Changes in neurokinin A (NKA) airway responsiveness with inhaled frusemide in asthma

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Abstract

Background – Inhaled frusemide exerts a protective effect against bronchoconstriction induced by several indirect stimuli in asthma which could be due to interference of airway nerves. A randomised, double blind, placebo controlled study was performed to investigate the effect of the potent loop diuretic, frusemide, administered by inhalation on the bronchoconstrictor response to neurokinin A (NKA) and histamine in 11 asthmatic subjects.

Methods – Subjects attended the laboratory on four separate occasions to receive nebulised frusemide (40 mg) or matched placebo 10 minutes prior to bronchial challenge with NKA and histamine in a randomised, double blind order. Changes in airway calibre were followed as forced expiratory volume in one second (FEV₁) and responsiveness to the agonists was expressed as the provocative concentration causing a 20% fall in FEV₁ from baseline (PC₂₀).

Results – Compared with placebo, inhaled frusemide reduced the airway responsiveness to NKA in all the subjects studied, the geometric mean (range) values for PC₂₀NKA increasing significantly ($p < 0.001$) from 130.3 (35.8–378.8) to 419.9 (126.5–1000) µg/ml after placebo and frusemide, respectively. Moreover, a small but significant change in airway responsiveness to histamine was recorded after frusemide, their geometric mean (range) PC₂₀ values being 0.58 (0.12–3.80) and 1.04 (0.28–4.33) mg/ml after placebo and frusemide, respectively.

Conclusions – The decrease in airway responsiveness to NKA after administration of frusemide by inhalation suggests that this drug may interfere with the activation of neurotransmission in human asthma.

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metabisulphite,⁶ adenosine 5'-monophosphate (AMP),⁷ and bradykinin.⁸ Although the mechanisms underlying the protective effects of this drug against these different forms of provocation in asthma are not yet clear, an inhibitory effect on airway nerves has been suggested. In support of this, inhaled frusemide has been shown to inhibit the cough response induced by low chloride aerosols⁹ and to produce dose-dependent inhibition of the contractile response of airways smooth muscle induced by the stimulation of cholinergic and non-adrenergic non-cholinergic (NANC) nerves in guinea pigs.¹⁰

The peptide tachykinin, neurokinin A (NKA), exhibits a range of features which may be relevant to the pathophysiology of asthma, including contraction of airway smooth muscle, increased vascular permeability, mucus secretion, and activation of cholinergic neurotransmission.^{11,12} Immunocytochemical studies have demonstrated the presence of NKA and its related receptors within the human airways.^{13,14} When administered by inhalation to asthmatic subjects NKA elicits dose-related bronchoconstriction, with the asthmatic subjects being more responsive than normal individuals.^{15–19} The mode of action by which NKA elicits bronchoconstriction in asthma is not well understood. The bronchoconstrictor effect of nebulised NKA in asthmatic patients is inhibited by prior treatment with nedocromil sodium,^{17,20} suggesting that this response may be evoked indirectly rather than through direct stimulation of airway smooth muscle. In addition, there is recent evidence for an action of NKA in eliciting bronchoconstriction through activation of cholinergic pathways.²¹

We have therefore investigated the effect of prior administration of frusemide, given by inhalation, on the airways response of asthmatic subjects to NKA and to histamine which was included to evaluate the specificity of inhaled frusemide in the response.

Methods

SUBJECTS

Eleven asthmatic subjects (eight women) with a mean (SE) age of 34.5 (3.6) years referred to our chest clinic with stable asthma participated in the study (table 1). They were non-smokers and all were atopic as defined by positive skin prick tests (>3 mm weal response) to one or more of six common aeroallergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, wall pellitory grass, mixed grass pollens,

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A number of studies have accumulated evidence showing an effect of loop diuretics in bronchial asthma.¹ The inhalation of frusemide has been shown to protect the asthmatic airways against various bronchoconstrictor stimuli including allergen,² ultrasonically nebulised distilled water,³ exercise,⁴ cold air,⁵ sodium

Table 1 Demographic details of subjects studied

Subject no.	Sex	Age (years)	Baseline FEV ₁ (% predicted)	Atopy†	PC ₂₀ histamine (mg/ml)	PC ₂₀ NKA (µg/ml)
1	M	21	81	D-P	1.60	153.3
2	F	38	115	P	1.59	254.9
3	F	26	79	P	0.51	313.7
4	F	41	100	D	0.40	278.1
5	F	19	89	D-G	0.38	84.8
6	M	22	92	D-G-P	0.50	114.1
7	M	43	72	P	0.37	476.5
8	F	56	70	D	0.35	60.7
9	F	40	82	P	0.56	106.7
10	F	44	70	D	0.19	36.8
11	F	26	86	G	1.04	114.3
Mean (SE)		34.2(3.6)	85.1(4.1)		0.55*(0.19–1.60)	141.2*(36.8–476.5)

FEV₁ = forced expiratory volume in one second; PC₂₀ = provocation concentration producing a 20% fall in FEV₁.

* Geometric mean (range).

† Atopic, positive immediate skin test to one or more allergens: D = *Dermatophagoides*, P = *Parietaria* pollen, G = grass pollen.

cat fur, dog hair). At the beginning of the study all subjects were asymptomatic with a baseline forced expiratory volume in one second (FEV₁) of >70% of their predicted values. None had received oral or topical steroids, theophylline, antihistamines, or sodium cromoglycate within the preceding four weeks. Inhaled bronchodilators were discontinued for at least eight hours before each visit to the laboratory. Subjects were not studied within four weeks of an upper respiratory tract infection or exacerbation of their asthma and all visits to the laboratory were carried out at the same time of day and outside the pollen season.

The study was approved by the ethical sub-committee of the Department of Respiratory Diseases (University of Catania) and all subjects gave their informed consent.

BRONCHIAL PROVOCATION

Airway calibre was recorded before and during the provocation as FEV₁ using a dry wedge spirometer (Vitalograph, Buckinghamshire, UK), the better of the two consecutive measurements being used for analysis.

Histamine (Sigma Chemical Co, St Louis, Missouri, USA) and NKA (Peninsula Laboratories Ltd) were made up in 0.9% sodium chloride (1% albumin solution) to produce a range of increasing doubling concentrations of 0.03–16.00 mg/ml and 3.9–500 µg/ml, respectively. The aqueous solutions were administered as aerosols generated from a starting volume of 3 ml in a disposable Inspiron mini-nebuliser (C R Bard International, Sunderland, UK) driven by compressed air at 8 l/min. Under these conditions the nebuliser had an output of 0.48 ml/min and generated an aerosol with a mass median particle diameter of 4.7 µm.²² Wearing a nose clip, subjects inhaled the aerosolised solutions in five breaths from end-tidal volume to full inspiratory capacity via a mouth-piece as described by Chai *et al.*²³ Subjects were trained to take three seconds to reach full inspiratory capacity.

STUDY DESIGN

The study consisted of two distinct phases.

Phase 1

Subjects attended the laboratory on two separate occasions, at least 72 hours apart, to undertake concentration-response studies with inhaled histamine and NKA in the absence of any drug treatment.

On the first occasion, after 15 minutes rest, three baseline measurements of FEV₁ were made at intervals of three minutes followed by inhalation of 0.9% sodium chloride and further FEV₁ measurements were repeated at one and three minutes. Provided FEV₁ had not fallen by more than 10% of the baseline value, a histamine concentration-response study was carried out. After administration of each histamine concentration FEV₁ was measured at one and three minutes. Increasing doubling concentrations of histamine were inhaled at five minute intervals until FEV₁ had fallen by more than 20% of the post-saline baseline value. The fall in FEV₁ following each concentration of agonist was expressed as a percentage of the higher of the two post-saline baseline FEV₁ recordings. The percentage fall in FEV₁ was plotted against the cumulative concentration of agonist on a logarithmic scale and the provocation concentration required to produce a 20% decrease in FEV₁ from the post-saline baseline value (PC₂₀) was determined by linear interpolation. On the second occasion a bronchial provocation test with inhaled NKA was undertaken in a similar manner to that described for histamine. FEV₁ measurements were recorded one and three minutes after inhalation of each concentration of NKA and the corresponding PC₂₀ FEV₁ values derived.

Phase 2

Subjects attended the laboratory on four separate occasions, at least four days apart, to undertake concentration-response studies with histamine and NKA after receiving nebulised frusemide (Lasix, Hoechst, Frankfurt AM Main, Germany) or matched placebo administered double blind and in random order 10 minutes before challenge. The dosage of inhaled frusemide used in the present study and the timing of administration before bronchial challenge were chosen on the basis of previous

studies.^{7,8} Both the active and placebo solutions were freshly prepared by an independent investigator on the basis of a randomised code and then returned to the conducting physician to administer to the attending subject. On each occasion, after 15 minutes rest, three baseline measurements of FEV₁ were made at intervals of three minutes followed by inhalation of nebulised frusemide (10 mg/ml, 4 ml) or nebulised vehicle placebo consisting of 0.9% sodium chloride adjusted to the same pH and tonicity as the frusemide. The aerosol solutions were generated from a starting volume of 4 ml in an Inspiron mini-nebuliser driven by compressed air at 8 l/min and inhaled to dryness by deep tidal breathing over 7–9 minutes. The same nebuliser was used for all studies on all subjects. Further FEV₁ measurements were repeated at one, five, and 10 minutes after drug/placebo inhalation and dose-response studies with increasing concentrations of histamine and NKA were carried out in a similar manner to that described in phase 1.

DATA ANALYSIS

Results are expressed as mean (SE) unless otherwise stated and p values of <0.05 were accepted as the minimum level of statistical significance. Baseline values of FEV₁ before and after treatment were compared between and within study days by two-factor analysis of variance (ANOVA).

After nebulised frusemide a 20% fall in FEV₁ was not obtained in five subjects (nos 1–4 and 7) at the highest concentration of NKA administered, and therefore a minimal estimate was obtained by calculating the cumulative PC₂₀ on the next doubling concentration beyond the highest administered. Because of these censored data the results were compared for significance with Wilcoxon’s signed rank test. Values of PC₂₀ histamine and NKA following treatment with frusemide and placebo were log-transformed and compared by means of two-factor ANOVA.

Concentration ratios for the effect of frusemide against bronchoprovocation with each agonist were calculated individually by dividing the PC₂₀ value obtained after the active drug by that obtained after placebo and compared using the Wilcoxon’s signed rank test. The relationship between the airway responses to histamine and NKA was tested by least squares

Figure 1 Mean (SE) changes in provocation concentrations of NKA required to provoke a 20% decrease in FEV₁ (PC₂₀FEV₁) on baseline, placebo, and frusemide study days in 11 asthmatic subjects. *p<0.01 versus placebo.

linear regression analysis of their log-transformed PC₂₀ values. Linear regression analysis was also used to evaluate any relationship between the concentration ratios after the drug and the airway responses to histamine and NKA.

Results

There was no significant difference in mean (SE) baseline values of FEV₁ between any of the study days, with values ranging from 2.37 (0.19) l to 2.44 (0.18) l. Administration of frusemide did not cause any change in FEV₁ from baseline on either NKA or histamine study days. Mean values of FEV₁ following administration of frusemide were not significantly different from those after placebo when compared at all time points.

Inhaled histamine and NKA produced concentration-related falls in FEV₁. The geometric mean (range) of PC₂₀ values obtained were 0.55 (0.19–1.60) mg/ml and 141.2 (36.8–476.5) µg/ml for histamine and NKA, respectively (table 1). No significant correlation was observed between PC₂₀ values for histamine and NKA.

When compared with placebo, inhaled frusemide had a significant protective effect against the fall in FEV₁ produced by NKA. Frusemide produced a displacement of the NKA concentration-response curve to the right in all the subjects studied. For these subjects the geometric mean (range) PC₂₀NKA values increased 3.5-fold from 130.3 (35.8–378.8) µg/ml to 419.9 (126.5–1000) µg/ml after placebo and frusemide, respectively (p<0.01; fig 1, table 2). No correlation could be found between baseline airway reactivity and the protection of airway response to NKA after frusemide exposure.

In addition, inhaled frusemide elicited a small but significant protection against the airway response to histamine (p<0.05), the geometric mean (range) PC₂₀ histamine value increasing 1.7-fold from 0.58 (0.12–3.80) mg/ml to 1.04 (0.28–4.33) mg/ml after placebo and frusemide, respectively (fig 2, table 2). Finally, no relationship was found between the concentration ratios for the effect of frusemide against bronchoprovocation with NKA and histamine (p=NS; r=0.10).

Table 2 Effects of pretreatment with inhaled frusemide and placebo on airway NKA and histamine responsiveness

Subject no.	PC ₂₀ NKA (µg/ml)		PC ₂₀ histamine (mg/ml)	
	Placebo	Frusemide	Placebo	Frusemide
1	205.2	1000	3.80	4.33
2	169.6	1000	2.12	3.38
3	326.0	1000	0.19	0.28
4	250.4	1000	0.44	1.66
5	46.9	178.3	0.28	0.35
6	126.4	211.5	0.90	0.80
7	378.8	1000	0.24	1.80
8	35.8	126.5	0.44	1.45
9	109.4	210.0	1.25	0.90
10	61.2	384.6	0.12	0.39
11	121.1	177.3	0.86	0.86
Geometric mean	130.3	419.9	0.58	1.04
(range)	(35.8–378.8)	(126.5–1000)	(0.12–3.80)	(0.28–4.33)

Figure 2 Mean (SE) changes in provocation concentrations of histamine required to provoke a 20% decrease in FEV₁ (PC₂₀FEV₁) on baseline, placebo, and frusemide study days in 11 asthmatics. **p* < 0.05 versus placebo.

Discussion

This study confirms previous findings that inhaled NKA causes dose-related bronchoconstriction in asthmatic subjects.^{15–19} We have also shown that frusemide administered by inhalation elicits protection of the airways against bronchoconstriction provoked by NKA in all the asthmatic subjects studied. In addition, we have shown a small but significant change in airway responsiveness to histamine following frusemide exposure. The protection afforded by frusemide was twice as great against NKA as against histamine, which suggests involvement of several mechanisms.

The approximately 3.5-fold displacement of the concentration-response curves to the right with NKA is similar to that reported with other indirectly acting bronchoconstrictor stimuli and is similar to that observed after disodium cromoglycate or nedocromil sodium. In addition, the observation that inhaled frusemide and disodium cromoglycate/nedocromil sodium have little or no significant protective effect against methacholine-induced bronchoconstriction in asthma^{5–7,24} suggests that these drugs have indirect effects. There is now considerable evidence to support the hypothesis that frusemide has an inhibitory effect on neural pathways in the airways. Frusemide has been shown to inhibit the cough response induced by low chloride solutions in dogs²⁵ and normal volunteers,⁹ and both frusemide and bumetanide produce dose-dependent inhibition of the contractile response of airways smooth muscle induced by stimulation of cholinergic and non-cholinergic non-adrenergic nerves in guinea pigs.¹⁰ Similarly, Verleden *et al*²⁶ have recently shown that loop diuretics elicit a dose-dependent inhibition of cholinergic neurotransmission in human airways in vitro, possibly via an action on ion channels located on these nerves. Although the bronchoconstrictor activity of NKA is markedly inhibited by nedocromil sodium,^{17,20} the selective histamine H₁ receptor antagonist, terfenadine, does not appear to protect against NKA-induced bronchoconstriction.²⁷ Furthermore, anticholinergic blockade also affords protection against this agonist.²¹ These findings, together with the results of the present investigation, suggest that excitation of neural pathways may underlie NKA responses in human asthma. However,

the exact stimulus prompting the neural reflex activation remains to be clarified. A possibility may include an indirect activation of cholinergic reflexes through the production of prostaglandins. There is evidence in vitro that part of the biological effects of exogenously applied neuropeptides may result from the local release of bioactive prostaglandins.²⁸ Indeed, we have recently shown a significant decrease in airway responsiveness to NKA after cyclooxygenase blockade.²⁹

The contribution of alternative protective mechanisms of inhaled frusemide in NKA-induced bronchoconstriction must be considered. Prostaglandin release may be involved in the protective effects of frusemide. Loop diuretics have been shown to induce their effect in the kidney by the secondary production of the prostaglandins PGE₂ and PGI₂.^{30,31} In man frusemide stimulates the production of PGE₂ by increasing the availability for arachidonic acid³² and enhances the urinary excretion of PGI₂.³³ Since the inhibitory effect of loop diuretics on nerve activation in human airways in vitro is related to the release of endogenous cyclooxygenase products from the epithelium,²⁶ it is suggested that frusemide may afford airway protection by releasing epithelium derived PGE₂ and PGI₂, both of which are potent functional antagonists. Other in vitro experiments in the guinea pig trachea also suggest an influence of inhibitory prostaglandins.³⁴ In addition, PGE₂ has been shown to inhibit airway neurotransmission in human bronchial preparations.³⁵ Recent data by Pavord *et al*³⁶ and by us³⁷ support the view that the prevention of bronchoconstrictor stimuli by loop diuretics in human airways may be due to the local production of inhibitory prostaglandins. Thus, the inhibitory action of inhaled frusemide against NKA-induced bronchoconstriction is likely to be due to a suppressive action on the activation of neural pathways or to the local production of inhibitory prostaglandins with functional antagonistic effects.

In this study we have also shown a small but significant effect of frusemide against histamine-induced bronchoconstriction. Although this finding is in keeping with the data reported by Vaghi *et al*,³⁸ two previous studies failed to show any significant effect of frusemide on histamine responses in both adult and paediatric asthmatic subjects.^{39,40} There may be factors in the characteristics of these patients which affects their response to frusemide. The asthmatic subjects studied in the present investigation differ from those in the studies of O'Connor *et al*³⁹ and Mochizuki *et al*⁴⁰ for having a considerably higher degree of bronchial hyperresponsiveness. Moreover, Mochizuki *et al* found that inhaled frusemide afforded protection against bronchoconstriction only in the child with the highest degree of bronchial hyperresponsiveness. Bronchial hyperresponsiveness correlates with the extent of epithelial damage in human asthma. Damaged airway epithelium may alter the pharmacokinetic profile of inhaled loop diuretics, thus changing their local pharmacodynamic activity. It is therefore possible that higher drug

concentrations are penetrating deeper in the bronchial mucosa of those asthmatic subjects with the highest degree of bronchial hyper-reactivity so that a more significant response is seen.

Histamine elicits bronchoconstriction by stimulating specific receptors and, to a lesser extent, via cholinergic activation. In support of the ability of histamine to influence vagal airway tone, both atropine and inhaled ipratropium bromide^{41,42} have been shown to be effective inhibitors of histamine-induced bronchoconstriction through cholinergic blockade. Thus, as shown for NKA, the small protection of inhaled frusemide against histamine-induced bronchoconstriction may be due to a suppressive action on the activation of neural pathways or to the local production of inhibitory prostaglandins with functional antagonistic effects. However, the lack of correlation between the protective effect against NKA and histamine argues against this possibility.

In conclusion, our results demonstrate that inhaled frusemide produces protection against NKA-provoked bronchoconstriction in asthmatic subjects and indicate a role either for the inactivation of neural pathways or the local production of inhibitory prostaglandins. Whatever the mechanism, the present findings further support the view that the mechanism of action of NKA in asthma is likely to be indirect. However, further work is necessary to investigate the exact role and the mode of action of NKA in human airways.

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