The Effect of Pyridostigmine on Bronchial Hyperreactivity*

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We examined the effect of pyridostigmine (PY) at a dose of 30 mg orally three times a day on nonspecific bronchial hyperreactivity in ten normal nonsmokers (NNS), ten smokers (SM), and ten mild asthmatics (AS). We conducted a double-blind, placebo-controlled, crossover trial, randomly assigning subjects to receive either placebo (PL) or PY before undergoing bronchoprovocation challenge with eucapnic voluntary hyperventilation (EHV) using dry gas. Compliance with PY was confirmed by measuring red blood cell acetylcholinesterase (Achase) levels during both days of testing. While taking PL, the mean (±SEM) falls in FVC and FEV₁ after the bronchoprovocation were as follows: NNS, 1.0 percent (±0.6) FVC and 4.3 percent (±1.0) FEV₁; SM, 5.4 percent (±1.1) FVC and 2.7 percent (±1.3) FEV₁; AS, 5.3 percent (±2.3) FVC and 11.5 percent (±2.8) FEV₁.

Pyridostigmine (PY) belongs to a class of organic compounds called carbamates that bind reversibly to acetylcholinesterase (Achase) in synapses and erythrocytes, thereby temporarily inactivating this enzyme. Subsequently, there is an increased amount of acetylcholine (Ach) available to bind to nicotinic and muscarinic Ach receptor sites. It has also been shown recently that PY acts as an agonist for the nicotinic Ach receptor and increases the affinity of the receptor for Ach.1,2

Because of its ability to enhance cholinergic neurotransmission, PY has been used clinically for many years in the treatment of myasthenia gravis. Pyridostigmine has another intriguing application as a pretreatment against exposure to chemical warfare agents. Current US military doctrine dictates that soldiers take PY bromide 30 mg every 8 h in situations where there is a threat of nerve agent attack.3 Nerve agents, by irreversibly binding Achase as well as by having direct effects on central receptor-ion channel complexes, lead to a cholinergic crisis and often death in the untreated patient.4 There is strong evidence that the use of PY combined with current antidote therapy (injectable atropine and oximes) greatly reduces the morbidity and mortality associated with exposure to nerve agents. The theory behind this protective effect of PY is simply that the Achase that is reversibly bound to PY at the time of nerve agent exposure is inaccessible to the toxin; as the effect of PY diminishes, the Achase will become available to metabolize Ach, allowing nearly normal neuromuscular transmission. As studies of drug efficacy against nerve agents cannot be safely conducted in man, this prophylactic strategy is based on animal experiments.5,6

People who have been accidentally exposed to high concentrations of organophosphates (a situation mimicking nerve agent exposure) exhibit various manifestations of a cholinergic crisis.4 It is known that in these people, increased muscarinic activity causes excessive bronchial secretions and bronchospasm, and increases in nicotinic activity cause muscle fatigue and paralysis.

Pyridostigmine, through the various mechanisms previously mentioned, acts as a mild neuromuscular poison. We hypothesized that pretreatment with PY...

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The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army, Uniformed Services University of the Health Sciences, or the Department of Defense. The research was approved by the Walter Reed Army Medical Center Clinical Investigation Committee and the Human Use Committee, Institutional Review Board. All subjects enrolled into the study voluntarily agreed to participate and gave written informed consent. Funding for the study was supported by the Department of Clinical Investigation protocol 1742. Manuscript received April 21; revision accepted October 7.

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The mean decreases in FVC and FEV₁, while taking PY were as follows: NNS, 1.8 percent (±0.7) FVC and 4.3 percent (±0.8) FEV₁; SM, 3.8 percent (±1.4) FVC and 5.2 percent (±1.6) FEV₁; AS, 4.4 percent (±1.3) FVC and 11.8 percent (±2.8) FEV₁. Within each category, using a paired t test to compare the results on each day of testing, no statistically significant differences were noted. Pyridostigmine at the tested dose has no significant effect on nonspecific bronchial hyperreactivity in normal NNS, SM, or AS.

Ach = acetylcholine; Achase = acetylcholinesterase; AS = asthmatics; EHV = eucapnic voluntary hyperventilation; NNS = normal nonsmokers; PL = placebo; PY = pyridostigmine; SM = smokers

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Table 1—Descriptive Statistics

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Smokers</th>
<th>Asthmatics</th>
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</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>33.9 (±2.1)</td>
<td>36.8 (±2.2)</td>
<td>34.4 (±1.8)</td>
</tr>
<tr>
<td>Sex</td>
<td>8M/2F</td>
<td>4M/6F</td>
<td>8M/2F</td>
</tr>
<tr>
<td>FVC, % pred</td>
<td>106.4 (±3.6)</td>
<td>101.3 (±2.5)</td>
<td>101.6 (±1.9)</td>
</tr>
<tr>
<td>FEV₁, % pred</td>
<td>104.0 (±3.3)</td>
<td>97.7 (±2.4)</td>
<td>92.4 (±3.2)</td>
</tr>
<tr>
<td>FEF25-75, % pred</td>
<td>94.0 (±6.4)</td>
<td>85.5 (±6.6)</td>
<td>76.3 (±9.5)</td>
</tr>
</tbody>
</table>
would increase the response to nonspecific bronchial challenge and that this effect would be more pronounced in people with potential or preexisting bronchial hyperreactivity (eg, smokers\textsuperscript{2} and asthmatics).

**Materials and Methods**

**Subject Population**

A total of 30 subjects were enrolled, all of whom were older than 20 years and younger than 50 years of age. Ten subjects were entered in each of the following groups: normal controls, asymptomatic smokers, and mild asthmatics. All subjects had normal baseline screening spirometry. Normal control subjects were lifelong nonsmokers or previous smokers with less than a five-pack-year history of smoking and no tobacco use in the previous five years. In addition, they had no respiratory symptoms, no history of asthma or other chronic respiratory illness, no upper respiratory tract infections for the previous three weeks, and no other confounding medical problems (heart disease, obesity, malnutrition, etc).

The smoking group used at least one half package of cigarettes (ten cigarettes) per day for the previous year, had no new respiratory symptoms for the previous three weeks, and had no other medical problems. Patients with mild asthma were identified either retrospectively by chart review or prospectively at the time of referral to the Pulmonary Clinic for evaluation of symptoms consistent with asthma or exercise-induced bronchospasm. They had normal results of baseline screening pulmonary function tests; in addition, they had a “qualifying” eucapnic voluntary hyperventilation (EVH) challenge that demonstrated the presence of a reversible obstructive ventilatory defect with a fall in FEV\textsubscript{1}, of at least 9 percent as compared with baseline. Asthma patients also had sufficiently mild conditions that they did not require the regular use of medications to carry on their routine activities of daily living. We chose to study patients with mild asthma for two reasons. For safety considerations, we did not want to expose people with more severe disease to a bronchoprovocation challenge after taking PY. From a more practical standpoint, the military prescreen individuals with asthma in that these people are either not allowed to enter active duty or if on active duty would not be deployed into a combat environment.

**Evaluations**

Each subject was given either four 30-mg tablets of PY or four placebo (PL) tablets. They were instructed to take these pills at 6 AM, 2 PM, and 10 PM on the day prior to the first challenge, and at 6 AM on the day of the challenge. Study subjects then underwent an evaluation of bronchial reactivity by performing a bronchoprovocation challenge with EVH. This test has been described previously and is well tolerated by patients and normal subjects.\textsuperscript{14} Following baseline spirometry, the subject breathed a compressed gas mixture (21 percent O\textsubscript{2}, 74 percent N\textsubscript{2}, and 5 percent CO\textsubscript{2} at essentially 0 percent relative humidity) at a target minute ventilation of 30 times his baseline FEV\textsubscript{1}, for 6 min. The inspired gas mixture was delivered at ambient temperature, which was held between 22° and 24°C. Spirometry was repeated immediately after and at 5, 10, and 30 min following challenge, and then every 15 to 30 min until the FEV\textsubscript{1} was within 10 percent of the prechallenge value. Each test consisted of at least three forced maneuvers with the greatest value of FEV\textsubscript{1}, taken to represent airflow at that time. The lowest FEV\textsubscript{1} observed postchallenge was used to determine the fall in FEV\textsubscript{1}.

A second EVH challenge test was performed on a subsequent day (no later than six weeks after the first test), while the subject took the crossover treatment. A comparison of the degree of bronchial reactivity was made between the first and second challenge tests. If the subject showed an increase in bronchial reactivity of 50 percent (eg, the FEV\textsubscript{1}, changes from a 10 percent decrease with PL to a 15 percent decrease while receiving PY), the subject was considered to be sensitive to the effects of PY. In addition, serum levels of RBC Achase were drawn each day of bronchoprovocation to ensure compliance with and to assess the effect of PY therapy.

**Statistical Analyses**

Within each category (normal nonsmokers [NNS], smokers [SM], and asthmatics [AS]), we used a paired t test to compare the effect of PL and PY on the response to EVH. A p value of 0.05 was considered to be statistically significant. One-way analysis of variance was used to evaluate the effect of PY on decreases in RBC Achase activity between categories. Again, a p value of 0.05 was considered to be statistically significant. The effect of PY on nonspecific bronchial hyperreactivity, as manifested by the difference between changes in FVC and FEV\textsubscript{1}, after EVH or bronchoprovocation caused by PY. Group mean data are presented as an arithmetic average ± the SEM.

**Results**

Descriptive statistics for the subjects enrolled in each category are presented in Table 1 (data expressed as arithmetic means ± SEM). All subjects had normal results of baseline screening spirometry. The RBC Achase levels (reported in milliliters per milliliter) were measured for all subjects on both days of testing. Five samples could not be analyzed; subsequently, comparisons could be made on only 25 of 30 subjects. The mean decrease in RBC Achase level for subjects while taking PY (as compared with their baseline while taking PL) was 21.4 percent. Within each category, the mean decreases in RBC Achase activity were as follows: NNS (seven subjects), 17.6 percent (± 6.6); SM (nine subjects), 24.5 percent (± 9.7); AS (nine subjects), 21.5 percent (± 11.4). These differences were not statistically significant among groups (p = 0.89). Pyridostigmine also had no effect on baseline spirometry (ie, prechallenge FVC and FEV\textsubscript{1} were not different within groups comparing values obtained on each day of testing). The mean percentage decline in FVC and FEV\textsubscript{1} after bronchoprovocation challenge and differences between responses while taking PY and PL (expressed in terms of 95 percent confidence intervals) are reported in Table 2. Pyridostigmine had essentially no effect on the response to EVH in any of the groups tested. The largest difference in mean percentage of decline in FEV\textsubscript{1} occurred in the smoking subjects (Fig 1); this difference was neither statistically significant (p = 0.21) nor clinically significant.

**Discussion**

We chose to use EVH as the method for bronchoprovocation challenge because it would best stimulate the environment that troops would be exposed to (eg, exercise/hipernea in a dry environment). Though bronchoprovocation with inhaled methacholine is cer-
Table 2—Mean Percentage Decline in Spirometry Following Eucapnic Voluntary Hyperventilation

<table>
<thead>
<tr>
<th></th>
<th>FVC (%)</th>
<th>FEV₁ (%)</th>
</tr>
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<tbody>
<tr>
<td><strong>Normal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (PL)</td>
<td>1.00 (±0.58)</td>
<td>4.28 (±1.05)</td>
</tr>
<tr>
<td>Pyridostigmine (PY)</td>
<td>5.35 (±0.72)</td>
<td>4.27 (±0.84)</td>
</tr>
<tr>
<td>PY-PL</td>
<td>0.79</td>
<td>-0.01</td>
</tr>
<tr>
<td>95% CI</td>
<td>-0.76 to 2.33</td>
<td>-1.77 to 1.75</td>
</tr>
<tr>
<td>p value</td>
<td>0.28</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>Smoker</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>2.39 (±1.10)</td>
<td>2.70 (±1.33)</td>
</tr>
<tr>
<td>Pyridostigmine</td>
<td>3.82 (±1.36)</td>
<td>5.22 (±1.63)</td>
</tr>
<tr>
<td>PY-PL</td>
<td>1.43</td>
<td>2.52</td>
</tr>
<tr>
<td>95% CI</td>
<td>-2.79 to 5.64</td>
<td>-1.71 to 6.74</td>
</tr>
<tr>
<td>p value</td>
<td>0.46</td>
<td>0.21</td>
</tr>
<tr>
<td><strong>Asthmatic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>5.35 (±2.29)</td>
<td>11.51 (±2.79)</td>
</tr>
<tr>
<td>Pyridostigmine</td>
<td>4.45 (±1.35)</td>
<td>11.77 (±2.77)</td>
</tr>
<tr>
<td>PY-PL</td>
<td>-0.90</td>
<td>0.26</td>
</tr>
<tr>
<td>95% CI</td>
<td>-4.03 to 2.24</td>
<td>-2.54 to 3.06</td>
</tr>
<tr>
<td>p value</td>
<td>0.53</td>
<td>0.84</td>
</tr>
</tbody>
</table>

It has been well documented that cholinergic neurotransmission plays a significant role in determining bronchial smooth muscle tone (and therefore airways resistance) in normal humans.¹⁴⁻¹⁶ Previous reports demonstrating the effects of Achase inhibitors on the respiratory system have been somewhat contradictory. In 1971, Ringquist and Ringquist¹⁷ reported an increase in airways resistance following intramuscular injection of neostigmine. This response was dose dependent. In contrast, De Troyer and Borenstein¹⁸ published a report in 1981 looking at the effects of intramuscular PY in four normal subjects and in eight patients with myasthenia gravis. They found no changes in airway resistance in either group. However, normal subjects received only 2 mg of PY intramuscularly—a very low dose.

Shale and his associates¹⁹ studied the effects of PY on respiratory function in 20 patients with myasthenia gravis. Eight had evidence of airways obstruction at baseline; six of these patients had extensive pulmonary function testing. After taking a PL inhaler and 60 or 120 mg of PY orally, airways resistance as measured by whole body plethysmography increased significantly. Interestingly, inhaled ipratropium bromide prevented the effect of PY on airways obstruction. In 1988, Liggett et al.²⁰ described three patients with COPD and myasthenia gravis whose respiratory symptoms worsened with PY, and subsequently improved with inhaled ipratropium bromide. To our knowledge, there are no other studies in the English literature regarding the effects of PY on respiratory function, either in normal subjects or in people with known airway hyperreactivity.

This study reveals that PY at a dose of 30 mg orally three times a day has no significant effect on nonspecific bronchial hyperreactivity as measured by EVH.

![Graph](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21672/)

**Figure 1.** Placebo vs pyridostigmine. Mean percentage decline following eucapnic voluntary hyperventilation (EVH).
in normal subjects, smokers, and mild asthmatics. This
dose of PY was chosen for study because it is the
dose that soldiers would use in the setting of a possible
nerve agent exposure. Though this study was initiated
well before the onset of hostilities in the Gulf region,
Operation Desert Storm provided a unique opportu-
nity for the Army to retrospectively assess the clinical
effect of PY on a largely healthy population.31 Though
about one half of the approximately 40,000 soldiers
surveyed after taking PY experienced some symptoms
that they felt were attributable to the drug, only 1
percent sought medical attention. From a respiratory
standpoint, only three soldiers complained of “wors-
ening of acute bronchitis,” and one soldier with a
history of asthma had bronchospasm that seemed to
be temporally related to the administration of PY. This
analysis, despite its shortcomings, supports the con-
tention that PY at this dose does not cause a significant
impairment in respiratory function in normal subjects.

Previous studies have suggested a dose-dependent
increase in airways resistance and/or a worsening in
lung function induced by PY if subjects had some
degree of obstructive lung disease. 17,19,20 It is quite
possible—even probable—that had we used a higher
dose of PY or investigated subjects with more severe
asthma, we would have detected a clinically significant
increase in airway hyperreactivity and/or a decline in
baseline spirometry values. These issues were not
particularly relevant to our study as individuals with
asthma are generally not allowed to enter or remain
on active duty in the US Army. This may warrant
further investigation, however, as some patients with
myasthenia gravis will certainly have obstructive lung
disease and require higher doses of PY to control their
symptoms.

We conclude that PY pretreatment does not pose a
risk of causing or aggravating mild asthma. This is
supported by anecdotal survey of experience during
the Gulf War.

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REFERENCES
1 Rickett DL, Glenn JF, Houston WE. Medical defense against
2 Wachtel RE. Comparison of anticholinesterases and their effects
on acetylcholine-activated ion channels. Anesthesiology 1990;
77:496-503
3 Pyridostigmine pretreatment for nerve agents. US Army Acad-
emy of Health Sciences Field Circular 9-48, 26 March 1987
4 Dunn MA, Sidell FR. Progress in medical defense against nerve
agents. JAMA 1989; 262:499-502
5 Caldwell RW, Lowensohn HS, Chrysanthis MA, Nash CB.
Interactions of pyridostigmine with cardipulmonary systems
and their relationships to plasma cholinesterase activity. Fundam
Appl Toxicol 1989; 12:432-41
6 Kluwe WM, Page JG, Tofa JD, Riddler WE, Chung H. Phar-
macological and toxicological evaluation of orally administered
pyridostigmine in dogs. Fundam Appl Toxicol 1990; 14:40-53
7 Ellin RI, Kaminski A. Carbamoylated enzyme reversal as a
means of predicting pyridostigmine protection against soman. J
Pharm Pharmacol 1989; 41:633-36
8 Maxwell DM, Brecht KM, Lenz DE, O’Neill BL. Effect of
carbamoylcholine inhibition on carbamate protection against
soman toxicity. J Pharmacol Exp Ther 1988; 246:986-91
9 Sidell FB. Soman and sarin: clinical manifestations and treat-
ment of accidental poisoning by organophosphates. Clin Toxicol
1974; 7:1-17
10 Cerveri I, Bruschi C, Zoia MC, Zanen P, Maccarini L, Grassi,
M, et al. Distribution of bronchial nonspecific reactivity in the
11 Phillips YI, Yaeger JJ, Laube BL, Rosenthal RR. Eucapnic
voluntary hyperventilation of compressed gas mixture: a simple
system for bronchial challenge by respiratory heat loss. Am Rev
Respir Dis 1985; 131:31-5
12 Eliasson AH, Phillips YY, Rajagopal KR, Howard RS. Sensitivity
and specificity of bronchial provocation testing: an evaluation of
four techniques in exercise-induced bronchospasm. Chest (in
press)
13 Kennedy SM, Burrows B, Vedal S, Enarson DA, Chan-yueng
M. Methacholine responsiveness among working populations:
relationship to smoking and airway caliber. Am Rev Respir Dis
1990; 142:1377-83
14 Widdicombe JG, Kent DC, Nadel JA. Mechanism of broncho-
constriction during inhalation of dust. J Appl Physiol 1962;
17:613-16
15 Ingram RH, Wellman JJ, McFadden ER, Mead J. Relative
contributions of large and small airways to flow limitation in
normal subjects before and after atropine and isoproterenol. J
Clin Invest 1977; 59:696-703
16 Hensley MJ, O’Cain CF, McFadden ER, Ingram RH. Distribution
of bronchodilation in normal subjects: beta agonist vs
17 Ringquist I, Ringquist T. Changes in respiratory mechanics in
myasthenia gravis with therapy. Acta Med Scand 1971; 190:509-
18
18 De Troyer A, Borenstein S. Acute changes in respiratory
mechanics after pyridostigmine injection in patients with
19 Shale DJ, Lane DJ, Davis JF. Air-flow limitation in myasthenia
gravis: the effect of acetylcholinesterase inhibitor therapy on
20 Liggitt SB, Daughaday CC, Senior RM. Ipratropium in patients
with COPD receiving cholinesterase inhibitors. Chest 1989;
90:210-12
21 Keeler JR, Hurst CG, Dunn MA. Pyridostigmine used as a
nerve agent pretreatment under wartime conditions. JAMA
1991; 266:690-95