Effect of Inhaled Amiloride on the Bronchial Response to Methacholine and Cold Air Hyperventilation Challenges*

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Inhaled amiloride has been recently demonstrated to have an effect on the decline of pulmonary function in patients with cystic fibrosis. Other diuretics have been demonstrated to provide protection against bronchoconstriction in asthmatic subjects. We report on the effect of inhaled amiloride on cold air hyperventilation challenge (CAHC) and methacholine challenge in asthmatics. We studied nine subjects with mild-moderate asthma in a double-blind, placebo-controlled, crossover study. Our results showed amiloride did not significantly protect against the bronchoconstriction induced by CAHC. Inhaled amiloride did not affect FEV,

in the hour after inhalation, and there was no significant difference between placebo or amiloride on the dose of methacholine causing a 20 percent fall in FEV. Inhaled amiloride appears not to have a profile of action as previously seen with inhaled furosemide.

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ANOVA = analysis of variance; CAHC = cold air hyperventilation challenge; PEFR = peak expiratory flow rate; PGE = prostaglandin E

Many asthmatic subjects develop bronchospasm with exercise. Furthermore, hyperventilation will simulate exercise-induced bronchospasm, which can be further enhanced by the inhalation of subzero air.\textsuperscript{1,2} Cold air hyperventilation challenges (CAHC) have been shown to significantly correlate with nonspecific bronchial hyperreactivity.\textsuperscript{3} The mechanism by which the indirect bronchoconstrictive challenges, such as CAHC and exercise, induce bronchospasm is not known. Hyperosmolarity of the mucosal fluid, secondary to loss of water, may result in mast cell degranulation and release of mediators, and it has been proposed as a possible mechanism for exercise-induced bronchoconstriction.\textsuperscript{4,5} Although exercise challenge has been shown to cause a significant systemic increase in mast cell mediators, CAHC has not.\textsuperscript{6,7}

It has long been known that cromolyn sodium protects against bronchoconstriction induced by indirect challenges such as hypertonic and hypotonic solutions, exercise, cold air, metabisulfite, and also allergen challenges, although the mechanism of protection is not known. Inhalation of furosemide has been shown to attenuate the bronchoconstriction induced by many of these same indirect challenges in asthmatic subjects.\textsuperscript{6-12}

Furosemide has been shown to inhibit the basolateral Na\textsuperscript{+}-K\textsuperscript{+}-2Cl\textsuperscript{−} co-transport mechanism in tracheal epithelial cells.\textsuperscript{9,10} The mechanism of protection afforded by furosemide against indirect challenges is unknown, but it has been proposed to be related to a stabilization of the change in the osmotic milieu of the airway mucosa or an inhibition of ion flux in airway smooth muscle in response to the mediators released from mast cells.\textsuperscript{13} Furosemide does not protect against bronchoconstriction when given systemically, suggesting a local effect when given by inhalation.\textsuperscript{9} Neither cromolyn sodium\textsuperscript{10} nor furosemide\textsuperscript{11,12} protect against methacholine-induced bronchoconstriction.

Amiloride, another diuretic agent, inhibits several sodium transporters, including sodium-entry channels in epithelium, and the universal Na\textsuperscript{+}/H\textsuperscript{+} exchanger. Aerosolized amiloride has been shown to have beneficial effects in cystic fibrosis, thought to be, in part, due to sodium transport inhibition.\textsuperscript{14} Inhaled amiloride has been shown to attenuate the bronchoconstricition induced by specific antigen challenges in guinea pigs, both in vivo\textsuperscript{15} and in vitro.\textsuperscript{16}

The purpose of this study was to determine if inhaled amiloride would attenuate the bronchoconstrictive effects of CAHC. In addition, the effect of amiloride on pulmonary function and on the bronchoconstrictive effect of methacholine was determined.

METHODS

Nine subjects with mild to moderate asthma were selected for study (Table 1). Each subject was required to be free of respiratory infection symptoms for 6 weeks prior to the study, and all subjects were required to be nonsmokers. Methylnaphthine and caffeine products were stopped for 24 h, antihistamine therapy was stopped for 3 days, and treatment with inhaled beta\textsubscript{2}-agonists was stopped for 8 h before each visit. Subjects also did not use oral or inhaled steroids or inhaled cromolyn sodium during the study or for 1 week prior to their individual starting date. The protocol was approved by the Creighton University Institutional Review Board, and all patients signed an informed consent. Exclusion criteria included a history of hypertension, renal disease, heart disease, or the use of any other medication except standard antiasthmatic preparations.
On the screening day (Table 2), each subject underwent a routine pulmonary function test (PFT) that included FVC, FEV₁, peak expiratory flow rate (PEFR), and FEV₂₀⁻₇₅ percent measurements. Following this, the subject underwent a CAHC of 5 min duration, as previously described. The minute ventilation (Ve) for the challenge was determined by multiplying the baseline FEV₁ by 20, and was kept constant at each of the remaining CAHC study days. The PFT values were recorded at 3, 6, 9, 12, and 15 min following the CAHC. Each subject was required to have a baseline FEV₁ of >70 percent of predicted and a fall in FEV₁ post-CAHC of >15 percent to qualify.

On study day 2, after baseline PFTs were recorded, the subject inhaled 3.5 ml of a solution of 3 mg of amiloride hydrochloride (Merck, Sharpe, and Dohme) dissolved in 10 ml of one-third strength normal saline solution (10⁻³ M, pH 7.0). Amiloride was not soluble in normal saline solution, and doses greater than 3 mg were relatively insoluble even using one-third strength normal saline solution. Larger concentrations of amiloride can be dissolved in distilled water, but concerns for bronchoconstriction induced by hypotonic solutions limited the final concentration of soluble amiloride in a less hypotonic solution. Aerosolization of 3.5 ml of solution required 10 min using a nebulizer (DeVilbiss model 646, DeVilbiss Co, Somerset, Pa) and a slow, deep breathing technique. Immediately following the inhalation, PFTs were recorded every 15 min for 1 h.

The next four study days (Table 2) were set up in a double-blind, crossover manner for the study drug and a randomized assignment for the challenges. On each of the four visits, baseline PFTs were recorded before the inhalation of placebo (normal saline solution) or amiloride. Fifteen minutes after inhalation of the solution, PFTs were again recorded and used as baseline measurements. A CAHC or methacholine challenge followed.

Methacholine challenges were performed following a method previously described. The dilution increments of methacholine were 0.1, 0.2, 0.6, 2.0, 6.0, 20.0, and 60.0 mg/ml. The inhalation procedure included five slow inhalations of methacholine from functional residual capacity to inspiratory capacity at each step without breath holding until a 20 percent decrease in FEV₁ was obtained. This was followed by an additional six inhalations of 60.0 mg/ml, if required. The total possible amount of methacholine inhaled at the completion of the test was 804.5 breath units. The mean output of each nebulizer for one breath is 0.031 ml.

**Statistical Analysis**

Differences in baseline FEV₁ were determined by analysis of variance (ANOVA). Baseline and postamiloride FEV₁ values were compared using ANOVA. The data from the CAHC from the three study days on which it was performed, including the screening visit 1, were compared via ANOVA, and any statistically significant changes were compared with the Student's paired t test. Since a dose-response curve for CAHC was not performed, the analysis was done with the percent fall FEV₁ at each time period after drug. The methacholine PD20 was calculated, and a Student's paired t test was used to analyze these data. A p value of 0.05 or less was considered significant. The results are expressed using a mean and standard deviation.

**Results**

The clinical data for the subjects are listed in Table 1. The baseline FEV₁ of each study day was not significantly different, and is shown in Figure 1, expressed as percent of FEV₁, on study day 1. An analysis demonstrated that amiloride had no effect on FEV₁ values, expressed as percent of baseline, up to 1 h after inhalation on study day 2 (Fig 2). The calculated PD20 for methacholine on the amiloride treatment day was 38.2 ± 47.6 breath units and on the

**Table 1—Clinical Features**

<table>
<thead>
<tr>
<th>Patient/ Sex/Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>Asthma Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/21</td>
<td>187.5</td>
<td>87.75</td>
<td>Theophylline, albuterol</td>
</tr>
<tr>
<td>2/M/23</td>
<td>160</td>
<td>67.5</td>
<td>Theophylline, metaproterenol</td>
</tr>
<tr>
<td>3/M/25</td>
<td>185</td>
<td>92.7</td>
<td>Albuterol</td>
</tr>
<tr>
<td>4/M/24</td>
<td>177.5</td>
<td>78.75</td>
<td>Albuterol</td>
</tr>
<tr>
<td>5/M/24</td>
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<td>Albuterol</td>
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<tr>
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<td>190</td>
<td>101.25</td>
<td>Theophylline, metaproterenol, cromolyn</td>
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<tr>
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<td>90</td>
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</tr>
<tr>
<td>9/M/25</td>
<td>175</td>
<td>76.5</td>
<td>Albuterol</td>
</tr>
</tbody>
</table>

**Table 2—Protocol**

- **Day 1** → Screening Cold Air Challenge (CAHC)
- **Day 2** → Amiloride
- **Day 3/4** → Placebo or Amiloride and CAHC
- **Day 5/6** → Placebo or Amiloride and Methacholine Challenge

**Figure 1.** Mean FEV₁ on each study as compared with screening day, expressed as mean ± SD.

**Figure 2.** Effect of inhaled amiloride on FEV₁ (mean ± SD) for 1 h after inhalation expressed as percent of baseline FEV₁ (mean ± SD).
placebo treatment day was 28.1 ± 58.5 breath units (Fig 3). As illustrated in Figure 4, the FEV₁ fell significantly at 3, 6, 9, 12, and 15 min after CAHC whether the subjects were treated with placebo or amiloride, and amiloride did not significantly attenuate bronchoconstriction. Bianco et al. used a similar method to determine the effect of furosemide on exercise. There were no adverse side effects or symptoms from the inhaled amiloride, including cough or diuresis.

DISCUSSION

Although amiloride has shown a protective effect against antigen-induced bronchoconstriction in guinea pigs, our study shows that it has no effect on the CAHC-induced bronchoconstriction in asthma subjects. Amiloride did not induce bronchodilation and did not significantly attenuate methacholine-induced bronchoconstriction.

Methacholine causes smooth muscle constriction directly. Antigen binds to IgE receptors to cause mast cell degranulation and mediator release, which causes bronchoconstriction. Exercise, ultrasonically neutralized distilled water, and possibly CAHC are thought to stimulate the release of mediators from mast cells to induce bronchoconstriction indirectly. Based on this, a distinction between indirect and direct bronchial hyperresponsiveness has been suggested. Furosemide has been found to attenuate the bronchoconstriction induced by a variety of indirect challenges, including CAHC in a similar study design, while this study shows amiloride does not.

In cystic fibrosis, amiloride has been shown to have a beneficial effect. Excess sodium absorption across a chloride impermeable epithelium is associated with the viscous airway secretions in cystic fibrosis. Amiloride blocks or inhibits sodium and volume absorption and thereby liquifies secretions.

The effect of amiloride in preventing bronchoconstriction in antigen challenges in animal models of asthma has been linked to its effects on sodium influx transport channels. The inhibition of sodium absorption attenuates contraction and electrical responses of airway smooth muscle in antigen challenge but does not block response to histamine, similar to the effect seen in a sodium-deficient medium.

One possible reason amiloride was not effective is the difference between the action of amiloride on Na⁺ channels and the Na⁺-K⁺-2Cl⁻ co-transporter that furosemide affects. Both amiloride and furosemide are diuretics that inhibit sodium transport channels. Furosemide's inhibition of the Na⁺-K⁺-2Cl⁻ co-transporter channel is on an electrically neutral chloride entry process with effects on chloride secretion but not sodium absorption. A decrease in intracellular chloride is the result. Amiloride, however, inhibits various sodium channels and prevents the absorption of sodium into the cell. Sodium inhibition by amiloride is not electrically neutral as is found with furosemide. It is not known whether these differences in ion channel inhibition can explain the different pulmonary effects seen with these drugs. It has also been hypothesized that ion inhibition stabilizes mast cells; however, CAHC has not specifically been shown to involve mast cells and mediator release, as its mechanism of inducing bronchoconstriction is unknown.

There are several additional possibilities to explain the result previously reported for the diuretic furosemide and that seen with amiloride in this report. Furosemide is relatively lipid-insoluble, which allows for prolonged mucosal surface action. Furosemide has greater protection to bronchoconstriction than bumetanide, a potent diuretic, but which has greater lipid solubility than furosemide. Due to the high

![Figure 3](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21665/)

**Figure 3.** Methacholine PD20 before and after amiloride. The asterisk indicates the mean value for the respective day.

![Figure 4](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21665/)

**Figure 4.** The graph shows the change from baseline (mean ± SEM) for FEV₁ after CAHC.
hydrophilicity of amiloride, we would expect the potential effect of amiloride on epithelial cell surface to be prolonged, as compared with furosemide. Intravenous furosemide causes release of prostaglandin E₂ (PGE₂), a potent bronchodilator.28 Whether inhaled furosemide has the potential to release intrapulmonary PGE₂ is not known, nor is information regarding the local release of prostaglandins with inhaled amiloride available. In addition, furosemide, but not amiloride, is a weak carbonic anhydrase inhibitor.29 Other carbonic anhydrase inhibitors are able to reduce bronchoconstriction induced by cold air hyperventilation.30 Recent evidence suggests inhaled furosemide modifies thermal heat lost during hyperventilation of frigid air, possibly through an effect on bronchial circulation.37

In this report, amiloride did not afford the protection against CAHC that has been shown previously using inhaled furosemide.9,12 This substantiates a report that showed 10 ml of inhaled amiloride (10⁻² M) did not block inhaled histamine challenge in asthmatics19 and suggests our results were not dependent on dose. Whether amiloride’s inability to protect against CAHC is unique to this particular challenge requires further evaluation using antigen and other indirect challenges.

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