sample size of 20 patients completing per protocol to show a 20% difference in maximum PNIF response (the primary outcome) to nasal AMP challenge for active treatments vs placebo. The coefficient of variation of maximum PNIF response with nasal AMP has been shown to be 5.1%.⁷ An overall, multifactorial, repeated-measures analysis of variance was performed, and where significant, this was followed by Bonferroni-corrected, multiple, pairwise testing with an α error of .05 (2 tailed).

Results

Study patients

There were 23 per protocol completers with 12 dropouts (male:female ratio, 1:1.3) with a mean (SEM) age of 42.1 (2.9) years, baseline PNIF of 129 (8.8) L/min, maximum decrease in PNIF in response to AMP of 38.2% (3.9%), time to a 20% or greater PNIF decrease of 10.4 (1.4) minutes, and forced expiratory volume in 1 second of 99.4% (2.7%). There were 6 patients with persistent sea-

sonal AR who were all skin prick positive to grass pollen, and 17 with persistent perennial AR who were all skin prick positive to house dust mite. There were 12 patients taking intranasal corticosteroids, 9 taking antihistamines, and 1 taking montelukast. Seven patients were required to use rescue intranasal cromoglycate during the study: in 5 cases on an occasional intermittent basis and in 2 cases on a more regular basis. In these 2 cases the last dose of rescue cromoglycate was always used at least 24 hours before AMP challenge, as per protocol.

AMP challenge

Prechallenge PNIF values were not significantly different between treatment groups, with a mean (SEM) value of 150(10) L/min for pooled placebo and pooled baseline. Data for absolute maximum percentage decrease in PNIF as change from placebo (ie, protection conferred with active treatment) are given in Table 1 and Table 2, respectively. No significant differences were seen in baseline values for each randomized treatment arm, showing a lack of any carryover effect. No significant differences were found between mean (SEM) placebo values for maximum percentage decrease at baseline, first dose, and last dose, which were 38.8% (4.0%), 36.8% (3.8%), and 34.7% (5.0%), respectively; thus, a pooled placebo value was used for comparison with active randomized treatments. The value for pooled baseline vs pooled placebo was not significantly different: 36.9% (3.8%) vs 37.7% (3.0%). Compared with placebo (Table 1), there was significant protection against AMP challenge after the first dose of fluticasone ($P < .05$), salmeterol ($P < .05$), and fluticasone-salmeterol combination ($P < .05$) but only after the last dose of fluticasone propionate ($P < .01$) and fluticasone-salmeterol combination ($P < .01$) (Fig 3). No significant difference was found in the degree of protection between active randomized treatments after the first dose, whereas fluticasone and fluticasone-salmeterol combination were significantly different from salmeterol after the last dose ($P < .05$). Moreover, a significant ($P < .05$) loss of protection was found comparing first vs last dose protection for salmeterol only.

Secondary outcomes

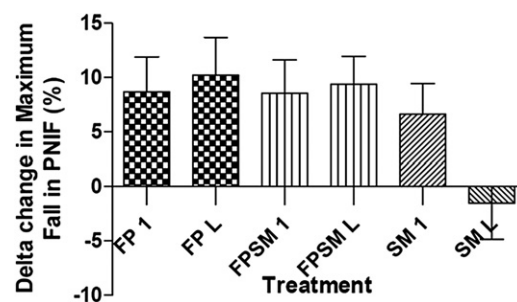
No significant difference was found in TNS4 score between pooled placebo vs pooled baseline (Table 2). In addition, no significant differences were found between placebo measurements at baseline, first dose, and last dose. The TNS4 score was significantly reduced for both first and last dose with the fluticasone-salmeterol combination (mean differences, 0.74; 95% confidence interval [CI], 0.17-1.31; $P < .05$; and 0.87; 95% CI, 0.23-1.51; $P < .05$; respectively). The TNS4 score was also significantly reduced after the last dose of fluticasone (mean difference, 0.65; 95% CI, 0.03-1.27; $P < .05$), but this did not significantly change after salmeterol. After the last dose, fluticasone-salmeterol combination was significantly superior to salmeterol (mean difference, 0.65; 95% CI, 0.03-1.27; $P < .05$).

No significant differences were observed in nasal nitric oxide, serum eosinophilic cationic protein level, or nasal airway resistance with active treatments compared with placebo (Table 2).

Table 2
PNIF response to adenosine monophosphate challenge, TNS4 score, nNO, serum ECP level, and R5^a

Variable	Pooled baseline	Pooled placebo	First dose of fluticasone	Last dose of fluticasone	First dose of fluticasone-salmeterol combination	Last dose of fluticasone-salmeterol combination	First dose of salmeterol	Last dose of salmeterol
Maximum percentage	36.9 (3.8)	37.7 (3.0)	28.2 ^b (2.6)	26.7 ^b (3.2)	28.3 ^b (3.5)	27.4 ^b (3.7)	30.3 ^b (2.7)	38.4 (4.6)
PNIF decrease								
TNS4 score	2.4 (0.4)	2.0 (0.3)	1.7 (0.3)	1.4 ^b (0.3)	1.4 ^b (0.3)	1.3 ^b (0.3)	2.0 (0.3)	1.9 (0.3)
nNO, ppb	695.8 (623.3–776.6)	699.2 (619.8–788.8)	741.8 (660.1–833.6)	703.3 (619.8–798.1)	696.2 (593.8–816.3)	623.5 (533.5–729.0)	662.9 (574.7–764.7)	675.5 (614.6–742.4)
ECP, µg/L	10.6 (7.7–14.7)	9.9 (6.8–14.6)	8.6 (6.2–12.1)	8.6 (6.2–12.1)		10.2 (7.1–14.5)		10.4 (7.5–14.4)
R5, %	337 (41)	290 (54)	243 (26)	243 (26)		251 (20)		330 (50)

Abbreviations: ECP, eosinophilic cationic protein; nNO, nasal nitric oxide; PNIF, peak nasal expiratory flow; R5, nasal airway resistance at 5 Hz; TNS4, total nasal symptom 4.

^aData are presented as mean (SEM) or mean (95% confidence interval).^bSignificant difference ($P < .05$).**Fig. 3.** Maximum percentage decrease in PNIF (as change from placebo) in response to adenosine monophosphate challenge after the first and last doses of each treatment arm (shown as means and SEM) (ie, a change from placebo in a positive direction denotes significant protection). FP 1 indicates first dose of fluticasone; FP L, last dose of fluticasone; SM 1, first dose of salmeterol; SM L, last dose of salmeterol; FPSM 1, first dose of fluticasone-salmeterol combination; and FPSM L, last dose of fluticasone-salmeterol combination.

Disease-specific Mini-RQLQ score was significantly different after 1 week of treatment with fluticasone and fluticasone-salmeterol combination for global, nasal, and practical symptoms (Table 3). The global difference after fluticasone-salmeterol combination was also clinically relevant (ie, >0.4) (mean difference, 0.52; 95% CI, 0.27–0.78; $P < .001$). No significant differences were found between active treatments.

Discussion

The results of the present study show that for the primary outcome of nasal AMP challenge, significant protection was afforded after single and chronic dosing with intranasal fluticasone and fluticasone-salmeterol combination, whereas for salmeterol protection was seen after single but not chronic dosing. The loss of protection after chronic dosing with LABAs alone is in keeping with previous data with bronchial AMP challenge in asthmatic patients, which has shown no additive protection in addition to inhaled steroids.^{26–28} This loss of protection on chronic dosing with LABAs can be explained by β_2 -adrenoceptor downregulation and associated subsensitivity on airway mucosal mast cells, as has been demonstrated on peripheral blood lymphocytes.^{29–31} In asthmatic patients there is a greater propensity for terbutaline-induced subsensitivity of response for AMP protection on mast cells than for methacholine protection on airway smooth muscle.³²

Thus, the presence of sustained AMP protection in the present study with fluticasone-salmeterol combination is presumably due to the effect of fluticasone moiety because no difference in response was found when comparing fluticasone-salmeterol combination vs fluticasone alone, indicating that there was no additive response on chronic dosing with the 2 separate moieties. However, even after single dosing we did not see any additive response in that the effect of fluticasone-salmeterol combination was less than the combined effects of fluticasone and salmeterol alone. In vitro and ex vivo data have suggested that salmeterol may potentiate the effect of fluticasone due to ligand-independent glucocorticoid receptor activation.^{33,34} To properly assess the potential for putative synergy between the fluticasone and salmeterol components when given together would have required evaluation of a lower dose of fluticasone-salmeterol combination (eg, 100/50 µg), compared with a higher dose of fluticasone propionate alone (eg, 500 µg). We may have reached a ceiling response for AMP protection at the 500-µg dose of fluticasone propionate. In other words if we had evaluated a lower dose of fluticasone propionate, 100 µg, we may have been able to show some additivity in response when adding a single dose of salmeterol, 50 µg. In this regard, we have previously demonstrated a significant dose response effect on nasal AMP challenge when comparing 2 weeks of aqueous intranasal fluticasone, 100 vs

Table 3
Jupiter mini rhinoconjunctivitis quality of life questionnaire scores^a

Variable	Pooled baseline	Pooled placebo	Last dose of fluticasone	Last dose of fluticasone-salmeterol combination	Last dose of salmeterol
Global	1.35 (0.2)	1.35 (0.2)	1.0 (0.2) ^b	0.8 (0.1) ^b	1.0 (0.2)
Activity	1.3 (0.2)	1.4 (0.2)	1.0 (0.2)	0.8 (0.1)	1.0 (0.2)
Practical	1.8 (0.2)	1.7 (0.2)	1.0 (0.2) ^b	1.0 (0.2) ^b	1.0 (0.2) ^b
Nose	1.8 (0.2)	1.7 (0.2)	1.1 (0.1) ^b	1.1 (0.2) ^b	1.3 (0.2)
Eye	0.9 (0.2)	1.1 (0.2)	0.5 (0.1)	0.6 (0.1)	0.7 (0.2)
Other	1.2 (0.3)	1.2 (0.3)	0.8 (0.3)	0.8 (0.2)	0.9 (0.3)

^aGlobal mean values for all domains are shown along with each separate domain. Data are given as mean (SEM).

^bSignificant difference ($P < .05$).

400 μ g, in AR; indeed, the lower dose did not confer any significant protection. Interestingly, there appears to be little difference when comparing our chronic dosing protection against nasal AMP challenge with 500 μ g of fluticasone propionate via spacer (10.2% protection) to the protection described by Barnes et al⁶ with 400 μ g of fluticasone propionate aqueous spray (12.9% protection). It was for this reason that we elected to use the higher 500- μ g dose of intranasal fluticasone propionate in the present study, especially because we were using a pressurized aerosol hydrofluoroalkane formulation spacer device, which would deliver finer particles than a conventional aqueous spray (ie, one might expect the intranasal deposition of larger particles on the inferior turbinates to be much lower with the spacer compared with aqueous spray). The decision to use the pressurized aerosol with a spacer was based on having to blind the study using available formulations of fluticasone-salmeterol combination and salmeterol, which are obviously not available in aqueous form.

We were somewhat surprised not to see any difference when comparing single and chronic dosing protection with intranasal fluticasone. For example, in a study looking at the sequential time course protection against bronchial AMP with inhaled fluticasone propionate, 1,500 μ g/d, there was increasing protection seen during a period of 4 weeks.³⁵ Perhaps we did not achieve maximal protection after 1 week of long-term dosing. However, we observed a similar degree of protection against nasal AMP challenge after 1 week of intranasal fluticasone compared with 4 weeks with intranasal aqueous mometasone furoate (10.2% vs 9.6% PNIF protection, respectively).⁵

Aside from the primary outcome we also demonstrated significant improvements in secondary outcomes, including nasal symptoms and quality of life on long-term dosing with fluticasone and fluticasone-salmeterol combination but not salmeterol, in keeping with the expected topical effect of intranasal corticosteroid on allergic nasal airway inflammation. Because our study was not powered on these secondary outcomes, we would not expect to be able to differentiate between the 2 treatment arms of fluticasone-salmeterol combination and fluticasone. Nonetheless, we were able to show a magnitude of response on the TNS4 and RQLQ, which exceeded the minimum clinically important differences for these outcomes.²⁴

In a previous study by Parameswaran et al,¹⁶ intranasal metered-dose aerosol formulations of placebo, fluticasone propionate, 50 μ g twice daily, salmeterol, 25 μ g twice daily, or fluticasone-salmeterol combination for 1 week each were compared on nasal allergen-induced responses. Significant effects of both fluticasone and salmeterol alone were found on eosinophils and albumin in nasal lavage fluid, suggesting that salmeterol may confer persistent anti-inflammatory activity after chronic dosing, but there was no additive response with the combination for any outcomes. However, they did not evaluate single-dose effects as we did in our study, and so it is not possible to ascertain whether subsensitivity of response on allergen challenge had occurred with the salmeterol moiety after chronic dosing, unlike in our study, where we showed

marked loss of protection between the first and last dose effect on AMP challenge.

We acknowledge our study has several limitations, especially the relatively short duration of treatment and the lack of power to determine differences on secondary outcomes of symptoms and disease-specific quality of life. At first sight our results suggest that salmeterol may not be an effective treatment for AR. However, we suggest that further studies may be indicated to look at more prolonged therapy with fluticasone-salmeterol combination in patients with more severe persistent AR, in particular using a lower dose of fluticasone.

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