Effect of inhaled frusemide on responses of airways to bradykinin and adenosine 5'-monophosphate in asthma

K Rajakulasingam, R Polosa, M K Church, P H Howarth, S T Holgate

Abstract

Background – Inhaled frusemide exerts a protective effect against bronchoconstriction induced by several indirect stimuli in asthma. This effect could be caused by interference with neural pathways. The effect of inhaled frusemide on bronchoconstriction induced by inhaled bradykinin, which is thought to cause bronchoconstriction via neural mechanisms, was studied and compared with the effects of adenosine 5'-monophosphate (AMP) which probably produces its airway effects by augmenting mast cell mediator release and interfering with neural pathways.

Methods – Patients first underwent AMP and bradykinin challenges. They were then studied in a randomised, placebo controlled, double blind fashion. Ten asthmatic subjects, studied on four days, were pretreated with inhaled frusemide (40 mg) or placebo for 10 minutes, five minutes before challenge with increasing concentrations of nebulised AMP or bradykinin.

Results – On the open visit days the provocative concentrations required to reduce forced expiratory volume in one second (FEV₁) by 20% from baseline (PC₂₀) for AMP and bradykinin were 16-23 (1-42-67-16) and 2-75 (0-81-6-6) mg/ml. There was a significant correlation between baseline AMP and bradykinin PC₂₀ values. For AMP the geometric mean PC₂₀ values following pretreatment with inhaled frusemide and matched placebo were 80-97 (9-97->400-0) and 14-86 (2-6-104-6) mg/ml respectively (95% CI 0-49 to 0-98). For bradykinin the geometric mean PC₂₀ values following pretreatment with inhaled frusemide and matched placebo were 13-22 (2-53->16-0) and 2-52 (0-45-5-61) mg/ml respectively (95% CI 0-43 to 1-01). Frusemide afforded 5-45 and 5-24 fold protection against AMP and bradykinin-induced bronchoconstriction respectively. Furthermore, there was a significant correlation between protection afforded to the airways against AMP and bradykinin.

Conclusions – These data suggest that inhaled frusemide affords protection against bradykinin-induced bronchoconstriction which is comparable to that against AMP, supporting a common mechanism of action for frusemide.

The loop diuretic frusemide, when administered by inhalation, protects the asthmatic airways against various bronchoconstrictor stimuli such as allergen, ultrasonically nebulised distilled water, exercise, cold air, sodium metabisulphite, and adenosine 5'-monophosphate (AMP). The mechanisms underlying the protective effects of this drug against these different forms of provocation in asthma are not understood. Based on structure-activity studies with other loop diuretics the protective efficacy of inhaled frusemide is thought not to involve the ATP-dependent Na⁺-K⁺-Cl cotransport. Other possibilities include the capacity of this drug to locally generate prostaglandin E₂ (PGE₂) with functional antagonistic effects, a suppressive action on airway mast cells, and inhibition of neural pathways. In support of an effect on airway nerves, frusemide has been shown to inhibit the cough response induced by low chloride aerosols, and both inhaled 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 independent of cyclooxygenase production in guinea pigs. Bradykinin is produced by the action of kallikreins on high molecular weight kininogen. Inhaled bradykinin induces bronchoconstriction in a dose-dependent fashion in asthmatic subjects. In vivo structure-activity studies have suggested that bradykinin produces bronchoconstriction by stimulating β₂ receptors. Studies in guinea pigs indicate an important role for tachykinin release from sensory neurones as a major pathway mediating the constrictor effects of bradykinin in this species. Bradykinin-induced bronchoconstriction is partially blocked by inhaled ipratropium bromide, and Ichinose et al have shown that FK 224, a neurokinin 1 (NK₁) antagonist, affords eight fold protection against bradykinin-induced bronchoconstriction in asthma. Furthermore, nedocromil sodium and disodium cromoglycate, two drugs known to interfere with sensory nerve fibre discharge, also afford protection against this mediator. On the other hand, both the selective histamine H₁ receptor antagonist, terfenadine, and the potent inhibitor of cyclooxygenase, flurbiprofen, exert only minimal protective effects on bradykinin-induced bronchoconstriction in asthma.
The effect of inhaled frusemide on bradykinin-induced bronchoconstriction has not been reported. In this study we have investigated the effect of inhaled frusemide against bronchoconstriction provoked by bradykinin and compared it with that of AMP which probably produces its airway effects by augmenting mast cell mediator release and excitation of neural pathways. We have also assessed the degree of protection afforded by frusemide against both stimuli as, if a similar degree of protection was found, this would support a common mechanism of action for this drug in the airways.

**Methods**

**Subjects**

Ten asthmatic subjects (eight males) with a mean (SE) age 25.5 (2.4) years participated in the study. All subjects were non-smokers with atopic asthma as judged by at least one weal > 3 mm on skin prick testing with *Derma-topagoides pteronyssinus*, house dust, mixed grass pollen, cat fur, and feathers (Bencard, Brentford, Middlesex, UK). Their baseline FEV₁ was > 75% of predicted values and none were receiving oral corticosteroids or theophylline, but five were on low dose inhaled beclometasone (table 1). Treatment with inhaled β₂ agonists was withdrawn at least eight hours before each visit to the laboratory, although subjects were allowed to continue inhaled corticosteroids as usual. Patients 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. The study was approved by the Southampton University and Hospitals ethical subcommittee and written informed consent was given by all subjects involved in the study.

**Bronchial provocation tests**

**Bronchial challenge with adenosine 5'-monophosphate and bradykinin**

Adenosine 5'-monophosphate (AMP) (Sigma Chemical Co, St Louis, USA) was made up in 0.9% sodium chloride in a series of doubling concentrations ranging from 0.39 to 400 mg/ml (4.48–1151.4 mmol/l). Bradykinin triactic acid (Nova Biochem, Nottingham, UK) was dissolved in phosphate buffered saline (pH 7.4) to produce a concentration range of 0.03–16 mg/ml (0.028–15.09 mmol/l). To avoid loss of bradykinin through oxidation and adherence to plastic surfaces the stock solution was stored at 4°C before use and broncho-provocation was performed within 30 minutes of preparing the dilutions. The solutions were administered as aerosols generated from a starting volume of 3 ml in a Cirrus Mininebuliser (Intersurgical, Middlesex, UK) driven by compressed air at 8 l/min. Under these conditions the nebuliser generates an aerosol with a mass median particle diameter of 4.1 µm.

Bronchial challenge was performed with a technique modified from that of Chai et al. Measurements of FEV₁ were made using a dry wedge spirometer (Vitalograph, Buckinghamshire, UK). Before challenge, after 15 minutes rest, three baseline measurements of FEV₁ were made at three minute intervals and the highest value recorded. Subjects then inhaled nebulised 0.9% sodium chloride (saline) taking five slow breaths from functional residual capacity (FRC) to full inspiration, and FEV₁ measurements performed at one and three minutes, the higher value being recorded. If this value was 10% of the initial FEV₁, then bronchial provocation with AMP or bradykinin was undertaken. Increasing concentrations of agonists were inhaled at five minute intervals until the FEV₁ had fallen by > 20% of the post-diluent baseline value, or until the highest concentrations of agonist had been administered. The percentage fall in FEV₁, from post-diluent baseline was plotted against the cumulative concentration of agonist administered, and the provocative concentration of agonist required to produce a 20% fall in FEV₁ from the post-diluent baseline (PC₉₀ FEV₁) derived by linear interpolation of the last two points.

**Study design**

The study was divided into two phases.

**Phase 1**

Subjects attended the laboratory on three separate occasions at least 72 hours apart to undertake concentration response studies with inhaled AMP, bradykinin, and histamine in the absence of any drug treatment in order to establish the baseline level of response.

**Phase 2**

Subjects attended the laboratory on four occasions, separated by at least 72 hours, to undertake concentration response studies with inhaled AMP and bradykinin after nebulised frusemide or matched nebulised vehicle placebo. These were administered in a double blind and random fashion. On each occasion, after 15 minutes rest, three baseline measurements of FEV₁ were recorded at intervals of three minutes. This was followed by inhalation of nebulised frusemide (10 mg/ml, pH 9, osmolarity 289 mosmol/kg) or matched placebo (pH 9, osmolarity 298 mosmol/kg). The aerosol solutions were generated from a start-

**Table 1 Characteristics of subjects**

<table>
<thead>
<tr>
<th>Subject no</th>
<th>Age (years)</th>
<th>PC₂₀ histamine (mg/ml)</th>
<th>FEV₁ (% predicted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>0.23</td>
<td>96.0</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>0.5</td>
<td>105.0</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>0.5</td>
<td>86.0</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>1.0</td>
<td>95.0</td>
</tr>
<tr>
<td>5</td>
<td>23</td>
<td>0.25</td>
<td>98.0</td>
</tr>
<tr>
<td>6</td>
<td>33</td>
<td>1.3</td>
<td>78.0</td>
</tr>
<tr>
<td>7</td>
<td>18</td>
<td>2.4</td>
<td>82.0</td>
</tr>
<tr>
<td>8</td>
<td>32</td>
<td>0.44</td>
<td>94.0</td>
</tr>
<tr>
<td>9</td>
<td>38</td>
<td>0.27</td>
<td>105.0</td>
</tr>
<tr>
<td>10</td>
<td>21</td>
<td>1.2</td>
<td>102.0</td>
</tr>
<tr>
<td>Mean (SE)</td>
<td>25.5 (2.4)</td>
<td>0.60</td>
<td>94.1 (3.1)</td>
</tr>
</tbody>
</table>

* Geometric mean.
Effect of inhaled frusemide on bradykinin-induced bronchoconstriction

Table 2  PC<sub>20</sub> values (mg/ml) for AMP and bradykinin (BK)

<table>
<thead>
<tr>
<th>Subject no</th>
<th>Baseline PC&lt;sub&gt;20&lt;/sub&gt;AMP</th>
<th>Baseline PC&lt;sub&gt;20&lt;/sub&gt;BK</th>
<th>Post-placebo PC&lt;sub&gt;20&lt;/sub&gt;AMP</th>
<th>Post-placebo PC&lt;sub&gt;20&lt;/sub&gt;BK</th>
<th>Post-frusemide PC&lt;sub&gt;20&lt;/sub&gt;AMP</th>
<th>Post-frusemide PC&lt;sub&gt;20&lt;/sub&gt;BK</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>43-72</td>
<td>80-5</td>
<td>&gt;400-0</td>
<td>4-4</td>
<td>2-6</td>
<td>6-8</td>
</tr>
<tr>
<td>2</td>
<td>12-8</td>
<td>2-66</td>
<td>20-69</td>
<td>0-81</td>
<td>0-45</td>
<td>2-53</td>
</tr>
<tr>
<td>3</td>
<td>22-38</td>
<td>7-11</td>
<td>85-1</td>
<td>6-6</td>
<td>3-7</td>
<td>1-10</td>
</tr>
<tr>
<td>4</td>
<td>11-92</td>
<td>6-11</td>
<td>9-9</td>
<td>5-85</td>
<td>3-38</td>
<td>&gt;1-60</td>
</tr>
<tr>
<td>5</td>
<td>18-3</td>
<td>22-25</td>
<td>70-31</td>
<td>1-9</td>
<td>2-64</td>
<td>7-7</td>
</tr>
<tr>
<td>6</td>
<td>17-88</td>
<td>15-55</td>
<td>122-38</td>
<td>5-38</td>
<td>4-4</td>
<td>&gt;1-60</td>
</tr>
<tr>
<td>7</td>
<td>13-04</td>
<td>12-02</td>
<td>153-26</td>
<td>1-47</td>
<td>0-63</td>
<td>&gt;1-60</td>
</tr>
<tr>
<td>8</td>
<td>20-84</td>
<td>49-75</td>
<td>5-9</td>
<td>2-01</td>
<td>5-61</td>
<td>7-42</td>
</tr>
<tr>
<td>9</td>
<td>1-42</td>
<td>2-6</td>
<td>1-08</td>
<td>1-63</td>
<td>3-36</td>
<td>1-331</td>
</tr>
<tr>
<td>10</td>
<td>67-16</td>
<td>104-6</td>
<td>&gt;400-0</td>
<td>5-41</td>
<td>4-78</td>
<td>&gt;1-60</td>
</tr>
<tr>
<td>Geometric mean (range)</td>
<td>16-23 (1-42-67-16)</td>
<td>7-5 (2-6-104-6)</td>
<td>8-97 (9-97-400-0)</td>
<td>2-75 (0-81-6-6)</td>
<td>2-52 (0-45-5-61)</td>
<td>1-322 (2-53-16-0)</td>
</tr>
</tbody>
</table>

Results

Baseline values of FEV<sub>1</sub> did not differ significantly between any of the six study days, the mean values being 3-68 (0-2), 3-63 (0-2), 3-58 (0-19), 3-5 (0-2), 3-6 (0-18), and 3-61 (0-24) for open AMP and bradykinin days, AMP-active frusemide and AMP-placebo frusemide days, and bradykinin-active frusemide and bradykinin-placebo frusemide treatment days, respectively. There were no significant differences in baseline values of FEV<sub>1</sub> after placebo or frusemide between any of the study days.

All 10 subjects exhibited bronchial responsiveness to inhaled AMP and bradykinin, the geometric mean (range) concentrations required to produce a 20% decrease in FEV<sub>1</sub> (PC<sub>20</sub> FEV<sub>1</sub>) values being 16-23 (1-42-67-16) and 2-75 (0-81-6-6) mg/ml, respectively. On a molar basis AMP was therefore approximately 17-9 (4-0-48-3) fold less potent than bradykinin in reducing FEV<sub>1</sub>. A significant correlation was observed between baseline PC<sub>20</sub>AMP and bradykinin values (r = 0-58, p < 0-05). However, there was no correlation between bronchial responsiveness to histamine and either AMP or bradykinin.

Inhaled frusemide had a significant protective effect against the fall in FEV<sub>1</sub>, produced by AMP, the geometric mean (range) PC<sub>20</sub> increasing from 14-86 (2-6-104-6) after placebo to 80-97 (9-97-400-0) mg/ml after frusemide (95% CI 0-49 to 0-98) (fig 1, table 2). The same dose of frusemide was also effective in protecting against bradykinin-induced bronchoconstriction, the geometric mean (range) PC<sub>20</sub> increasing from 2-52 (0-45-5-61) mg/ml after placebo to 13-22 (2-53-16-0) mg/ml following frusemide pretreatment (95% CI 0-43 to 1-01) (fig 2, table 2). When expressed as concentration ratios frusemide afforded 5-45 fold (> 2-5 doubling dilution) and 5-24 fold (> 2-5 doubling dilution) protection of the Airways against AMP and bradykinin respectively. A significant correlation existed for the capacity of frusemide to protect against bronchoconstriction provoked by AMP and by bradykinin (p = 0-05, r<sub>s</sub> = 0-51).

Discussion

This study confirms previous findings that inhaled bradykinin and AMP both caused dose-related bronchoconstriction in asthmatic subjects. Within the group a significant correlation existed between the baseline PC<sub>20</sub> values for AMP and bradykinin. We have also shown that frusemide administered in an inhaled dose of about 28 mg (of which about 10% reaches the Airways) produced an approx-
approximately fivefold protection against bronchoconstriction induced by both AMP and bradykinin. Since these agonists are considered to produce bronchoconstriction by differing mechanisms — bradykinin involving sensory nerve stimulation and tachykinin release, and AMP augmenting mast cell mediator release and, to a lesser extent, via neural stimulation — the mechanism of protection afforded by frusemide is puzzling.

The lack of correlation between histamine and either bradykinin or AMP bronchial reactivities supports indirect mechanism(s) of action for bradykinin and AMP. Three subjects (nos 2, 3, and 8) have shown more than a doubling concentration difference in PC20 for AMP on baseline and placebo days (table 2). We have previously shown, however, that coefficients of repeatability were within single doubling concentrations for AMP and bradykinin.\(^6\)\(^9\)

Since the original description by Bianco et al of the inhibitory effect of inhaled but not oral frusemide against exercise-induced asthma,\(^3\)
further studies have shown that it affords little or no protection against histamine-induced bronchoconstriction and only minor protection against methacholine. In providing protection against different "indirect" stimuli such as allergen, fog, exercise, cold air, sodium metabisulphite, AMP, and now bradykinin, however, the inhibitory effect must embrace a mechanism or mechanisms common to all these stimuli.

Frusemide is known to produce some of its effect in the kidney by the secondary production of endogenous prostanoids and in bovine tracheal mucosa it produces PGE₂. Furthermore, a recent study has shown that indomethacin reduced the protective effect of frusemide against exercise-induced asthma, suggesting release of prostaglandins such as PGE₂ and PGI₂ which are both potent functional antagonists. Both human airways and pulmonary vascular endothelial cells are rich sources of PGI₂ and PGE₂. In asthma inhaled PGI₂ has been shown to afford short term protection against stimuli such as exer-
cise,27 fog,27 and PGD,28 in the absence of any consistent bronchodilator effect. Similarly, inhaled PGE, protects against the contractile effect of sodium metabisulphite and metha-
choline in asthma.29 Another loop diuretic, bumetanide, which is also known to induce the generation of prostanoids and act via the Na-
K-Cl cotransporter mechanism in renal tubules, has not been shown to afford protection against AMP.7 This indicates that the airway effects of frusemide may be mediated through pathways independent of those shared with bumetanide. However, the lack of effect of bumetanide might also have been due to its pharmacokinetic properties.

The bronchoconstrictor actions of AMP and Bradykinin are inhibited by disodium cromoglycate and nedocromil sodium18,20 and attenu-
ated by anticholinergic agents.13,31,32 suggesting that excitation of neural pathways may under-
lie these responses. Furthermore, FK 224, a neurokinin 1 antagonist, has been shown to afford protection against bradykinin-induced bronchoconstriction in asthma.33 Mast cell re-
lease of histamine plays no significant part in bradykinin-induced bronchoconstriction in asthma.18 While antihistamines have been shown to afford protection against AMP-
induced bronchoconstriction,31,34 this protection was incomplete and additional pathways are likely to be involved. One possibility is that vagal reflexes may contribute directly to the bronchoconstriction induced by inhaled pur-
ines in asthma, as suggested by Pauwels et al.35 Furthermore, Polosa et al have shown that inhaled irtrapatrom bromide affords a sig-
nificant 2-5 fold protection against AMP-
induced bronchoconstriction in asthma.31 However, the exact stimulus prompting the vagal reflex activation remains to be clarified. Possibilities include a direct effect of purines in the stimulation of cholinergic reflexes, or indirect activation through the release of mast cell mediators such as histamine and prosta-
glandins. In support of the ability of histamine to influence vagal airway tone, both atropine and inhaled hexamethonium bromide have been shown to be effective inhibitors of hista-
mine-induced bronchoconstriction through cholinergic and ganglionic blockade respect-
ively.36,37

Peachell et al have shown that, in immuno-
logically activated human lung mast cells, ad-
enosine not only enhances histamine release but also potentiates production of prosta-
noids.38 Furthermore, in asthma, cyclo-
 oxygenase blockade has been shown to inhibit the bronchoconstrictor response provoked by inhaled purines.39,40 As the effects of prosta-
noids on airway calibre are known to be medi-
ated in part by vagal reflexes,41 it is possi-
ble that adenosine-induced production of prostanoids from mast cells contributes to bronchoconstriction via cholinergic pathways. Some evidence has accumulated to indicate that inhaled frusemide might alter neural activity in the airways. In healthy subjects inhaled frusemide inhibits cough responses induced by low chloride aerosols.42 Further evidence derives from the observation that both frusemide and bumetanide inhibit the airway smooth muscle contraction induced by stimulation of non-cholinergic non-adrenergic nerves independent of cyclooxygenase produc-
tion.12

Our observations of a similar degree of pro-
tection produced by frusemide against the air-
ways response to inhaled AMP and brady-
kinin, and the finding of a significant correlation between the degree of protection against both stimuli, suggest that inhaled fru-
semide may act in a manner different from that of the other "indirect" stimuli. Since many of the other "indirect" stimuli also involve neural reflexes to a greater or lesser extent, this seems the most likely component of the bronchoconstrictor response that is fru-
semine sensitive.

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