

Effect of salbutamol on nasal symptoms and mast cell degranulation induced by adenosine 5' monophosphate nasal challenge

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Summary

Background In addition to its well-known functional agonism at the level of β_2 adrenergic receptors on airways smooth muscle cells, salbutamol appears to have additional protective effects, possibly through an inhibition of mast cell activation.

Objective The aim of this study is to provide the first evidence *in vivo* of inhibition of human mast cell activation by salbutamol.

Methods Nine atopic subjects received placebo and salbutamol (5 mg/mL) 15 min before an adenosine 5' monophosphate (AMP) nasal provocation in a double-blind crossover study design. The nasal lavage was collected from these subjects prior to or 3, 5, 15 or 30 min after the AMP nasal challenge, and concentrations of histamine and tryptase in the nasal lavage were measured.

Results AMP nasal provocation produced considerable sneezing and induced a transient increase in histamine and tryptase release with peak values achieved at 3 min after the challenge in all the subjects studied. Compared with placebo, salbutamol significantly attenuated the release of histamine and tryptase induced by AMP challenge ($P = 0.048$ and 0.020 , respectively). Moreover, the AMP-induced sneezing was also inhibited by pre-treatment with salbutamol ($P = 0.004$).

Conclusions Intranasal salbutamol attenuates nasal symptoms and inhibits histamine and tryptase release caused by AMP nasal provocation thus supporting the hypothesis that salbutamol may play an additional protective role in the airways by inhibiting mast cell activation.

Keywords adenosine challenge, allergic rhinitis, histamine, salbutamol, tryptase

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Introduction

Inhaled β -agonists are the most widely prescribed bronchodilator drugs for the symptomatic treatment of acute exacerbation of asthma and chronic obstructive pulmonary disease [1]. Their effect is normally mediated by functional agonism at the level of β_2 adrenergic receptors expressed on the surface of airway smooth muscles. Besides their well-known activity as bronchodilators, β -agonists appear to exert effective inhibition on mediator release from human airway mast cells [2, 3]. β_2 adrenergic receptors have also been identified on a variety of inflammatory cells including eosinophils, neutrophils, lymphocytes, macrophages, and mast cells [3, 4]. Several research groups have shown that β_2 -adrenoceptor agonists inhibit the release of histamine, prostaglandins (PGD), cysteinyl leukotrienes, and pro-inflammatory cytokines (including granulocyte macrophage colony-stimulating factor, IL-5, and TNF- α) from human mast cells following IgE stimulation [5–12]. That β_2 -adrenoceptor agonists may exert their suppressive action on

human lung mast cell degranulation was also corroborated in allergen bronchoprovocation models of clinical asthma [13, 14].

Adenosine 5' monophosphate (AMP) is a purine nucleotide, which is known to provoke bronchoconstriction when inhaled by subjects with asthma by stimulating the cell surface A2B adenosine receptors expressed on airway mast cells with subsequent release of pre-formed and newly formed mediators in allergic individuals [15]. In support of this view, we have previously shown that bronchial and nasal provocation with AMP elicits a substantial increase in histamine level in the bronchial and nasal lavage fluid (NLF) of subjects with asthma and allergic rhinitis (AR) [16–18]. Phillips et al. [19] have demonstrated that nebulized salbutamol displaced the concentration–response curves for methacholine, histamine, and AMP to the right. The difference between the results for AMP and those for histamine and methacholine were statistically significant and it was concluded that whereas salbutamol protected against methacholine and histamine induced-bronchoconstriction by functional agonism, the additional greater bronchoprotective response against AMP was clearly unrelated to functional agonism but could be ascribed to an inhibitory modulation of mast cell functions by the drug. However, direct evidence *in vivo* for the suppressive action of salbutamol on human airway mast cell degranulation is missing.

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By employing nasal provocation with adenosine as a convenient *in vivo* model of airway mast cell mediator release in allergic individuals [17, 18], we sought to demonstrate that the protective effect of salbutamol is likely to be ascribed to an inhibition in mast cell degranulation by measuring histamine and tryptase levels in the NLF of individuals with AR who were pre-treated intranasally with salbutamol.

Methods

Subjects

We studied nine atopic subjects (six males (M), three females (F); age range 18–35 years) with seasonal AR. All atopic subjects reported typical hayfever symptoms in April and May and a positive skin prick test response and specific IgE of class 2 or more to either grass pollen or *Parietaria judaica* (Pharmacia CAP System; Pharmacia Diagnostics AB, Uppsala, Sweden). None of the subjects studied was taking any form of medication at the time of the study, and none had ever received specific immunotherapy. No subject was admitted to the study within 6 weeks of a respiratory tract infection. None of the subjects studied had nasal polyposis, chronic nasal obstruction, or other serious illness. Subjects were informed about the purpose of the investigation, and all signed forms indicating their consent to participate. The study protocol was approved by the local ethics committee.

Study design

Subjects attended on two separate occasions, at least 1 week apart, to receive either 5 mg/mL (3 mL) salbutamol administered as an aerosol (Salbutamol, Valeas S.p.A., Milano, Italy) or normal saline (placebo) prior to nasal challenge with AMP (6.5 mg – administered as a single dose) in a randomized double-blind, placebo-controlled study design. The dosage of salbutamol used in this investigation and the timing of administration before challenge were chosen on the basis of previous studies that had been shown to effectively reduce the bronchospastic response to AMP in subjects with asthma [19].

Treatment solutions were freshly prepared on each visit by a technician who was blind to the study rationale and aerosolized from a nebulizer (DeVilbiss 646, Somerset, PA, USA) connected to a nasal adaptor. At each study visit, sequential pre-challenge lavages were performed bilaterally three times prior to administration of the study treatments to reduce the baseline level of histamine. Further nasal lavages were performed at 3, 5, 10, 15, and 30 min after AMP challenges to evaluate changes in histamine and tryptase levels. The numbers of sneezes were counted for up to 3 min after AMP challenge. Nasal blockage and rhinorrhoea could not be recorded in a reliable way because of the nasal lavage procedure. All visits to the laboratory were carried out at the same time of day and outside the pollen season.

Adenosine 5' monophosphate nasal challenge

Nasal challenges with AMP were performed in the morning, 15 min after nebulization of placebo or salbutamol. AMP (Sigma Chemicals, St Louis, MO, USA) was dissolved in 0.9% w/v normal saline to achieve a concentration of 50 mg/

mL, divided into small aliquots and frozen at -20°C . Saline and AMP solutions for nasal challenge were kept at room temperature for 1 h before provocation. AMP was sprayed using a hand-held pump spray calibrated to deliver $130 (\pm 9) \mu\text{L}$ per puff. The pump spray was placed in one of the nostrils, while occluding the contralateral nostril, and activated once during quiet inspiration. The procedure was then repeated in the opposite nostril. At the concentration used, this method delivered a total dose of approximately 6.5 mg AMP equally divided between the two nostrils. This dose of 6.5 mg was chosen on the basis of our previous dose-response studies with AMP in rhinitic subjects; this dose elicited nasal symptoms in all the subjects studied [17, 18].

Nasal lavage and sample processing

The lavage technique has been previously described in detail [17]. In brief, each subject was instructed to tilt his/her neck backwards, to hold his breath and to refrain from swallowing. Pre-warmed normal saline (2.5 mL/nostril) was then instilled into each nostril and, after 10 s, the subject flexed his neck and expelled the mixture of mucus and saline into a conical polypropylene tube placed on ice. Samples were immediately filtered to remove mucus and centrifuged at $400g$ for 10 min at 4°C . The supernatant was separated from the cell pellet by pipetting, concentrated in centrifugal filter units (Amicon Ultra-15, Millipore, Watford, UK), aliquoted into Eppendorf tubes and stored at -70°C for subsequent analysis.

Levels of histamine and tryptase in nasal lavage fluid

Histamine level in concentrated NLF was assayed by using a commercially available histamine ELISA kit (Immuno Biological Laboratories, Hamburg Germany), whereas tryptase was measured by a fluorescence immunoassay method (UniCap[®], Pharmacia Diagnostics AB, Uppsala, Sweden). Samples were assayed according to the instructions of the manufacturers. The lowest detectable levels for histamine and tryptase were 0.5 and 1.0 ng/mL, respectively. Salbutamol (final dose, 5 mg/mL) did not interfere with the immunoassay measurement of histamine or tryptase in the lavage fluid samples.

Analyses of data

Data shown are mean ($\pm$ SEM) values of all subjects in each group. Numbers of sneezes and the basal, peak, or area under the curve in the first 30 min ($\text{AUC}_{0-30\text{ min}}$) values of histamine and tryptase concentrations between placebo- and salbutamol-treated days were compared using paired *t*-test. A *P*-value <0.05 was considered significant. All statistical analyses were performed using the Statistical Package for Social Science (SPSS, Chicago, IL, USA) for Windows version 11.0.

Results

Sneeze count

Nasal provocation with AMP produced considerable sneezing in subjects with AR. The AMP-induced sneezing was significantly reduced by pre-treatment with salbutamol, the

Table 1. Effect of pre-treatment with salbutamol or placebo on AMP-induced sneezing in subjects with allergic rhinitis

Subject no.	Placebo	Salbutamol
1	8	3
2	4	2
3	5	2
4	3	1
5	7	5
6	2	2
7	6	4
8	3	1
9	2	2
Mean	4.4	2.4
SEM ($\pm$)	(± 0.7)	(± 0.4)

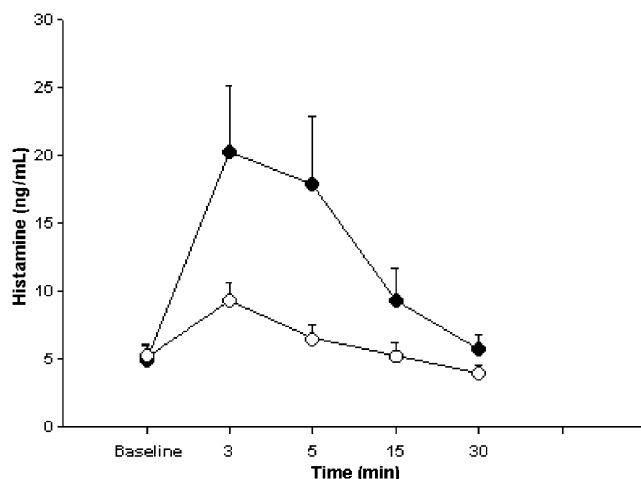
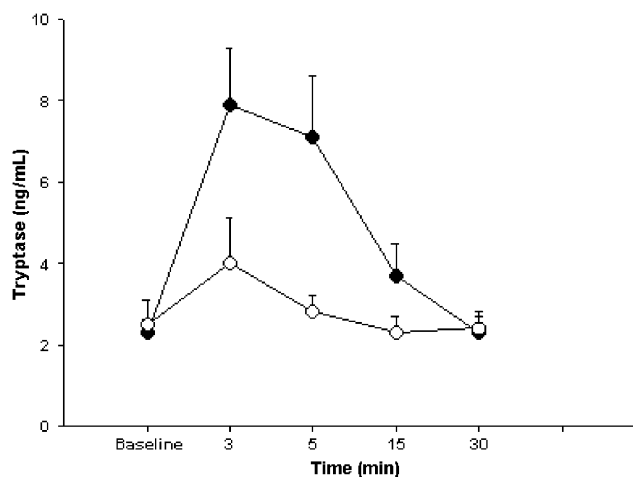
Data shown are numbers of sneezes after AMP nasal challenge.
AMP, adenosine 5' monophosphate.

mean ($\pm$ SEM) number of sneezes being 4.4 (0.7) and 2.4 (0.4) in placebo- and salbutamol-treated days, respectively ($P = 0.004$) (Table 1).

Histamine levels in nasal lavage fluid

In subjects with AR treated with placebo, nasal provocation with AMP elicited a significant rise in histamine levels with a peak response at 3 min (4.1-fold over basal, $P = 0.004$). In the same subjects, pre-treatment with salbutamol significantly reduced the peak increase in histamine levels induced by AMP challenge ($P = 0.048$) without affecting the basal histamine levels (Fig. 1). The mean ($\pm$ SEM) baseline histamine levels were 5.0 (± 1.0) and 5.1 (± 0.9) ng/mL on placebo- and salbutamol-treated days, respectively. The mean ($\pm$ SEM) peak histamine levels at 3 min after nasal challenge were 20.3 (± 4.8) and 9.3 (± 1.3) ng/mL on placebo- and salbutamol-treated days, respectively.

In addition to the evaluation of peak responses at 3 min, to assess the effect of salbutamol and placebo on AMP-induced

**Fig. 1.** Time course of histamine concentrations in nasal lavage fluid (NLF) after nasal challenge with adenosine 5' monophosphate in placebo-treated (closed symbol) or salbutamol-treated (open symbol) days in subjects with allergic rhinitis ($n = 9$). Data shown are mean $\pm$ SEM of histamine concentrations in the NLF.**Fig. 2.** Time course of tryptase concentrations in nasal lavage fluid (NLF) after nasal challenge with adenosine 5' monophosphate in placebo (closed symbol) or salbutamol-treated (open symbol) days in subjects with allergic rhinitis ($n = 9$). Data shown are mean $\pm$ SEM of tryptase concentrations in the NLF.

release of histamine and tryptase over time, the $AUC_{0-30 \text{ min}}$ was also calculated. Similar to peak responses, a significant difference in the $AUC_{0-30 \text{ min}}$ values was observed between placebo- and salbutamol-treated days ($P = 0.048$); the mean ($\pm$ SEM) AUC values decreasing from 10.8 (± 2.4) to 5.5 (± 0.6) ng/mL/min, after placebo and salbutamol, respectively.

Tryptase levels in nasal lavage fluid

Similarly, in subjects with AR treated with placebo, nasal provocation with AMP elicited a significant rise in tryptase levels with a peak response at 3 min (3.4-fold over basal, $P = 0.004$). In the same subjects, pre-treatment with salbutamol significantly reduced the peak increase in tryptase levels induced by AMP challenge ($P = 0.020$) without affecting the basal tryptase levels (Fig. 2). The mean ($\pm$ SEM) baseline tryptase levels were 2.3 (± 0.3) and 2.5 (± 0.6) ng/mL on placebo- and salbutamol-treated days, respectively. The mean ($\pm$ SEM) peak tryptase levels at 3 min after nasal challenge were 7.9 (± 1.4) and 4.0 (± 1.1) ng/mL on placebo- and salbutamol-treated days, respectively.

When the effect of AMP on tryptase release was expressed as $AUC_{0-30 \text{ min}}$ values, there was also a significant difference between placebo- and salbutamol-treated days ($P = 0.042$) with a mean ($\pm$ SEM) AUC values of 4.3 (± 0.7) and 2.6 (± 0.4) ng/mL/min, respectively.

Discussion

By employing nasal provocation with adenosine as a simple model of airway mast cell mediator release in allergic individuals, we provide for the first time direct evidence *in vivo* for the suppressive action of salbutamol on human airway mast cell degranulation. In the present study, the levels of histamine and tryptase in the NLF of individuals with AR, who were challenged with adenosine, were substantially reduced by intranasal salbutamol. We have also

shown that administration of topical salbutamol reduces nasal symptoms in almost all the allergic subjects challenged with AMP.

Although the mode of action of AMP in provoking symptoms in AR is not known with certainty, it is likely that stimulation of specific cell surface A_{2B} adenosine receptors expressed on airway mast cells will provoke subsequent degranulation [15, 20]. *In vitro*, adenosine is known to enhance mediator release from immunologically primed human airway mast cells [21, 22]. These *in vitro* data are compatible with the results of the present and previous study *in vivo* [17, 18], which clearly show that adenosine elicited a prompt increase in histamine and tryptase levels in the NLF of all the subjects studied. These changes in mediator levels were associated with considerable sneezing. In the same way, the airways of allergic asthmatics are known to respond to endobronchial instillation of AMP with bronchial narrowing, and this is coupled with a rise in histamine levels in the bronchoalveolar lavage fluid [16]. Thus, potentiation of mast cell mediator release may account for the nasal symptoms provoked by nasal challenge with AMP in AR.

In the present study, AMP-induced sneezing was attenuated when subjects received intranasal salbutamol, thus supporting the view of an inhibitory effect of the drug on mediator release from airway mast cells. To the best of our knowledge this is the first study to show a protective effect of intranasal salbutamol on nasal symptoms. This is in agreement with an early study in which allergen-induced nasal symptoms were significantly attenuated when allergic subjects received topical fenoterol [23, 24]. Recently, a placebo controlled-study has shown oral salbutamol to be effective in seasonal grass pollen-induced AR, reducing symptoms, use of antihistamines, and the increase in serum IgE that occurred during the grass-pollen season [25]. Taken together these findings indicate that topically applied salbutamol and related molecules may have beneficial effects in alleviating symptoms of AR, but larger studies are needed to confirm our observations and to define the characteristics of the patients who would benefit most from such therapeutic approach.

This ability of salbutamol to attenuate AMP-induced sneezing might be explained by the substantial reduction in the level of histamine and tryptase immediately after AMP challenge. This is in keeping with the observation that β_2 -adrenoceptor agonists inhibit the release of histamine from human lung mast cells following IgE stimulation [5–12]. Likewise, in a bronchoprovocation model of clinical asthma, the additional bronchoprotective response against adenosine by inhaled salbutamol in asthma [19] could be ascribed to an inhibitory modulation of mast cell functions by the drug. Our study demonstrates for the first time that the suppressive action of salbutamol on human airway mast cell is because of a substantial reduction in histamine and tryptase levels in the NLF of individuals with AR challenged with adenosine. In contrast to our findings, Proud et al. [26] reported that treatment with a single dose of salmeterol (0.1 mg) had no effect on activation of mast cells during the immediate response to allergen challenge, specifically salmeterol did not affect the concentrations of histamine, tryptase and PGD₂, and sneeze counts. It is likely that the apparent discrepancy between our current study and that of Proud is because of the

different doses of β_2 -agonist used: Salmeterol (0.1 mg) was used in the study by Proud whereas we administered approximately 10 mg of salbutamol from a nebulizer connected to a nasal adaptor to allow for greatest receptor occupancy by the drug.

The cellular mechanism by which salbutamol attenuates the release of histamine and tryptase induced by AMP challenge is not known with certainty. A plausible mechanism of action for salbutamol is through the activation of β_2 -adrenoceptors leading to elevation of cyclic AMP (cAMP) levels, which reduces Ca²⁺ release from intracellular stores and/or prevents Ca²⁺ influx from the extracellular compartment. It has been shown that the degree of inhibition of anti-IgE induced histamine release is well correlated with the elevation of intracellular cAMP levels after 10 min of salbutamol treatment in cultured human mast cells [27]. Several authors have reported that cAMP blocked the immunologically induced increase of Ca²⁺ in rodent mast cells [28–30]. In addition, studies in different smooth muscle types have demonstrated that stimulation of β_2 adrenoceptors may decrease inositol 1,4,5-triphosphate-induced Ca²⁺ release from the sarcoplasmic reticulum [31, 32], thus inhibiting intracellular mechanisms of cell degranulation. Thus, although further investigations on highly purified human airway mast cells are necessary before definitive conclusions can be drawn, these data suggest that the protective effect of topical salbutamol on adenosine-induced responses in AR may be ascribed to activation of β_2 -adrenoceptor leading to increases in cAMP, which then modulates intracellular levels of Ca²⁺ and results in a reduction in mast cell degranulation.

We conclude that topical salbutamol is effective against AMP-induced nasal responses in subjects with AR. Taking into account the notion of airway mast cell mediators contributing to the nasal response of AMP, the present findings support the hypothesis that intranasal salbutamol plays a protective role possibly by inhibition of mast cell activation and subsequent release of mast cell mediators. Further work is required in order to clarify the molecular mechanism by which salbutamol produces this novel anti-inflammatory activity and to validate whether this has any relevance to a possible therapeutic benefit in AR.

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