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Effect of short- and long-acting inhaled β_2-agonists on exhaled nitric oxide in asthmatic patients

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Effect of short- and long-acting inhaled β_2-agonists on exhaled nitric oxide in asthmatic patients. D.H. Yates, S.A. Kharitonov, P.J. Barnes. ©ERS Journals Ltd 1997.

ABSTRACT: Increased concentrations of exhaled nitric oxide (NO) occur in patients with asthma, and exhaled NO may be useful for assessing the effect of drug therapy on airway inflammation. β_2-agonists have been proposed to have both pro-inflammatory and anti-inflammatory effects. We therefore assessed exhaled NO after β_2-agonists in asthmatic patients.

Two randomized, double-blind, placebo-controlled studies were conducted. Firstly, exhaled NO was measured in 18 asthmatics (9 taking inhaled glucocorticosteroids (GCS)) before and after nebulized salbutamol (5 mg), or identical placebo (0.9% saline). Exhaled NO and forced expiratory volume in one second (FEV_1) were measured at 15 min intervals for 1 h (Study 1). Secondly, the effect of 1 week of treatment with the long-acting β_2-agonist, salmeterol (50 μg *b.i.d.*), added to either budesonide (800 μg *b.i.d.*) or placebo, was studied in eight mild asthmatic subjects (Study 2). Exhaled NO was measured by a chemiluminescence analyser, adapted for on-line recording.

In Study 1, exhaled NO showed no significant change at any time-point in patients not taking inhaled GCS. In asthmatics on inhaled GCS, exhaled NO increased compared to placebo at 15 and 30 min, but this did not reach statistical significance. In Study 2, treatment with salmeterol increased FEV_1, but exhaled NO levels were not significantly changed, either after budesonide treatment (143±35 to 179±67 ppb), or after placebo (201±68 to 211±65 ppb).

Our results confirm that single high dose salbutamol does not increase exhaled nitric oxide in asthmatics not taking inhaled glucocorticosteroids. Salbutamol may increase exhaled nitric oxide in asthmatics taking inhaled glucocorticosteroids. However, regular use of salmeterol resulted in no change in exhaled nitric oxide, either used alone or in combination with inhaled glucocorticosteroids.
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Nitric oxide (NO) is a short-lived radical that is produced endogenously within the airways. It is an important signalling molecule in the regulation of airway function [1]. NO can be measured in the exhaled air of humans, either directly in expired air [2], or indirectly by collection in NO-impermeable bags [3, 4], but all methods show significant correlation [5]. Direct measurement of exhaled NO has been suggested as a rapid and noninvasive approach to the investigation of airway inflammation [5, 6].

NO is formed by the enzyme NO synthase (NOS) from the amino acid L-arginine. At least three isoforms of NOS have been identified, including an inducible form of NOS (iNOS or type II NOS). Type II NOS is rapidly induced by proinflammatory cytokines and by endotoxin in a variety of cells, including pulmonary macrophages and airway epithelial cells [1, 7]. Asthma is a chronic inflammatory disease, in which there is evidence of the secretion of multiple cytokines, including interferon-γ (IFN-γ), interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) [8]. These cytokines increase airway epithelial cell expression of iNOS *in vitro* [9], and an increased expression of iNOS-like immunoreactivity has been observed in the airway epithelium of patients with asthma [10], suggesting that airway NOS expression is increased in asthma.

Levels of exhaled NO are higher in asthmatic than in normal subjects [2, 3]. Allergen-induced late asthmatic reactions are associated with an elevation of exhaled NO [11], and expired NO levels fall during treatment of acute asthma, presumably accompanying the improvement in airway inflammation [12]. Treatment with inhaled and oral glucocorticosteroids (GCS) reduces levels of exhaled NO in asthmatic patients, presumably reflecting an inhibitory effect of inhaled steroids on airway cytokine production or iNOS expression in airway inflammatory cells [2, 13, 14]. Evidence from several recent studies, thus, suggests that measurement of exhaled NO may be an indirect measure of airway inflammation in asthma, and may provide a way of monitoring disease activity and assessing therapy.

$Beta_2$-agonists are the most effective drugs available for the treatment of acute exacerbations of asthma [8]. The effect of β_2-agonists on airway inflammation is controversial. *In vitro,* β_2-agonists inhibit the release of histamine and leukotrienes from mast cells, which

participate in the acute allergic inflammatory response [15], and inhaled β_2-agonists may also stabilize mast cells *in vivo* [16, 17]. β_2-agonists, however, have little or no beneficial effect on the chronic inflammatory response which underlies airway hyperresponsiveness and chronic asthma.

Currently, there is concern regarding potentially detrimental effects of high-dose short-acting β_2-agonists in asthma [18, 19]. These effects could possibly be mediated by a negative interaction between β_2-agonists and GCS. Due to the prolonged action and greater potency of the long-acting β_2-agonists, it is possible that any such effect may also apply to the long-acting β_2-agonists.

To examine these issues, we studied the effect of a short- and a long-acting β_2-agonist on exhaled NO in asthmatic subjects, both treated and not treated with inhaled GCS. The acute effect of the short-acting β_2-agonist, salbutamol, and also the short-term effect of the long-acting β_2-agonist, salmeterol, were studied. In the first study, mild asthmatic subjects who did not usually use inhaled GCS were examined, as well as more severe asthmatic subjects, who required regular inhaled GCS for maintenance of good asthma control. In the second study, the effect of regular salmeterol treatment was examined in patients treated in a cross-over manner, with and without a therapeutic dose of inhaled GCS.

Methods

Subjects

Thirty nonsmoking subjects with a clinical diagnosis of asthma, based on American Thoracic Society (ATS) criteria [20] were recruited (21 males and 9 females; mean (±SEM) age 29±2yrs). Twenty subjects were recruited into Study 1, and 10 into Study 2. Five patients took part in both studies, after a wash-out period of at least 3 months. All gave written informed consent to participate in the studies, which were approved by the Royal Brompton Hospital Ethics Committee. All subjects had symptoms of variable wheeze and dyspnoea, and none had suffered an asthma exacerbation or respiratory tract infection within 6 weeks before study. No subject was using any antiasthma medication other than inhaled GCS and salbutamol as required. Baseline forced expiratory volume in one second (FEV_1), for all subjects was >65% predicted, and for all subjects the provocative concentration of methacholine producing a 20% fall in FEV_1, (PC_{20}) was <16 $mg \cdot mL^{-1}$. Patients using regular long-acting inhaled β_2-agonists were excluded, as were patients using more than four puffs of as-required salbutamol per day in the week prior to the study, and those with poor asthma control (*i.e.* frequent symptoms of wheeze or chest tightness, or any nocturnal awakening due to asthma). Patients did not consume any caffeinated beverage or inhaled or oral medication, including β_2-agonist, in the 6 h before each study visit.

Methods

Each subject was screened before enrolment. After recording a clinical history and examination of the patient, atopic status was assessed by measuring skin-prick tests using six common allergens applied to the forearm (house dust mite, cat dander, grass pollen, *Aspergillus fumigatus*, histamine and glycerinated saline as positive and negative controls). Skin-prick tests were considered positive if one or more wheal >3 mm larger than the negative control was recorded.

FEV_1 was measured using a dry wedge spirometer (Vitalograph, Buckingham, UK). Three forced expiratory manoeuvres were performed, with the patient wearing a noseclip, and the best result recorded and compared with predicted values. Airway responsiveness was measured by methacholine challenge. Increasing concentrations of methacholine (0.063 to 32 $mg \cdot mL^{-1}$) were nebulized using a dosimeter (Mefar, Brescia, Italy) with an output of 100 μL per actuation. Doubling concentrations of methacholine (five breaths of 1 s, with breath-holding time of 6 s) were inhaled, and FEV_1 was measured 2 min after the last inhalation, until a maximum fall in FEV_1 of 20% from baseline control inhalation (0.9% saline solution) was achieved, or the maximum concentration was inhaled. PC_{20} was calculated by interpolation of the logarithmic dose-response curve.

Exhaled NO was measured using our original technique as reported previously [2]. After resting quietly for 15 min, exhaled NO was sampled using a chemiluminescence analyser (Dasibi Environmental Corporation Model, Glendale, CA, USA), sensitive to NO from 2 to 4,000 parts per billion (ppb) (by volume), and altered to allow on-line recording of exhaled NO concentration. This feature obviates the need for collection in a reservoir, with its variable loss of reactive NO. The sampling rate of the analyser was 250 $mL \cdot min^{-1}$ for all measurements. The analyser was calibrated daily using certified NO mixtures (90 and 400 ppb) in nitrogen (BOC Special Gases, Surrey Research Park, Guildford, UK). Ambient air NO was recorded before and after each subject was studied.

Whilst seated and wearing a noseclip, subjects performed a slow vital capacity manoeuvre over 30–75 s into wide-bore Teflon™ tubing, with a flow of approximately 1 $L \cdot min^{-1}$ (exhaled NO-obstructed). This manoeuvre results in increased oropharyngeal pressure, and closure of the soft palate, thereby diminishing the nasal NO component [21]. Three successive reproducible recordings were made at 2 min intervals, and the highest of the readings was recorded as the exhaled NO level. Results were displayed on a chart recorder and compared with the signals generated from the calibration mixtures of NO in nitrogen. Area under the curve (AUC) was highly correlated with the peak value (r=0.89); peak values were therefore used in all calculations [2]. All measurements were made by a single observer (SAK), who had no knowledge of the treatment of individual patients.

Study protocols

Study 1. The study was of a double-blind, randomized, placebo-controlled, cross-over design. After a screening visit, during which skin-prick tests, exhaled NO, baseline spirometry and methacholine PC_{20} were assessed, subjects were allocated two study visits exactly 1 week apart. Two groups of asthmatic subjects were selected: those with mild asthma, not requiring inhaled GCS for control of asthma symptoms; and those with more severe asthma, who received regular GCS therapy.

Each study visit took place at an identical time of day. The protocol was as follows: after resting quietly for at least 15 min, baseline NO and FEV_1 measurements were performed, followed immediately by inhalation of either 5 mg nebulized salbutamol (Glaxo, Greenford, UK) or 0.9% saline control *via* a Model CR 60 Medic-Aid nebulizer (Horsham, UK) over a 12 min period. A noseclip was worn during inhalation. Exhaled NO and FEV_1 were measured immediately after cessation of nebulization, and thereafter at 15 min intervals for 1 h. The subsequent study visit was identical, except that the alternative nebulized treatment was used.

Study 2. Mild asthmatic subjects not usually requiring inhaled GCS were selected for this study. After a screening visit in which atopic status, exhaled NO, baseline spirometry and methacholine PC_{20} were assessed, subjects were randomized to either regular budesonide (800 μg *b.i.d. via* a dry powder delivery system (Turbohaler)) or identical placebo control (Astra Draco, Sweden). Subjects inhaled either budesonide or identical placebo for 3 weeks. Salmeterol (50 μg *b.i.d. via* dry powder inhaler (Diskhaler; Glaxo, Greenford, UK)) was added to the last week of each treatment period, as open label. Subjects attended once a week, at the same time of day, for spirometry and exhaled NO measurement. FEV_1 was measured exactly 12 h after the last salmeterol inhalation. Subsequently, after a wash-out period of at least 3 weeks, the alternative treatment was administered in an identical manner.

Compliance was assessed by counting remaining Turbohaler doses and the number of used salmeterol discs.

Statistical analysis

All mean exhaled NO concentrations are reported in ppb and the corresponding standard error of the mean (SEM). PC_{20} values were analysed after $\log_{10}$ conversion by unpaired t-tests, and results are expressed as geometric means and geometric SEM. Comparisons of the mean expired NO concentrations among groups were made using the Mann-Whitney rank sum test. A p-value of less than 0.05 was considered significant.

Results

Study 1

Subjects. Of the 20 patients recruited, all completed the study. The results of two patients were excluded from the analysis of NO levels because of poorly reproducible technique. One patient was excluded from the group taking inhaled GCS, and one from those not taking inhaled GCS. Subject characteristics are presented in Table 1.

Baseline FEV_1 was lower in subjects taking inhaled GCS (91±6% pred) than in subjects not taking inhaled GCS (94±5% pred), although mean PC_{20} values showed no significant difference (table 1).

FEV1. In patients not taking inhaled GCS (n=10), FEV_1 rose from 3.72±0.31 L (94±5% pred) to a maximum of 4.08±0.3 L (104±5% pred; p<0.05 compared with saline control) (fig. 1a). In patients taking inhaled GCS, mean FEV_1 also rose with salbutamol treatment from 3.33±0.19 L (91±6% pred) to 3.71±0.18 L (98±5% pred; p<0.05

Table 1. – Study 1: characteristics of subjects studied

Pt No.	Sex	Age yrs	FEV_1 % pred	PC_{20} mg·mL^{-1}	Atopy	Medication
Patients not taking inhaled corticosteroids						
1	F	29	92	0.41	+	*p.r.n.* Salb
2	M	37	65	1.22	-	*p.r.n.* Salb
3*	M	30	86	0.30	+	*p.r.n.* Salb
4	M	26	100	2.62	+	*p.r.n.* Salb
5	M	28	88	0.64	+	*p.r.n.* Salb
6	M	21	118	-	+	*p.r.n.* Salb
7	M	39	78	0.09	+	*p.r.n.* Salb
8	F	26	93	1.50	+	*p.r.n.* Salb
9	F	37	83	1.22	-	*p.r.n.* Salb
10	M	28	100	0.16	+	*p.r.n.* Salb
Mean			94	0.58‡		
SEM			5	1.40		
Patients taking inhaled corticosteroids						
11	F	26	88	0.30	+	Bud 800 μg *b.i.d.*
12*	F	23	84	0.47	+	Bud 400 μg *b.i.d.*
13	M	36	65	1.74	+	Bud 400 μg *b.i.d.*
14	M	25	80	0.61	+	Bud 1.2 mg *b.i.d.*
15	F	22	75	6.35	-	Bud 200 μg *b.i.d.*
16	M	63	76	0.93	+	Bud 800 μg *b.i.d.*
17	F	24	92	1.50	+	Bud 800 μg *b.i.d.*
18	M	24	96	0.06	+	Bec 200 μg *b.i.d.*
19	M	27	87	0.23	+	Bud 800 μg *b.i.d.*
20	M	26	99	2.62	+	Bud 800 μg *b.i.d.*
Mean			91	0.74‡		
SEM			6	1.60		

*: withdrawn from the study; ‡: geometric mean SEM. Pt: patient; F: female; M: male; FEV_1: forced expiratory volume in one second; % pred: percentage of predicted value; PC_{20}: provocative concentration of methacholine causing a 20% fall in FEV_1 from baseline; +: positive; -: negative; Salb: salbutamol; Bud: budesonide; Bec: beclomethasone.

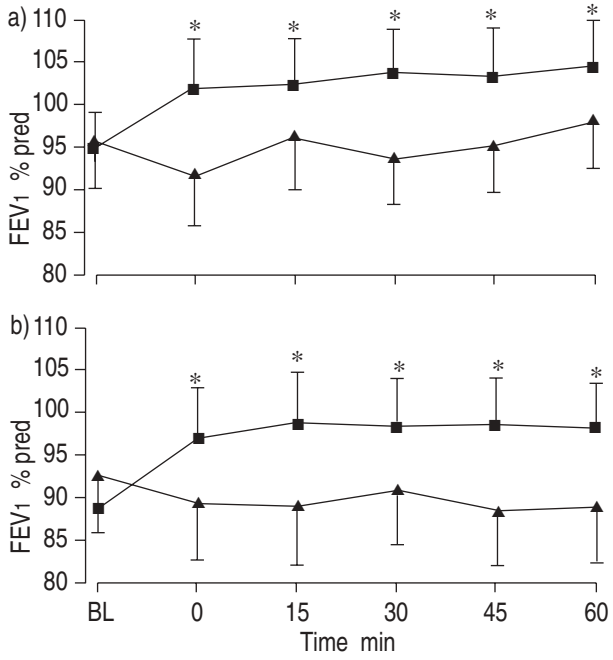

Fig. 1. – Effect of nebulized salbutamol 5 mg (—■—) and 0.9% saline placebo (—▲—) on forced expiratory volume in one second (FEV_1): a) in nine asthmatic subjects not taking inhaled glucocorticosteroids; and b) in nine asthmatic patients taking regular inhaled glucocorticosteroids. BL: baseline; % pred: percentage of predicted value. *: p<0.05, compared to placebo.

compared with saline control) (fig. 1b). Patients not taking inhaled GCS achieved 104% of predicted FEV1 after 5 mg of salbutamol, whereas patients on inhaled GCS achieved only 98% predicted FEV1, possibly reflecting greater asthma severity. A maximal response was achieved immediately after nebulization (T0), with only minor changes thereafter.

Exhaled NO. NO was detected in the exhaled air of all subjects studied, and there was a trend towards mean baseline exhaled NO being higher in patients not taking inhaled GCS (205±40 ppb) than in patients using inhaled GCS therapy (144±61 ppb), although this did not reach statistical significance, presumably due to the small number of subjects. Mean baseline exhaled NO levels were not significantly different between any study visit (205±37 *vs* 204±44 ppb in patients not using inhaled GCS, and 124±38 *vs* 164±85 ppb in patients using inhaled GCS).

Patients not using inhaled GCS. In patients not taking inhaled GCS, exhaled NO levels did not change significantly either with salbutamol inhalation or with placebo (fig. 2a). Very little variation from baseline was observed.

Patients using regular inhaled GCS. In patients treated with regular inhaled GCS, much greater variation in response was observed than in patients not using regular inhaled GCS. After salbutamol inhalation, exhaled NO did not change significantly compared to placebo, although the trend was for an increase rather than the minor decrease which had been observed in patients not using inhaled GCS (fig. 2b).

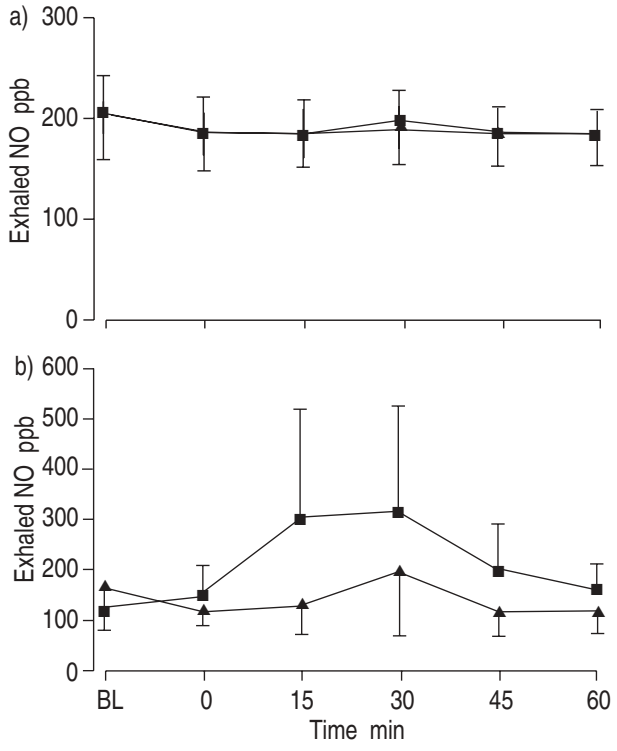

Fig. 2. – Effect of nebulized salbutamol 5 mg (—■—) and 0.9% saline placebo (—▲—) on exhaled nitric oxide (NO): a) in nine asthmatic subjects not taking inhaled glucocorticosteroids; and b) in nine asthmatic patients taking regular inhaled glucocorticosteroids. BL: baseline.

Table 2. – Study 2: characteristics of subjects studied

Pt No.	Sex	Age yrs	FEV1 % pred	PC20 mg·mL^{-1}	Atopy	Medication
Patients not taking inhaled corticosteroids						
1	M	27	83	2.00	+	*p.r.n.* Salb
2	M	26	80	0.33	+	*p.r.n.* Salb
3	F	24	86	16.00	+	*p.r.n.* Salb
4	M	30	100	8.70	+	*p.r.n.* Salb
5	M	30	82	0.31	+	*p.r.n.* Salb
6	M	39	97	1.84	+	*p.r.n.* Salb
7	M	25	109	2.60	+	*p.r.n.* Salb
8	M	25	106	0.44	+	*p.r.n.* Salb
9*	F	26	96	5.00	+	*p.r.n.* Salb
10*	M	26	104	0.58	+	*p.r.n.* Salb
Mean			92	1.3‡		
SEM			4	1.5		

*: withdrawn from the study; ‡: geometric mean and geometric SEM. For definitions see legend to table 1.

Study 2

Subjects. Of the 10 mild asthmatic subjects enrolled, eight completed the study. Two were withdrawn, one because of the development of an upper respiratory tract infection during the placebo arm of the study, and another due to hospital admission for an unrelated illness. Results from these subjects were excluded from the analysis. Subject characteristics are presented in table 2.

FEV1. At baseline, FEV1 did not differ significantly between budesonide and placebo treatment periods (91±4.1

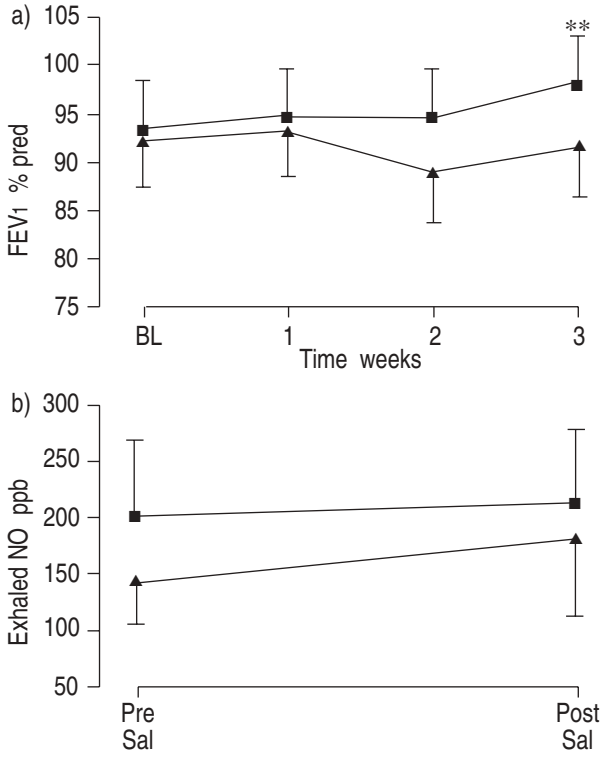

Fig. 3. – a) Effect of 3 weeks of treatment with regular inhaled budesonide (800 μg *b.i.d.*) (—■—) identical placebo (—▲—) on forced expiratory volume in one second (FEV1). b) Effect of 1 week of additional treatment with inhaled salmeterol (50 μg *b.i.d.*) on exhaled nitric oxide (NO) in eight mild asthmatic subjects; BL: baseline; % pred: percentage of predicted value; Sal: salmeterol. **: $p<0.01$, compared to placebo.

and 93±5.5% pred, respectively). Budesonide treatment over 2 weeks resulted in an increase in FEV_1 compared to placebo, which fell just short of statistical significance by the second week (fig. 3a). Salmeterol treatment resulted in a further increase in FEV_1, but this did not reach statistical significance compared with values from the previous week, either with placebo or with budesonide treatment.

Exhaled NO. A trend towards a fall in exhaled NO was seen with budesonide treatment (201±68 to 143±35 ppb), although this fell short of statistical significance. Salmeterol treatment during the third week of treatment did not significantly alter exhaled NO, when used in addition to either placebo or to budesonide therapy (fig. 3b).

Discussion

In this study, a single high dose of salbutamol did not significantly affect exhaled NO in mild asthmatic patients not taking inhaled GCS. In more severe asthmatics requiring regular inhaled glucorticosteroid (GCS) for asthma symptom control, high-dose salbutamol resulted in an increase in exhaled NO at 15 and 30 min compared to placebo, but this difference did not reach statistical significance. This observation is difficult to interpret, and needs to be confirmed with larger numbers of subjects. However, the pattern of response appeared different. Regular salmeterol therapy had no significant effect on exhaled NO levels in mild stable asthmatics, used either in conjunction with, or in the absence of, inhaled GCS treatment. The lack of change in exhaled NO observed both with short-acting and long-acting β_2-agonists, despite significant bronchodilatation, confirms previous findings showing that the levels of exhaled NO measured are independent of the presence of bronchoconstriction [2, 12].

The change in exhaled NO observed with high-dose inhaled salbutamol in the asthmatic subjects not using inhaled GCS was small and identical to that seen with saline control. Variation in exhaled NO in these patients was similar between salbutamol and placebo groups. In contrast, asthmatic subjects using inhaled GCS showed a much more variable response. An overall rise in exhaled NO occurred at every time-point, although this failed to reach statistical significance, possibly because of the limited number of subjects studied or due to individual differences in response. The increase in FEV_1 resulting from salbutamol inhalation was of similar magnitude irrespective of inhaled GCS usage, and thus cannot account for alteration in exhaled NO. We do not believe this was due to differences in individual technique, as the withdrawal rate was equal in each group, and occurred before the study code was broken. The significance of this observation is as yet uncertain, but should be explored in future studies and using larger numbers of subjects. Using salmeterol, no difference in exhaled NO was observed in the same subjects before and after 1 week of regular treatment at therapeutic dosage, either with or without inhaled GCS.

The cellular source of NO in exhaled air is as yet uncertain. In humans, NO is formed by at least three isoforms of NOS. Constitutive isoforms (endothelial nitric oxide synthase (eNOS) or type III NOS, and neuronal nitric oxide synthase (nNOS) or type I NOS) are basally expressed in many cells in the airways, and release picomoles of NO within seconds or minutes in response to receptor stimulation. An inducible form of nitric oxide synthase (iNOS or type II NOS) is rapidly induced by proinflammatory cytokines and by endotoxin in a variety of cells, including macrophages and airway epithelial cells [1, 7]. Activation of iNOS results in the production of much larger amounts of NO than after activation of constitutive isoforms. Inducible NOS is inhibited by GCS, whereas type I NOS is unaffected [22].

In normal individuals, there is evidence of high concentrations of NO in the nasopharynx, and this may contribute to NO in exhaled air in normal and asthmatic individuals [23]. NO is also derived from the lower respiratory tract, and can be measured in intubated patients and *via* direct bronchoscopic sampling [21]. Nasopharyngeal NO is unlikely to account for more than a minor component of exhaled NO in the present study, as the subjects all wore noseclips and performed a slow expiratory manoeuvre. In normal individuals, there is evidence for expression of constitutive NOS in the alveolar epithelium, which may account for the low levels measured in normal subjects [24]. Immunohistochemical studies of bronchial biopsies in asthmatic patients have demonstrated increased iNOS expression in asthmatic patients compared to normal subjects [10], and inhalation of a selective iNOS inhibitor, aminoguanidine, decreases exhaled NO in asthmatic but not in normal subjects [25]. This suggests that exhaled NO in asthma is likely to derive mainly from iNOS in the airway epithelial cell, rather than from constitutive NOS expression.

It is possible that airway inflammatory cells may also be involved, including macrophages, or nonadrenergic noncholinergic (NANC) nerves [7]. Two recent studies, however, failed to demonstrate significant immunoreactivity for iNOS expression outside airway epithelial cells [10, 24], and capsaicin, a potent NANC stimulator, failed to increase exhaled NO in normal subjects [26].

We studied asthmatic subjects who were treated and not treated with inhaled GCS, because we were interested in the potential proinflammatory effects of β_2-agonists when used either alone or in conjunction with inhaled GCS. There is some evidence that high concentrations of β_2-agonists may interfere with the anti-inflammatory effect of steroids in animal and human lung *in vitro* through a direct protein-protein interaction between the GCS receptor and the transcription factor cyclic adenosine monophosphate response element (CRE)-binding protein (CREB), which is activated by the β_2-agonist [27]. The clinical extrapolation of this interaction may be that high doses of β_2-agonists may increase airway inflammation or may result in an increased requirement for steroid. That this may occur *in vivo* has been suggested in a recent clinical study, where treatment for 3 weeks with an inhaled GCS (budesonide, 800 μg *b.i.d.*) inhibited the airway response to allergen, whereas similar treatment with an inhaled β_2-agonist (terbutaline, 1,000 μg *t.i.d.*) had no such inhibitory effect. However, when terbutaline was combined with budesonide, no protective effect of the GCS was found,

suggesting that the β_2-agonist had interfered with its anti-inflammatory effect [28].

Our results suggest that neither a high concentration of short-acting β_2-agonist, nor a longer treatment with a long-acting β_2-agonist, decrease exhaled nitric oxide in asthmatic patients not using inhaled glucocorticosteroids. This is consistent with the lack of anti-inflammatory effect of β_2-agonists in asthma [17]. Whether no change occurs with use of short-acting β_2-agonists in patients using regular inhaled glucocorticosteroids appears less certain, as our results were less conclusive, although no effect was noted with the long-acting β_2-agonist salmeterol in patients treated with budesonide. Our results did not demonstrate an increase in airway inflammation with β_2-agonist use. Our studies were, however, only performed over a short time period, and a longer treatment period may be necessary for any potential proinflammatory effect to develop [18, 29]. Further studies are needed, using larger numbers of patients and over longer periods, to investigate this issue. In this context, exhaled nitric oxide may prove a useful tool for the investigation of airway inflammation, including drug interactions, in the future.

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