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Protective effects of inhaled ipratropium bromide on bronchoconstriction induced by adenosine and methacholine in asthma

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ABSTRACT: Although adenosine-induced bronchoconstriction is mainly due to mast cell mediator release, vagal reflexes have also been implicated in this response.

We have investigated the effect of a specific muscarinic-receptor antagonist, ipratropium bromide, on methacholine- and adenosine-induced bronchoconstriction in a randomized, placebo-controlled, double-blind study of 12 asthmatic subjects. Airway response was evaluated as forced expiratory volume in one second (FEV_1).

Inhaled ipratropium bromide (40 μg), administered 20 min prior to bronchoprovocation, increased the provocation dose of inhaled methacholine and adenosine required to reduce FEV_1 by 20% from baseline (PD_{20}) from 0.11 to 0.79 mg ($p<0.01$) and from 0.57 to 1.27 mg ($p<0.01$), respectively. The mean baseline FEV_1 values after administration of ipratropium bromide were significantly higher than after placebo administration ($p<0.05$). However, there was no correlation between the degree of bronchodilatation and dose-ratios for methacholine and adenosine.

The findings of the present study implicate vagal reflexes in the bronchospastic response induced by inhaled adenosine in asthma.

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The mechanism by which adenosine and its related nucleotide, adenosine 5'-monophosphate (AMP), cause bronchoconstriction when inhaled by atopic [1, 2] and non-atopic [1, 3] asthmatic subjects remains to be clarified. The fact that mast cell degranulation plays a primary role in initiating the bronchospastic response induced by adenosine in asthma is supported by the observation that this nucleotide or analogues active on A_2-purinoceptors enhance immunoglobulin E (IgE)-triggered mediator release from human lung tissue [4] and peripheral blood basophils [5]. PEACHELL *et al.* [6], working with human lung mast cells, have also shown an enhancing effect of adenosine on leukotriene C_4 and histamine release.

Both cromolyn sodium and nedocromil sodium, drugs with "mast cell stabilizing" properties [7], protect the airways of asthmatic subjects against bronchoconstriction provoked by inhaled adenosine and AMP [8, 9]. The observation that selective histamine H_1-receptor antagonists greatly inhibit the bronchospastic effect induced by inhaled AMP in asthmatic subjects [2, 3, 10] suggests an important role for histamine as a mediator of purine-induced bronchoconstriction in asthma. However, the incomplete protection afforded by the antihistamine drug, terfenadine, against bronchoconstriction provoked by AMP could not be improved by increasing the drug dose from 180 to 600 mg [2]. Since we [11] and other authors [12] have recently demonstrated that cyclooxygenase blockade may significantly inhibit part of the contractile response to purines in human asthma, the antihistamine-resistant reduction in forced expiratory volume in one second (FEV_1) provoked by AMP may be due to production of newly generated bronchoconstrictor mediators, such as prostaglandin D_2 and thromboxane A_2.

An alternative explanation is that adenosine may partially provoke reflex bronchoconstriction. Using rabbit bronchial smooth muscle, GUSTAFSSON *et al.* [13] have demonstrated an enhancement of the constrictor response to transmural nerve stimulation by what they considered to be an A_2-purinoceptor mediated effect of adenosine. An inhibitory effect of atropine on the adenosine-induced contractile responses has been shown in the rat [14]. In human asthma, evidence for an atropine sensitive component is controversial; MANN *et al.* [15] reporting that muscarinic blockade with ipratropium bromide failed to greatly affect the airways response to inhaled adenosine, whereas OKAYAMA *et al.* [16] reported an inhibitory effect.

The present study was designed to establish whether stimulation of vagal reflexes could account for some of the bronchoconstrictor properties of adenosine in bronchial asthma. The anticholinergic drug ipratropium bromide was used to test the effect of post-synaptic cholinergic blockade on adenosine- and methacholine-induced bronchoconstriction in a group of subjects with stable asthma.

Methods

Subjects

The study was performed on 12 asthmatic patients (7 males) with a mean (SEM) age of 29 (±3.2) yrs (table 1). All patients had a history of dyspnoea, with wheezing or chest tightness on exposure to airborne allergens, and they were atopic as defined by positive immediate skin prick tests (>2 mm wheal response) to one or more common aeroallergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, mixed grass pollen, wall pellitory pollen and cat fur). All were nonsmokers with normal chest X-rays and had no previous history of other respiratory diseases. At the beginning of the study, all patients were asymptomatic with FEV_1 values not less than 70% of their predicted normal values and none was receiving oral corticosteroids or theophylline. Sodium cromoglycate and antihistamines were discontinued at least 3 weeks prior to the study. Inhaled bronchodilators were withheld for at least 12 h prior to each visit to the department, although the patients were allowed to continue taking their inhaled corticosteroids as usual. Patients were not studied within 4 weeks of an upper respiratory tract infection or exacerbation of their asthma.

Table 1. – Characteristics of subjects studied

Subject no.	Sex	Age yrs	Height m	FEV_1 % pred	Treatment
1	M	21	1.65	81	S, B
2	F	34	1.60	105	S
3	F	43	1.59	90	S
4	F	35	1.55	79	S
5	F	53	1.60	77	S
6	M	16	1.61	101	S, SCG
7	M	29	1.70	102	S
8	M	20	1.74	80	S, B
9	M	34	1.65	81	S, B
10	M	19	1.80	84	S, SCG
11	F	27	1.55	100	S
12	M	22	1.62	110	S
Mean		29.4	1.64	90.8	
SEM		±3.2	±0.02	±3.5	

S: salbutamol, *p.r.n.*; B: beclomethasone, 3 puffs *b.i.d.*; SCG: sodium cromoglycate, 2 puffs *q.i.d.*; FEV_1: forced expiratory volume in one second.

The provocative dose of adenosine producing a 20% fall in FEV_1 from the post-saline baseline value ($PD_{20}FEV_1$) was previously measured in order to select patients for the study. Patients with a value of PD_{20} adenosine lower than 0.5 mg were accepted.

The protocol was approved by the Ethics Review Committee of the Department of Respiratory Disease (University of Catania) and consent was obtained from each subject after the nature and reason of the study had been explained in detail.

Bronchial challenge

The airways response to bronchial provocation was measured as a change in FEV_1 by means of a Fleisch pneumotacograph (Pulmonary system 47120A, Hewlett-Packard, Waltham, MA, USA). On each challenge day, methacholine (Sigma Chemical Co., St. Louis, MO, USA) and adenosine (Sigma Chemical Co., St. Louis, MO, USA) were freshly prepared in 0.9% NaCl to produce a range of doubling doses of 0.015–8.00 mg and 0.03–4 mg, respectively.

Increasing doubling doses were administered as an aerosol generated from a starting volume of 2 ml in a DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, PA, USA) driven by compressed air at an airflow of 12 $l \cdot min^{-1}$; the output for each breath was 16.66 µl and the particle size mass diameter was 1.5–3.5 µm). Each dose was administered by 15 tidal breaths standardized by a microcomputer (Mefar, Bologna, Italy) only during inhalation by means of a microcomputer linked to a solenoid valve. The inhalation (0.8 s) and exhalation (1.6 s) times had previously been standardized.

FEV_1 was measured at 1, 3 and 5 min intervals after each inhalation and thereafter every 5 min until a value higher than the previous one was obtained. The test was stopped when FEV_1 had fallen by at least 20% of the post-saline value. The percentage decrease in FEV_1 value was plotted against the dose of agonist administered on a logarithmic scale; from each curve the log of PD_{20} was calculated.

Study design

This study consisted of two phases. In the first phase, patients attended the department on two separate occasions to undertake dose-response studies with methacholine and adenosine in the absence of any drug treatment (open study days). On the first occasion, after 15 min rest, three baseline measurements of FEV_1 were made at intervals of 3 min, followed by inhalation of control solution (0.9% NaCl) and repeat FEV_1 measurements at 1, 3 and 5 min, the higher value being recorded. Provided that FEV_1 did not fall by >10% of the baseline value an adenosine dose-response study was carried out to derive the PD_{20} value as already described. On the second occasion, a bronchial provocation test with inhaled methacholine was undertaken in a similar manner and the corresponding PD_{20} values derived.

In the second phase of the study, patients attended the laboratory on four occasions, not less than 72 h apart, to undertake dose-response studies with inhaled methacholine and AMP after ipratropium bromide or matched placebo, administered double-blind and in random order. On each occasion three baseline measurements of FEV_1 were recorded at intervals of 3 min, followed by inhalation of ipratropium bromide (Boehringer Inghelheim, Inghelheim, FRG) in a dose of 40 μg or matched placebo. Ipratropium bromide and placebo were delivered as pressurized aerosols *via* metered dose inhalers (MDIs). The inhalation of pressurized aerosols was supervised by the same investigator (who was blinded as to the purpose of the study) throughout the trial. The MDI was held 2 cm from the mouth and pressed during a slow deep inspiration with a breathholding pause of 10 s; the second administration followed 30 s after the first puff. Twenty minutes after inhaling the active drug or matched placebo, a dose-response study with one of the two agonists was performed. Provided that FEV_1 did not fall by >10% of the post-drug baseline value following 0.9% NaCl inhalation, increasing doubling doses of agonist were inhaled until FEV_1 had fallen by >20% of the post-saline value.

Statistical analysis

Figures refer to mean±SEM unless otherwise stated and the $p<0.05$ level of significance was accepted. Pre- and post-treatment baseline values of FEV_1 prior to bronchial challenges were compared within and between study days by two-factor analysis of variance (ANOVA). Since the post-saline FEV_1 values after ipratropium bromide were significantly greater than those after placebo, the agonists' bronchospastic effects were expressed as percentage fall from the post-drug saline value. The percentage fall in FEV_1 was plotted against the cumulative dose of bronchoconstrictor agonist on a log scale and the provocation dose required to produce a 20% decrease in FEV_1 from post-drug saline value (PD_{20}) determined by linear interpolation.

The repeatability of the challenge procedure with inhaled methacholine and adenosine in these patients was determined according to the method of ALTMAN and BLAND [17].

AMP and methacholine PD_{20} values were log transformed to normalize their distribution and compared by means of two-factor ANOVA. The protective effect of ipratropium bromide against bronchoprovocation with each agonist was expressed as dose-ratios, which were derived by dividing the PD_{20} value post-ipratropium by that post-placebo. The relative potency of ipratropium bromide in protecting against adenosine- and methacholine-induced bronchoconstriction was analysed by comparing the respective dose-ratios using the Wilcoxon signed rank test.

Relationship between dose-ratios and the degree of bronchodilation following active treatment was investigated by least-squares linear regression analysis.

Results

There were no significant differences in the mean baseline and post-saline values of FEV_1 between open and placebo study days. However, following inhalation of ipratropium bromide 40 μg, mean baseline values of FEV_1 were significantly greater than those obtained after placebo ($p<0.05$) on both methacholine and adenosine study days (table 2).

Table 2. – Baseline FEV_1 values (*l*) after placebo (P) and ipratropium bromide (IB) treatment

	Methacholine study day		Adenosine study day	
Subject no.	Post-P	Post-IB	Post-P	Post-IB
1	2.67	3.16	2.58	2.92
2	2.81	2.97	2.90	2.90
3	2.41	2.32	2.21	2.40
4	2.16	2.33	2.22	2.47
5	1.88	2.17	2.07	2.12
6	2.81	2.84	2.77	2.69
7	3.99	3.10	4.00	3.91
8	3.50	3.58	3.40	3.74
9	2.74	2.89	2.65	2.97
10	3.99	4.45	3.80	4.46
11	3.05	3.39	3.02	3.27
12	3.28	3.41	3.26	3.46
Mean	2.94	3.05	2.91	3.11
SEM	±0.19	±0.18	±0.18	±0.20

FEV_1: forced expiratory volume in one second.

For the twelve subjects studied the geometric mean (95% confidence limit (CL)) PD_{20} values obtained on the open study days for adenosine and methacholine were 0.45 (0.34–0.58) and 0.07 (0.03–0.14) mg, respectively. The challenge procedure with AMP was found to be repeatable, there being a coefficient of repeatability of 1.5 doubling doses. The inhalation test was repeatable to within a single doubling dilution in 10 of the 12 subjects receiving AMP.

After placebo and ipratropium bromide both adenosine and methacholine provoked dose dependent falls in FEV_1. For the 12 subjects studied the geometric mean (95% CL) doses of adenosine and methacholine required to produce a 20% decrease in FEV_1 after placebo were 0.57 (0.37–0.88) and 0.11 (0.05–0.24) mg, respectively, (table 3). Inhaled ipratropium bromide had a small but significant protective effect against the fall in FEV_1 produced by adenosine, the geometric mean (95% CL) PD_{20} increasing from 0.57 after placebo to 1.27 (0.93–1.74) mg after active treatment ($p<0.01$) (table 3). Inhaled ipratropium bromide was more effective in protecting against methacholine, the geometric mean (95% CL) PD_{20} increasing from 0.11 after placebo to 0.79 (0.45–1.36) mg ($p<0.01$) (table 3). Thus, when expressed as dose-ratios ipratropium bromide afforded a 2.2 and 7.2-fold protection of the airways against adenosine and methacholine, respectively.

Table 3. – Methacholine and adenosine PD_{20} (mg) values after placebo (P) and ipratropium bromide (IB) treatment

Subject no.	Methacholine PD_{20}		Adenosine PD_{20}	
	Post-P	Post-IB	Post-P	Post-IB
1	0.03	0.12	0.25	0.62
2	0.44	0.98	1.90	1.90
3	1.00	1.18	1.53	1.60
4	0.05	1.25	0.72	2.08
5	0.66	1.98	0.60	2.05
6	0.18	0.42	0.61	2.12
7	0.13	1.30	0.33	1.29
8	0.09	1.18	1.01	1.16
9	0.03	1.14	0.49	0.77
10	0.03	1.03	0.25	0.56
11	0.07	1.19	0.56	1.90
12	0.05	0.18	0.25	0.86
Geometric mean	0.11	0.79*	0.57	1.27*
95% CL	(0.05–0.24)	(0.45–1.36)	(0.37–0.88)	(0.93–1.74)

*: $p<0.01$ *vs* placebo. PD_{20}: provocative dose of adenosine or methacholine producing a 20% fall in forced expiratory volume in one second from baseline; CL: confidence limit.

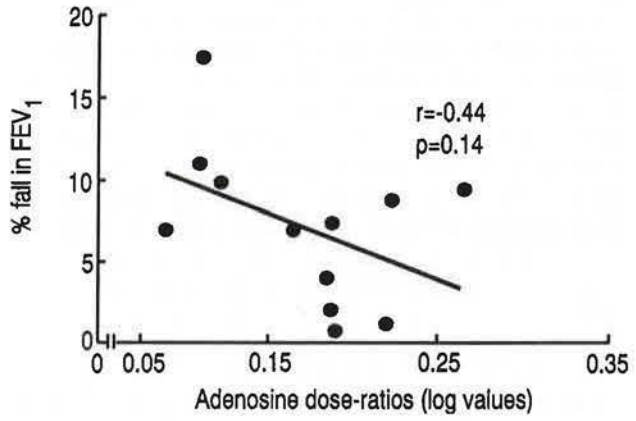

Fig. 1. – Correlation plot between the percentage bronchodilation by inhaled ipratropium bromide and the dose-ratio for adenosine. FEV_1: forced expiratory volume in one second.

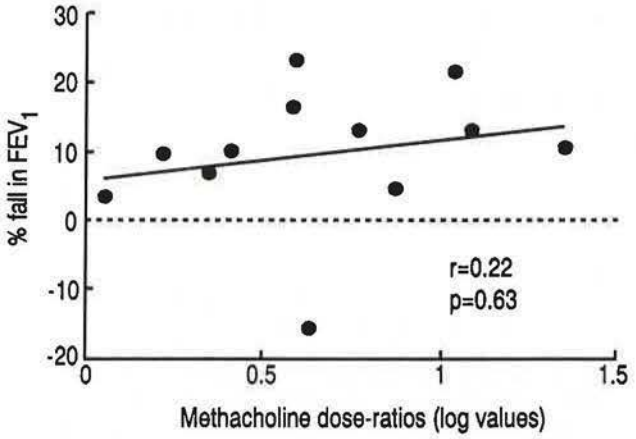

Fig. 2. – Correlation plot between the percentage bronchodilation by inhaled ipratropium bromide and the dose-ratio for methacholine. FEV_1: forced expiratory volume in one second.

The difference between adenosine and methacholine dose-ratios was significant ($p<0.01$). There was no correlation between degree of bronchodilation induced by active treatment and dose-ratios for adenosine ($p=0.14$; $r=-0.44$) (fig. 1) and methacholine ($p=0.63$; $r=0.22$) (fig. 2).

Discussion

This study demonstrates that inhaled adenosine provokes dose-related bronchoconstriction in asthmatic subjects and confirms the findings of previous studies with this agonist [1–3]. In addition, we have shown that premedication with the anticholinergic drug ipratropium bromide in a dose that caused significant reduction of the airway response to methacholine, is able to afford a significant twofold protection to the airways against the constrictor effect of adenosine. This suggests that cholinergic pathways mediate a component of the bronchoconstriction induced by adenosine in asthma.

In the group of asthmatics studied, inhaled ipratropium bromide resulted in a significant bronchodilatation as shown by mean increases in FEV_1 of 5 and 7% over basal values on the adenosine and methacholine study days, respectively. This confirms previous findings that the airways in asthma possess intrinsic vagal tone [18, 19]. However, the degree of bronchodilatation did not correlate with the degree of bronchoprotection on both adenosine and methacholine study days, thus suggesting that the protective effect of ipratropium bromide in reducing the contractile airway responses to the agonists is probably the result of an efficient competitive muscarinic receptor blockade [20] rather than a nonspecific functional antagonism.

The mode of action of adenosine-induced bronchoconstriction in asthma remains to be clarified. Current evidence strongly supports a central role for the airway mast cell in the response to adenosine. *In vitro*, adenosine potentiates the release of beta-hexosaminidase and histamine from the granules of immunologically stimulated human [4] and rodent mast cells [21]. *In vivo*, the potent and selective H_1-histamine receptor antagonists, terfenadine and astemizole have been shown to greatly reduce the bronchospastic response of asthmatic airways to inhaled AMP [2, 3, 10], thus suggesting that a major component of bronchoconstriction provoked by adenosine and AMP occurs through the enhanced release of histamine from preactivated airway mast cells.

Recently, PEACHELL *et al.* [6] have demonstrated that in immunologically stimulated human lung mast cells adenosine not only enhances histamine release, but also potentiates prostanoid generation. To investigate the potential role of spasmogenic prostanoids on adenosine and AMP challenge in asthma, potent cyclooxygenase inhibitors have been used. Using this approach we [11], and others [12], have demonstrated that contractile, newly-generated mediators may contribute to the antihistamine-resistant reduction in FEV_1 provoked by inhaled purines.

An alternative possibility is that neural reflexes may contribute to the bronchoconstrictor action of inhaled adenosine and AMP in asthma. The findings of the present study corroborate previously reported observations by OKAYAMA *et al.* [16] who found significant attenuation of adenosine-induced bronchoconstriction in asthmatics following administration of nebulized atropine and lignocaine. Although demonstrating a significant displacement of adenosine concentration-response curve towards the right following pretreatment with ipratropium bromide, MANN *et al.* [15] concluded that the small effect observed on the adenosine response represented minimal functional antagonism secondary to the bronchodilatory effect of the drug. In our view, these authors failed to provide data to support such conclusions and indeed they demonstrated that premedication with ipratropium bromide is able to afford a small but significant 1.6-fold protection to the airways against the constrictor effect of adenosine, which is comparable with the degree of protection (2.2-fold) achieved in our study.

Although functional antagonism cannot be thoroughly excluded, the lack of correlation between bronchodilation and bronchoprotection in the present study supports the view that a small component of the bronchoconstrictor effect of adenosine is mediated through cholinergic mechanisms. Another factor that needs to be considered when comparing such studies relates to the heterogeneous nature of the asthmatic response to anticholinergics. Anticholinergic drugs have shown a marked variability in efficacy between subjects undergoing bronchial challenges with histamine [22], allergen [23], cold air [24] and exercise [25]. Interestingly, in all of these cases no clear relationship between the bronchodilator effect and the protective effect of anticholinergic agents against bronchoconstrictor stimuli was demonstrated, thus supporting the view that the responsiveness to antimuscarinic drugs is different with regard to the bronchodilator and the bronchoprotective effect as suggested in this study. Thus, the pharmacological specificity of ipratropium bromide and its capacity to inhibit adenosine-induced bronchoconstriction to a certain degree implicate some involvement of the vagally-mediated pathways in the pathophysiology of the bronchospastic response of inhaled adenosine in asthma.

However, the exact stimulus prompting vagal reflex activation in adenosine-induced bronchoconstriction remains to be clarified. Possibilities include a direct effect of purine nucleosides in the activation of cholinergic reflexes and indirect interaction with vagal reflexes through the formation of histamine and prostanoids.

In a variety of animal models, adenosine has the capacity of enhancing the cholinergic responses by a direct interaction with specific prejunctional A_2 receptors [13, 26]. In addition, pharmacological studies in the rat suggest that adenosine-induced bronchoconstriction is the result of stimulation of adenosine receptors present on post-ganglionic vagal nerve endings [14].

Previous work has implicated the release of prostanoids in purine-induced bronchoconstriction in asthma [11, 12] and their effects on airway calibre are known to be mediated in part by vagal reflexes [27]. Hence, the findings of our present study might be interpreted as endogenous prostanoid production from mast cells by adenosine, producing some of its bronchospastic effect by activating cholinergic pathways.

In patients with asthma, the bronchoconstrictor response to inhaled histamine is due principally to the stimulation of histamine H_1-receptors on bronchial smooth muscle, with stimulation of vagal reflex pathways making some contribution [28]. It is, therefore, possible that histamine released endogenously from airway mast cells by adenosine, may in turn cause some of its bronchospastic responses by stimulating cholinergic pathways.

In conclusion, we have demonstrated a small but significant protective effect of ipratropium bromide on adenosine-induced bronchoconstriction. However, further studies are necessary to determine the exact mechanism of this protective effect.

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