Effects of Atropine on the Potentiation of Exercise-Induced Bronchospasm by Cold Air

Running Title: Cold Air, Atropine and Exercise-Induced Asthma

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ABSTRACT:

The role of vagal afferent activity in the cold air potentiation of bronchial obstruction after exercise in asthma was assessed by exercising 9 asthmatic subjects who breathed air at ambient and sub-freezing temperatures both before and after cholinergic blockade. Lung volumes and maximal expiratory flow-volume curves with air and with 80% helium - 20% oxygen were obtained before and 5 to 10 minutes after each exercise challenge. Isovolume comparisons of maximal expiratory flow rates with the two gases were used to assess relative contributions of large and small airways to flow limitation. Exercise under ambient conditions resulted in the expected airway obstruction and cold air exaggerated the response. Although in a few individuals pretreatment with atropine inhibited the ambient response, potentiation by subfreezing air continued to occur in all subjects. After atropine with ambient exercise, there was a consistent increase in the relative contribution of large airways to flow limitation while exercise with cold air resulted in a disproportionate increase in the contribution of small airways. We conclude that the potentiating effects of cold air are local rather than vagally mediated and suggest that the immediate stimulus is related to cooling of intrathoracic airways. Both the greater response and the greater small airway contribution suggest a deeper penetration of incompletely conditioned air when exercise is performed in the cold.

Airway obstruction, thermal effects on airways, heat and water exchange,
INTRODUCTION

Previous experiments from this laboratory have demonstrated that when asthmatics inhaled subfreezing air while performing an exercise challenge, the post-exertional bronchospastic response was markedly accentuated even though the physical stress of exercise was unaltered by cold as measured by ventilation and heart rates (16). Since exercise alone, and cold air breathing at rest, produced considerably less alterations in pulmonary mechanics individually than when combined, we concluded that these two naturally occurring stimuli interacted positively.

Theoretically there are several possible mechanisms for the synergism of cold and exercise depending upon where in the tracheobronchial tree the frigid inspirate is conditioned. If it were to be fully conditioned (i.e., brought to body temperature and saturated with water vapor) before reaching the posterior pharynx, then combining the observation that the local application of ice to the face of normals can cause a fall in specific conductance (10) with the fact that the bronchoconstrictor effects of cold air breathing at rest in asthmatics can be abolished with atropine pre-treatment (15), one could postulate that the enhancement outlined above could have resulted from a superimposition of the exercise response upon a background of increased or increasing vagal efferent tone secondary to cold induced stimuli arising from sensory endings above the larynx. If, on the other hand, the hyperpnea of exercise overwhelmed the heat exchanging capacity of the upper air passages, then colder than normal air would reach the lower trachea or beyond. If this were to
happen, then direct and indirect thermal and/or vago-vagal reflex airway effects could occur depending upon the extent of the temperature derangements in the local environment. In an effort to sort this matter out, our first approach was to study the effects of cholinergic blockade upon the cold response. Our observations form the basis of this report.

METHODS

Nine asymptomatic, atopic individuals, 2 men and 7 women, with reproducible exercise-induced asthma previously documented in our laboratory, served as subjects. Their mean age was 22.0 ± 1.7 (S.D.) years. All had strong allergic histories, and none were smokers. All refrained from taking any medication for at least 12 hours prior to any study day. None had used glucocorticoids or cromolyn sodium for at least a month before these studies. Informed consent was obtained from each participant.

Lung volumes and maximal expiratory flow-volume (MEFV) curves were recorded in an integrated-flow, pressure-corrected plethysmograph (12) whose volume signal was statically calibrated prior to each set of measurements. This signal was pressure corrected by means of shaping a rapid step input of volume approximating a square wave (11). The pneumotachograph used to record flow at the mouth was linearized in the expiratory direction according to Finucane, et al. (7) and calibrated over a wide range of flows. Lung volumes were determined by Boyle's law (5), and the resulting data represent the mean of five determinations.
All signals were appropriately transduced and monitored using a multichannel time base recorder (Hewlett Packard 7700-D). Box volume and mouth flow signals were also displayed as the x-y coordinates of a Hewlett Packard 141 A storage oscilloscope, and the resulting curves were photographed. To compare MEFV curves, maximal flows (Vmax) at an absolute lung volume equal to 70% of the control total lung capacity (TLC) were used and given the notation Vmax iso. The mouth flow signal was also electronically integrated and used to derive one second forced expiratory volumes (FEV₁). Maximum forced exhalations were performed in triplicate, and the subject's best effort, as defined by the MEFV curve with the largest vital capacity and highest Vmax, was used for analysis.

Density dependence of maximal flow was assessed by having the subjects breathe a gas mixture of 80% helium and 20% oxygen (HeO₂) until the nitrogen concentration at the end of a maximal exhalation was less than 5% as determined by a Med-Science Electronics 300 AR Nitralyzer. These data were also obtained in triplicate, and the MEFV curves with the highest maximal flows of which vital capacities on air and HeO₂ matched exactly were chosen. The data from two subjects had to be excluded because their post-exercise studies did not meet these criteria. The degree of density dependence of Vmax was assessed as the ratio of Vmax HeO₂ to Vmax air at 50% of the vital capacity (4,13,22).

For each MEFV curve, volume history was standardized as follows: After 15 seconds of quiet breathing, a shutter in the mouthpiece was closed at functional residual capacity and the subjects panted at that
lung volume. They then exhaled to residual volume (RV) and inhaled maximally, but not forcefully, to TLC following which the MEFV curve was obtained during the subsequent forced exhalation.

Cold air was produced by having the subjects inspire through a heat exchanger which was externally cooled by circulating isopropyl alcohol which was maintained at a temperature of -35°C. The exchanger consisted of a heavily insulated, 76 cm long copper tube with an internal diameter of 6.5 cm, equipped with a 10.7 cm (ID) one-way valve on the inspiratory port. Inspired air temperatures in all experiments were continuously recorded by a thermocouple situated in the airstream within the exchanger and located 10 cm upstream from the mouth.

Our experiments were performed on two separate days. In the first series, we reconfirmed the effects of cold air breathing on the pulmonary mechanical response to exercise and studied its effects on density dependence. This was accomplished by having the subjects perform two bouts of exhausting leg work on a cycle ergometer while breathing air at ambient or subfreezing temperatures in a random fashion through duplicate heat exchangers, one of which was kept cold as described above, the other kept at room temperature. Upon completion of the initial study, the subjects were allowed to rest for at least 1.5 hours while their pulmonary function returned to pre-exercise levels. The exercise was then repeated using identical work loads, RPM, and durations for each individual. The mean workload was $800 \pm 225$ (S.D.) kpm, and the mean duration of exercise was $3.9 \pm 1.3$ (S.D.) minutes.
Exhausting leg work was used as the provocational stimulus because it was technically easier to have the subjects breathe through the ex-changers from a fixed seated position. Previous experience with this form of maximum work has demonstrated that it is a highly effective and reproducible means of inducing bronchospasm (16-19). Similarly, the duration of work, the interval between studies, and the number of studies that could be performed within a day had all been previously verified as being appropriate (8,13,16-19).

Pulmonary mechanics were measured before and 5 to 10 minutes after cessation of work. Again, prior experiments demonstrated that this time sequence would coincide with the maximal response (8,13,16-19).

On the second study day, the procedures followed were identical to the first, except that atropine sulfate was administered by nebulizer before exercise commenced. In order to determine that sufficient atropine was being given, dose-response relationships were determined for parasympathetic stimulation as follows. Specific conductance (SGaw) was measured in a constant volume plethysmograph by standard techniques (1,6), and a 0.01% solution of methacholine was aerosolized by a DeVilbiss 42 nebulizer. The greatest change seen in SGaw during the next 5 minutes was accepted as the response. The subjects were allowed to recover and then the effect of a 0.1% solution was determined.

Atropine was then administered by inhalation in sufficient quantities to block the response to a 1.0% concentration of methacholine. After this had been accomplished, pulmonary mechanics were measured as
above, following which the first exercise challenge of the day commenced. Again, cold and ambient conditions were randomized. When the subjects recovered from the effects of exercise, the presence of continued vagal efferent blockade was assessed by repeat challenge with the 1% methacholine solution and atropine was given as required to insure its persistence. After we established that cholinergic blockade was still in effect, the second exercise challenge of the day began.

The data were analyzed using a two-factor analysis of variance and paired t-tests.

RESULTS

During the course of the first day's studies, the ambient room temperature ranged from 22.8 to 25.0°C (mean = 24.3 ± 1.3°C (S.D.)). The air temperature in the cold experiments varied from -8.4 to -14.5 and averaged -10.5 ± 2.6°C.

The effects of exercise on pulmonary mechanics while breathing ambient and subfreezing air are summarized in Figure 1. Exercise under ambient conditions produced the expected airway obstruction. One-second forced expiratory volumes and Vmax iso fell significantly from control and RV rose. When cold air was inspired, the effects of exercise provocation were significantly enhanced, and the previously recorded alterations in lung function were markedly accentuated in both absolute (Figure 1) and relative terms (Figure 2). The data in Figure 1 also demonstrate that recovery between trials was complete in that the pre-exercise baselines for each variable were statistically identical.
Figure 3 summarizes the methacholine challenges in all of our subjects on the second study day. In the first experiment, SGaw fell significantly \((p < 0.001)\) with both the 0.01 and 0.1% concentrations. After the administration of 4 mg of atropine, 100 times the previously effective quantity of methacholine failed to change airway caliber. The third section of this figure demonstrates that vagal efferent blockade was still present before the second exercise challenge of day 2. An additional 2 mg of atropine were administered to produce this effect.

The air temperatures for the two experiments on the second study day were 25.6 ± 1.4 and -13.0 ± 1.1°C for the ambient and cold experiments, respectively. There were no significant between day differences in mean air temperatures for either experiment.

Figure 4 displays the absolute data when exercise was performed after atropine pretreatment. Although the baseline values are significantly greater than on day 1 because of the bronchodilation produced by atropine, airway obstruction still developed, and all variables changed significantly from their control values in both the ambient and cold air studies.

When the magnitude of the changes observed on days 1 and 2 were compared (i.e., studies with and without atropine), the following was found. Figure 5 demonstrates that cholinergic blockade failed to influence either the ambient or cold responses for \(\text{FEV}_1\) or \(\text{Vmax iso}\). However, atropine pretreatment did diminish slightly the rise in RV under both exercise conditions. The reason for this is that hidden in the mean data
is the fact that 3 of our 9 subjects had their ambient air responses to exercise nearly abolished. However, all nine subjects continued to develop bronchospasm when cold air was breathed. By comparing the hatched bars for each variable in Figure 5, it can be seen that cold air breathing continued to result in greater obstruction than did ambient air. (Mean baseline response differences: \( \text{FEV}_1 \) ambient \( = 0.59 \pm 0.48 \text{ L} \) (S.D.); cold \( = 0.83 \pm 0.47; p < 0.05 \); \( \dot{V}_{\text{max}} \) iso ambient \( = 1.48 \pm 1.16 \text{ L/sec} \); cold \( = 2.26 \pm 1.23; p < 0.05 \); RV ambient \( = 0.48 \pm 0.41 \); cold \( = 0.77 \pm 0.52; p < 0.05 \).

Figure 6 shows the individual density dependence data for the 7 subjects with reliable data. Prior to the administration of atropine, the results under ambient conditions are similar to those previously reported from our laboratory (13). In 4 subjects with the development of airway obstruction, density dependence decreased, suggesting an increase in the relative contributions of small airways to flow limitation. In the other 3 subjects, this index either increased or remained the same, suggesting an increase or no change in the relative contributions of large airways. Essentially, the same distribution was seen with cold air breathing.

In the right panel of this figure, it can be seen that when reflex effects were removed by cholinergic blockade, the effect on density dependence became more homogeneous. After atropine pretreatment, ambient air exercise now uniformly resulted in an increase in density dependence, and hence the relative contribution of large airways to flow limitation.
Density dependence ambient baseline = 1.31 ± 0.09 (mean ± S.D.); post exercise = 1.53 ± 0.10; t = 4.25; p < 0.01). By contrast, exercise while breathing cold air uniformly resulted in a decrease in density dependence and a shift toward an increase in the relative contributions of small airways. (Density dependence cold baseline = 1.45 ± 0.13; post exercise = 1.31 ± 0.13; t = 2.92; p < 0.05). Of note is the fact that when the effects of atropine on the density dependence ratio were examined by comparing the two ambient baselines, a significant reduction in this ratio was found. (No atropine baseline = 1.44 ± 0.19; atropine baseline = 1.31 ± 0.09; t = 2.47; p < 0.05).

DISCUSSION

The results of this study demonstrate that vagal efferent blockade does not prevent the potentiation of exercise-induced asthma produced by breathing cold air even though it can inhibit the ambient exercise response in selected individuals. Consequently, neither somatic afferent-vagal efferent, nor vago-vagal reflex pathways can be involved in the reaction, and direct or indirect effects at the local airway level must be playing a role. In order for this to occur, the hyperpnea of exercise must overcome the ability of the upper airways to heat and saturate the inspired air to body conditions so that colder, and hence drier, than normal air penetrates into the intrathoracic airways.

Although the available data in the literature demonstrate that during resting ventilation incoming air is almost fully conditioned before reaching the posterior pharynx (2, 3, 21), Cole has shown that
during maximum voluntary ventilation in normals, the temperature of the
gas in the trachea was only 28 to 30°C when air was inspired at tempera-
tures of 18 to 21°C (3). This effect occurred irrespective of whether
the subject was breathing through the nose or the mouth. Given this in-
formation, one would anticipate that the colder the inspired air, the
more exaggerated these differences would be expected to become.

If poorly conditioned air were to reach the lungs, the intratho-
racic airways would be called upon to heat and humidify it, and in so
doing they would lose heat through direct transfer to the gas and by
evaporative water loss. The extent to which this would occur would de-
pend upon the local thermal and water vapor gradients between the gas
and the mucosal surface. In this context, the investigation of Cole al-
luded to earlier is of further interest in that he found that the ex-
pired air began to give up heat and water to the mucosa in more distal
parts of the trachea than normal, presumably because the preceding in-
spiration had lowered the temperature of the airway surface at a deeper
level (3).

We suggest that abnormal airway cooling due to the need to con-
dition large volumes of air within the thorax during exercise may be
part of the reaction sequence by which physical exertion leads to airway
obstruction, and the inhalation of subfreezing air potentiates this re-
response by exaggerating the local thermal burden. In support of this
concept, we have recently shown that exercise-induced asthma can be to-
tally prevented from developing if the inspired air is pre-conditioned
so as to minimize heat flux from the mucosa (19 ).
We believe that cold air potentiation of the ambient response occurs for two reasons. Based upon the relative magnitudes of the heat capacity of air (0.304 cal/L/°C) and the heat of vaporization of water (0.58 Kcal/g), it is apparent that quantitatively the lower the temperature of the gas in the airway (and thus its water content), the greater the thermal burden imposed locally. For example, to fully condition air that enters the respiratory tract at ambient conditions (25°C with 50% relative humidity (11.53 mg H2O/L air)) during a minute ventilation of 60 L, the amount of heat given up by the mucosa would be approximately 1.35 Kcal/min. Sixteen percent of the total would be used to heat the air, and the rest would be expended in vaporizing water. In contrast, to fully condition dry air at -10°C, the energy cost would be 2.39 Kcal/min for the same level of ventilation.

The mechanism by which airway cooling would induce bronchoconstriction in asthmatics is unknown at present. Theoretically, a direct effect could result from mucosal injury or deformation, or stimulation of smooth muscle cells, but there are no data to support these hypotheses. Much more attractive is the possibility that mast cells within the surface of the mucosa could be directly stimulated to release the mediators of anaphylaxis by the thermal conditions of the environment as they are in the skin in response to cold. Again, no direct evidence is available, but it is intriguing that cromolyn sodium (a drug which stabilizes mast cell membranes) has been demonstrated to attenuate the post-exercise response.
To gain some insight into the predominant site of airway response, we examined density dependence of maximal expiratory flow rates. As in a previous study (13), the density dependence changes with ambient air exercise were heterogeneous, and this finding persisted when cold air was breathed. These observations have been interpreted as indicating various admixtures of local and vagally mediated effects in different individuals. Those subjects who increased this ratio or had high initial values that did not change had a much lesser response to ambient air exercise after cholinergic blockade, and the ambient response of those who had low initial values or decreasing ratios was much greater and unaffected by atropine. In contrast to the heterogeneity seen before atropine, after blocking doses of this drug were administered, all subjects increased their density dependence following the ambient air challenge and decreased this ratio with cold air exercise. These observations suggest that, after atropine, the relative contributions of large airways to flow limitation increased under ambient conditions, and that there was a disproportionate increase in the relative contributions of small airways to flow limitation after cold. Since there was significantly greater airway obstruction under the latter experimental conditions, we think it is likely that an increasing small airway, locally mediated response was added to that of the larger airways. This idea is consonant with there being greater penetration of incompletely conditioned gas more deeply into intrathoracic airways when subfreezing air is inhaled during exercise.
The above explanation for post-atropine differences in density dependence with ambient versus cold air exercise leaves us with an unanswered question concerning those subjects who decreased their density dependence after exercise without atropine. If local thermal effects of incompletely conditioned air account for both the magnitude and site of the responses, it is reasonable to ask why these same subjects became predominantly large airway responders to the same stimulus after atropine. We do not have a clear answer to this question. It is possible that the large airway dilatation produced by atropine resulted in sufficient decreases in air velocity at a given volume flow to allow the large airways to become a more efficient heat exchanger. If this occurred, greater mucosal cooling would occur in a more localized area in the tracheobronchial tree. Opposite in sign to the effects of decreasing air velocity is a less favorable mucosal surface area to gas volume ratio in dilated airways. Thus we can only speculate that the former effect predominates over the latter one, and that this in turn could account for the observations presented.
REFERENCES


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FOOTNOTE

1. These calculations were derived from the following formula:

$$ RHG = \dot{V}_E (T_I - T_E) H_{CA} + (W_I - W_E) H_V $$

where 
- $RHG$ = respiratory heat gain or loss
- $\dot{V}_E$ = minute ventilation in L
- $T_I$ = inspired air temperature °C
- $T_E$ = expired air temperature °C
- $H_{CA}$ = heat capacity of air in cal/L/°C
- $W_I$ = inspired concentration of water in g/L
- $W_E$ = expired concentration of water in g/L
- $H_V$ = heat of vaporization of water in Kcal/g
LEGENDS FOR FIGURES

Figure 1. The effects of exercise on pulmonary mechanics while breathing air at ambient and subfreezing temperatures. The data points are mean values and the brackets indicate one standard error. The solid circles and lines represent the ambient temperature studies while the open circles and broken lines are the cold data. FEV₁ = one second forced expiratory volumes; V̇_{max iso} = maximum flow at an absolute lung volume equal to 70% of control total lung capacity; RV = residual volume. The p values below each graph were derived from paired baseline response comparisons. The letters B and R represent the baseline and post-exercise response data, respectively.

Figure 2. Comparison of the relative effects of exercise on pulmonary mechanics while breathing ambient and cold air. The height of the bars represents mean data and the brackets one standard error. The open and hatched bars indicate the ambient and cold studies respectively. The pulmonary mechanical variables are the same as in Figure 1. The p values below each graph were obtained by paired comparisons.

Figure 3. Methacholine dose response curves before and after atropine. Specific conductance (SGAW) is the ordinate and methacholine concentration is the abscissa. The data points are mean values and the brackets represent one standard error. The arrow indicates the steps in the protocol outlined in the text.
Figure 4. The effects of exercise on pulmonary mechanics while breathing air at ambient and subfreezing temperatures after atropine pretreatment. The format is identical to Figure 1.

Figure 5. Comparison of the mean baseline-response differences for the ambient and cold air experiments with and without atropine pretreatment. The left and right hand pairs of bars in each graph are the ambient and cold experiments, respectively. The hatched and unhatched bars indicate studies with and without atropine. The data are mean values and the brackets one standard error. The p values are for comparisons with and without atropine and were derived from a two-factor analysis of variance.

Figure 6. Changes in density dependence under all experimental situations. The closed circles and lines indicate the ambient air experiments and the open circles and broken lines are the cold studies. The letters B and R below each graph represent baseline and post-exercise response data respectively. The p values below each graph were derived from paired baseline response comparisons.
NO ATROPINE

- AMBIENT = 24.3 ± 1.3°C
- COLD = -10.5 ± 2.6°C

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**Fig 1.**

Chart showing the following measurements:

1. FEV₁ (L)
   - Ambient: P < 0.001
   - Cold: P < 0.001

2. Vmax iso (L/sec)
   - Ambient: P < 0.005
   - Cold: P < 0.001

3. RV (L)
   - Ambient: P < 0.005
   - Cold: P < 0.001
AMBIENT = 24.3 ± 1.3°C
COLD = -10.5 ± 2.6°C

% CHANGE FROM BASELINE

FEV₁  Ŷmax iso  RV

P < 0.005  < 0.01  < 0.005

Fig 2
Fig 3

BEST AVAILABLE COPY

POST ATROPINE

- AMBIENT = 25.6 ± 1.4°C
- COLD = -13.0 ± 1.1°C

FEV₁ (L)

- AMBIENT P < 0.01
- COLD P < 0.001

V\text{max iso} (L/sec)

- AMBIENT P < 0.005
- COLD P < 0.001

RV (L)

- AMBIENT P < 0.01
- COLD P < 0.005
Fig 5

Fig 6
a. The views of the author do not purport to reflect the positions of the Department of the Army or the Department of Defense.

b. Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 and USAMRDC Regulation 70-25 on Use of Volunteers in Research.
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Airway obstruction; thermal effects on airways; heat and water exchange; maximum flow

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