Airway Hyperresponsiveness to Methacholine in Subjects With Spinal Cord Injury*

Effie Singas, MD; Marvin Lesser, MD; Ann M. Spungen, EdD; William A. Bauman, MD; and Peter L. Almenoff, MD, FCCP

Previously, we found that never-smokers with quadriplegia were hyperresponsive to aerosolized methacholine. To further explore the phenomenon, we compared responsiveness to methacholine in never-smokers with that of smokers and ex-smokers. We also evaluated responsiveness in subjects with high paraplegia (lesions at T-1 to T-6) or low paraplegia (lesions at T-7 and below). We found that smokers and ex-smokers with quadriplegia were hyperresponsive to methacholine (provocative concentration causing a 20% fall in FEV₁ = 1.9 mg/mL), and that the response was comparable to that found in never-smokers, revealing that hyperresponsiveness among never-smokers cannot be attributed to preinjury airway hyperreactivity that precluded cigarette use. In contrast, subjects with low paraplegia were not hyperresponsive to methacholine. Among subjects with high paraplegia, the three subjects demonstrating airway hyperresponsiveness had significantly lower FEV₁ (percent predicted). The findings support the hypothesis that airway hyperresponsiveness in subjects with quadriplegia represents loss of sympathetic innervation of the lung, thereby leaving intact unopposed bronchoconstrictor cholinergic activity. However, reduced lung volumes in these subjects also suggest the possibility that airway hyperresponsiveness is due to loss of ability to stretch airway smooth muscle by deep breathing.

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Abbreviations: FRC = functional residual capacity; PC20 = provocative concentration causing a 20% decrease in FEV₁; RV = residual volume; TLC = total lung capacity; VC = vital capacity

Pulmonary function studies in subjects with quadriplegia have consistently demonstrated restrictive ventilatory impairment; measurement of lung volumes has revealed significant reductions in vital capacity (VC), inspiratory capacity, and expiratory reserve volume. Because flow rates were reduced in proportion to the reduction in VC, the presence of an obstructive airway component was not suspected. Recently, however, it was observed that approximately 40% of subjects with cervical spinal cord injury without histories of reactive airway disease had significant bronchodilatory responses following inhalation of metaproterenol sulfate or ipratropium bromide, thereby revealing that resting airway tone is increased in a significant number of subjects. The amplified response to bronchodilators was postulated to be due to the loss of sympathetic airway innervation, which originates from the upper six thoracic ganglia, thereby leaving intact unopposed bronchoconstrictor parasympathetic (cholinergic) activity, which arises from the vagal nuclei of the brainstem and passes down the vagus nerves.

To further investigate airway responsiveness in subjects with quadriplegia, eight otherwise healthy individuals were challenged with increasing concentrations of aerosolized methacholine. All of the subjects demonstrated marked airway hyperresponsiveness, which was completely blocked by pretreatment with ipratropium bromide. However, since only nonsmokers were included in this prior study, the possibility existed that hyperresponsiveness represented a preinjury condition, whereby cigarette use was avoided because of preexisting airway hyperreactivity. Therefore, to address this concern, in the current study, the response to methacholine in smokers and ex-smokers with quadriplegia was compared with that found in never-smokers. In addition, we evaluated methacholine responsiveness in subjects with spinal cord injury and intact sympathetic innervation of the lungs (lesions at T-7 and below; low paraplegia) and in subjects whose lesions were at the site of origin of sympathetic fibers (lesions at T-1 to T-6; high paraplegia).

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Veterans Affairs Medical solution patients nebulization breath, had interaction (using chairs). Bronchial concentrations of lung percent predicted. Subsequently, thumbport. (AsthmaKit; None none L airway NY). Spirometry patient Yorba Linda, Calif). All patients were smoking and pulmonary function parameters and PC20 among the three groups (quadriplegia, high paraplegia, and low paraplegia). Fisher’s protected least squares differences was used for all post hoc pairwise comparisons among the groups. A probability level less than 0.05 was considered significant.

### Materials and Methods

**Patients**

Patients were recruited from the Spinal Cord Injury Service, Veterans Affairs Medical Center, Bronx, NY. Prior to their injury, none of the subjects had any history of pulmonary diseases or respiratory symptoms. None required assisted ventilation, and none had respiratory complaints or findings of recent or active pulmonary infections. None were receiving any medication known to affect airway tone or responsiveness. All subjects granted informed consent for the study, which was approved by the Institutional Review Board of the Medical Center.

**Bronchial Provocation Testing**

Spirometry was performed while subjects were seated in wheelchairs (using a Sensor Medics 2100 Automated Pulmonary Function Laboratory; Yorba Linda, Calif). Baseline values were obtained for each patient in compliance with current American Thoracic Society criteria. Results were expressed as absolute values and percent predicted based on spirometry standards established by Morris et al.

Initially, subjects inhaled five breaths of aerosolized normal saline solution from functional residual capacity (FRC) to their total lung capacity (TLC); normal saline solution was administered via a nebulizer (AsthmaKit; Diemolding Healthcare Division; Canastota, NY) driven by air at a flow rate of 8 L/min. Upon initiation of each breath, nebulization was achieved by manual occlusion of a thumbport. Subsequently, by the same methods, increasing concentrations of methacholine (Provocholine; Rouche Laboratories; Nutley, NJ) (0.025, 0.25, 2.5, 10, and 25 mg/mL) were administered. Spirometry was performed 5 min after each challenge or sooner if the patient experienced cough, shortness of breath, or chest tightness. The PC20 was defined as the provocative concentration of methacholine needed to cause a 20% decrease in FEV1. The study was terminated when either the PC20 or maximal concentration of methacholine was reached. A PC20 of less than 8 mg/mL was considered diagnostic for airway hyperresponsiveness.

**Statistical Methods**

The results are reported as mean±SD. An unpaired Student’s t test was used for comparisons within each subgroup. A one-way analysis of variance was used to compare differences in pulmonary function parameters and PC20 among the three groups (quadriplegia, high paraplegia, and low paraplegia). Fisher’s protected least squares differences was used for all post hoc pairwise comparisons among the groups. A probability level less than 0.05 was considered significant.

### Results

A total of 25 patients were studied, 11 with quadriplegia, 6 with high paraplegia, and 8 with low paraplegia. Patient profile and pulmonary function results for the three groups are shown in Table 1. Individual results for each group are shown in Tables 2 through 4. Age did not differ significantly among subjects with quadriplegia, high paraplegia, and low paraplegia (Table 1). The duration of injury was lowest in subjects with quadriplegia. Baseline FVC, FVC percent predicted, FEV1, and FEV1 percent predicted were significantly reduced in subjects with quadriplegia, whereas FEV1/FVC ratios were similar among the three groups. All ex-smoking and active smoking subjects with quadriplegia demonstrated marked hyperresponsiveness to methacholine, with a mean PC20 of 1.9 mg/mL (Table 2), which did not differ significantly from that obtained in the 4 never-smokers (mean PC20 of 3.60 mg/mL).

PC20 values differed markedly among the 6 subjects with high paraplegia (Table 3), with 3 being responsive and 3 being nonresponsive. In this group, there was no apparent relationship between PC20 values and level or completeness of injury. Both never-smokers were unresponsive, as was one ex-smoker. The FEV1 per-
cent predicted was significantly lower (p<0.02) among responders compared with nonresponders; the FVC percent predicted was also lower among responders, although the difference compared with nonresponders did not reach statistical significance. None of the subjects in the low paraplegia group had a significant response to methacholine (Table 4).

**DISCUSSION**

In the current study, we found that methacholine responsiveness in smoking and ex-smoking subjects with quadriplegia did not differ significantly from that found in never-smokers. The PC20 findings in the smoking and ex-smoking subjects (PC20=1.90 mg/mL) was nearly identical to that previously found in a group of never smokers (PC20=1.42 mg/mL). These findings strongly suggest that our previous findings of hyperresponsiveness in never smokers cannot be attributed to preinjury airway hyperreactivity that precluded cigarette use. Cumulative findings from our 2 studies reveal that all 19 subjects with cervical spinal cord injury were hyperresponsive to methacholine, suggesting that this is a consistent finding. In contrast, we found that all subjects with intact autonomic nerve supply to the lungs (low paraplegia) had normal responses to methacholine.

We have previously attributed hyperresponsiveness in subjects with quadriplegia to the loss of sympathetic innervation of the lung, which originates in the upper six thoracic segments of the spinal cord. Postganglionic fibers synapse in the middle and inferior cervical ganglia and the upper four thoracic ganglia and enter the lung at the hilum to intermingle with cholinergic nerves to form a dense plexus surrounding airways and vessels. In contrast, with cervical cord injury, parasympathetic (cholinergic) nerves, which arise in vagal nuclei in the brainstem and pass down the vagus nerves to synapse in ganglia situated in the airway walls, remain intact. Postganglionic fibers pass to target tissues, including airway smooth muscle. Effort stimulation of the vagus nerves causes bronchoconstriction; baseline activation presumably contributes to resting bronchomotor tone. Although there appears to be rudimentary, direct functional sympathetic innervation of human airway smooth muscle, bronchodilatory sympathetic fibers may modulate bronchomotor tone indirectly by interacting with parasympathetic ganglia cells in the peribronchial plexa to influence parasympathetic ganglionic neurotransmission. In dogs, in the presence of vagal bronchoconstriction, electric stimulation of thoracic sympathetic nerves inhibited the constriction of airways in the homolateral lung. In pithed guinea pigs, electric stimulation of thoracic spinal outflow inhibited histamine-induced bronchoconstriction. Of more direct relevance to subjects with cervical spinal cord injury, using guinea pigs, Hey et al found that electrical stimulation of structures within the rostral region of the dorsal medulla activated a vagal cholinergic bronchoconstriction pathway and simultaneously a sympathetic inhibitory input; bronchoconstriction was amplified when the sympathetic inhibitory pathway was blocked by injection of lidocaine at the level of C-1 to C-2 or by surgical section of the cord at that location.

**Table 3—Profile and Findings in Subjects With High Paraplegia**

<table>
<thead>
<tr>
<th>Subject/Age, yr</th>
<th>DOI, yr</th>
<th>LOI (Thoracic)</th>
<th>COI</th>
<th>SMK</th>
<th>FVC (%)</th>
<th>FEV1 (%)</th>
<th>FEV1/FVC</th>
<th>PC20</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/66</td>
<td>37</td>
<td>T5-8</td>
<td>I</td>
<td>Never</td>
<td>4.10 (102)</td>
<td>2.78 (100)</td>
<td>68</td>
<td>&gt;25</td>
</tr>
<tr>
<td>2/44</td>
<td>22</td>
<td>T-4</td>
<td>C</td>
<td>Active (20 yr)</td>
<td>2.94 (53)</td>
<td>2.50 (63)</td>
<td>85</td>
<td>4.22</td>
</tr>
<tr>
<td>3/64</td>
<td>41</td>
<td>T3-4</td>
<td>C</td>
<td>ES (40 yr)</td>
<td>3.83 (77)</td>
<td>3.34 (98)</td>
<td>87</td>
<td>&gt;25</td>
</tr>
<tr>
<td>4/60</td>
<td>36</td>
<td>T-4</td>
<td>C</td>
<td>ES (25 yr)</td>
<td>3.13 (64)</td>
<td>2.29 (66)</td>
<td>73</td>
<td>5.44</td>
</tr>
<tr>
<td>5/55</td>
<td>35</td>
<td>T4-6</td>
<td>C</td>
<td>Never</td>
<td>3.12 (73)</td>
<td>2.91 (93)</td>
<td>93</td>
<td>&gt;25</td>
</tr>
<tr>
<td>6/34</td>
<td>11</td>
<td>T-2</td>
<td>C</td>
<td>Active (8 yr)</td>
<td>4.45 (71)</td>
<td>3.76 (79)</td>
<td>84</td>
<td>4.42</td>
</tr>
</tbody>
</table>

*See Table 2 for explanation of abbreviations.

**Table 4—Profile and Findings in Subjects With Low Paraplegia**

<table>
<thead>
<tr>
<th>Subject/Age, yr</th>
<th>DOI, yr</th>
<th>LOI (Thoracic and Lumbar)</th>
<th>COI</th>
<th>SMK</th>
<th>FVC (%)</th>
<th>FEV1 (%)</th>
<th>FEV1/FVC</th>
<th>PC20</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/63</td>
<td>6</td>
<td>T10-12</td>
<td>C</td>
<td>ES (3 yr)</td>
<td>3.39 (74)</td>
<td>2.48 (70)</td>
<td>73</td>
<td>19.7</td>
</tr>
<tr>
<td>2/48</td>
<td>25</td>
<td>T-11</td>
<td>C</td>
<td>Active (8 yr)</td>
<td>4.09 (88)</td>
<td>3.16 (91)</td>
<td>77</td>
<td>20.7</td>
</tr>
<tr>
<td>3/46</td>
<td>25</td>
<td>L-1</td>
<td>I</td>
<td>Active (25 yr)</td>
<td>3.47 (68)</td>
<td>2.45 (64)</td>
<td>71</td>
<td>18.4</td>
</tr>
<tr>
<td>4/48</td>
<td>27</td>
<td>T12-L2</td>
<td>I</td>
<td>ES (4 yr)</td>
<td>4.15 (73)</td>
<td>2.95 (72)</td>
<td>71</td>
<td>10.8</td>
</tr>
<tr>
<td>5/35</td>
<td>12</td>
<td>L2-3</td>
<td>I</td>
<td>Never</td>
<td>5.01 (93)</td>
<td>3.94 (95)</td>
<td>79</td>
<td>&gt;25</td>
</tr>
<tr>
<td>6/42</td>
<td>24</td>
<td>T9-10</td>
<td>C</td>
<td>Active (50 yr)</td>
<td>3.15 (68)</td>
<td>2.47 (69)</td>
<td>78</td>
<td>&gt;25</td>
</tr>
<tr>
<td>7/54</td>
<td>35</td>
<td>T12-L1</td>
<td>C</td>
<td>ES (20 yr)</td>
<td>4.71 (96)</td>
<td>3.82 (108)</td>
<td>81</td>
<td>&gt;25</td>
</tr>
<tr>
<td>8/34</td>
<td>14</td>
<td>T-12</td>
<td>C</td>
<td>ES (2 yr)</td>
<td>5.03 (101)</td>
<td>3.89 (100)</td>
<td>77</td>
<td>&gt;25</td>
</tr>
</tbody>
</table>

*See Table 2 for explanation of abbreviations.
Alternately, methacholine hyperresponsiveness in subjects with cervical spinal cord injury may be due to alteration in pulmonary function parameters secondary to inspiratory and expiratory muscle dysfunction. In the current study, FVC, FVC percent predicted, FEV₁, and FEV₁ percent predicted were significantly lower in subjects with quadriplegia as compared to those with low paraplegia (Table 1). In addition, subjects with quadriplegia have lower TLC, TLC percent predicted, VC, VC percent predicted, and expiratory reserve volume, and a higher residual volume (RV) and RV percent predicted, whereas FRC and FRC percent predicted are not significantly altered.¹⁹-²¹ In normal subjects, bronchoconstrictor responses to methacholine were enhanced when subjects were studied at FRC minus 0.5 L and reduced at FRC plus 0.5 L.²² The investigators concluded that lung volume is a major determinant of the bronchoconstrictor response to methacholine in normal subjects. They suggested that changes in lung volume act to alter the forces of interdependence between airways and parenchyma that oppose airway smooth muscle contraction. In support, among normal and asthmatic subjects, when methacholine challenge studies were performed during tidal volume breathing, the response to methacholine in normal subjects was amplified and comparable to that found in asthmatic subjects.²³ In subjects with chronic bronchitis or asthma, responsiveness to methacholine correlated inversely with baseline FEV₁.²⁴-²⁶ Among healthy smokers and those with obstructive lung disease, the most dramatic response to methacholine (PC20 <1 mg/mL) correlated significantly with a lower FEV₁, FEV₁ percent predicted, FEV₁/FVC, FEV₁/FVC percent predicted, and lower maximal flow rates at 25% and 50% of FVC.²⁷ Responders also had an increase in RV and FRC, although the increase was not of statistical significance. In the current study, among subjects with high paraplegia, methacholine hyperresponsiveness was noted in the 3 individuals with the lowest FVC percent predicted and FEV₁ percent predicted. However, it is doubtful that reduction in FEV₁ or FVC is the sole explanation for hyperresponsiveness, because subject 8 in the quadriplegia group, who had the lowest PC20, had normal spirometry parameters. Also, subjects 1,3,4, and 6 in the low paraplegia group, who had PC20 values greater than 8 mg/mL, had reduced FVC percent predicted and FEV₁ percent predicted.

Inability of subjects with quadriplegia to inhale deeply may also affect bronchomotor responses to provocative agents. In normal subjects, deep inhalation to TLC induced bronchodilation or had no effect, whereas among symptomatic asthmatic subjects, the maneuver induced bronchoconstriction.²⁸-³⁵ Following methacholine-induced bronchoconstriction, normal subjects transiently reduce airway resistance, whereas asthmatic subjects have a diminished or absent effect.²⁹,³⁰ Of interest, the exaggerated effect of methacholine among normal subjects maintained at tidal volume breathing persisted for a short period after deep breaths were initiated.³¹ It has been suggested that the absence of bronchodilation with deep inhalation among asthmatics is caused by impairment of stretch of airway smooth muscle,²⁵,³⁶ possibly because of increased airway wall stiffness. Histologically, the airways from asthmatic subjects demonstrate increase in thickness of the epithelial, muscle, and submucosal layers.³⁴ The increase in muscle mass may contribute to excessive increase in resistance in response to bronchomotor stimuli.³⁵ Orehek et al.³⁶ noted among asthmatic subjects that the magnitude of fall of FEV₁ provoked by carbachol was greatest in patients in whom deep inspiration increased airway resistance.³⁶ The physiologic responses to deep inhalation and pathologic changes in airways have been attributed to peripheral airway obstruction due to chronic inflammation.³⁷ Similarly, among cigarette smokers, nonspecific airway hyperresponsiveness correlated with starting airway caliber and airway inflammation.²⁷ It is unknown if airways of subjects with quadriplegia are abnormal microscopically. However, it has been proposed that pulmonary compliance is reduced because of basilar microatelectasis secondary to altered mechanical properties of the chest cage.¹,³⁸

Regardless of the underlying cause for methacholine-induced hyperresponsiveness in subjects with quadriplegia, 40% and 48% of the subjects following inhalation of a β₂-agonist or ipratropium bromide,⁷,⁸ respectively, had a significant bronchodilatory response, demonstrating that resting airway tone is increased in a large percentage of these individuals. Underlying airway hyperreactivity may explain the high incidence of respiratory symptoms among subjects with quadriplegia, including chronic cough, wheezing (63%), sputum production (30%), occasional wheezing (30%), and cough (17%).⁹ Also, increased resting bronchomotor tone may contribute to the high incidence of atelectasis and pneumonia,⁴⁰,⁴¹ which are major causes of morbidity and mortality. Long-term treatment of subjects with quadriplegia with a bronchodilator may be postulated to reduce symptoms or pulmonary complications. Of some interest, long-term treatment with ibuprofen, a γ-aminobutyric acid (GABA) agonist frequently prescribed for muscle spasticity, appeared to prevent hyperresponsiveness to methacholine,⁴² without increasing FVC. The agent inhibits neurally induced cholinergic and peptidergic airway constriction.⁴³ In addition, subjects with quadriplegia may have accentuated airway responses following ex-
posure to irritants in their immediate environment, including cigarette smoke, fumes, and dusts.39

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