Pulmonary Afferents Are Not Necessary for the Reflex Inhibition of Human Inspiratory Muscles Produced by Airway Occlusion

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INTRODUCTION

Reflex responses to sudden inspiratory loads or airway occlusion, consisting of an initial short-latency inhibition and a subsequent increase of electromyographic (EMG) activity, have been recorded in a range of human inspiratory muscles with the use of both surface and intramuscular electrodes (Butler et al. 1995; Newsom Davis and Sears 1970; Plasman et al. 1987). The initial inhibitory response (IR) to loading, which may be an important protective reflex (Butler et al. 1995), is of particular interest because the equivalent response in limb muscles (the stretch reflex) usually consists of two phases of excitation with no intervening inhibition (e.g., Hammond 1956; Marsden et al. 1976; Matthews 1989). There has been much debate about the contribution of muscle and cutaneous afferents to the stretch reflex in limb muscles (e.g., Darton et al. 1985; Marsden et al. 1977), the likely spinal and supraspinal pathways (for review see Matthews 1991), and the different behavior of the reflex in different muscle groups (Lenz et al. 1983; Marsden et al. 1976).

Despite the difference between the responses in limb and respiratory muscles, the underlying mechanisms for it have been investigated in only two studies. When the surface of the upper airway was anesthetized (Butler et al. 1995; Newsom Davis and Sears 1970) or bypassed with a cuffed endotracheal tube (Butler et al. 1995), the reflex responses in inspiratory muscles were not abolished. This implies that these responses are produced either by intramuscular receptors (i.e., muscle spindles endings and tendon organs) and/or by intrathoracic receptors.

However, the possible role of intrapulmonary receptors in the production of the potent IRs in inspiratory muscles to sudden loading has never been assessed directly. Both inhibitory and excitatory reflex changes in inspiratory muscle activity have been ascribed to vagal afferents. Intrapulmonary receptors are known to contribute to a robust inhibition of inspiratory muscle activity in response to lung inflation via the Hering-Breuer inflation reflex (Knowlton and Larrabee 1946; van der Grinten et al. 1992; van Lunteren et al. 1988; Widdicombe 1954). Although this reflex is present in sleeping and anesthetized human subjects (Hamilton et al. 1988; Ibor et al. 1995; Widdicombe 1961), the regulation of respiratory activity in awake humans is less clear (Hamilton et al. 1988). In contrast, vagally mediated facilitation during eupnea has been reported for the diaphragm (Bartoli et al. 1975; Di Marco et al. 1981) and the parasternal intercostal muscles (De Troyer 1991). It is not possible to exclude the potential contribution of pulmonary afferents on the basis of the relatively short latency of the inhibition to sudden airway occlusion. Pulmonary afferents conduct at up to 35 m/s in the cat (Paintal 1973), so that there would be sufficient time for afferent conduction from the lung to the spinal cord and then back to the inspiratory muscles (see Butler et al. 1995). In the current study, the aim was to exclude the possibility of a contribution of intrapulmonary receptors to the reflex inhibition of inspiratory muscles to airway occlusion.

There is some evidence that rapidly adapting pulmonary afferents are not involved in the genesis of the reflex responses to airway occlusion. The IR was preserved after subjects inhaled nebulized lignocaine (4%) sufficient to abolish the cough reflex (Butler et al. 1995). This procedure would block the activity of some, but not necessarily all, the superficial rapidly adapting airway receptors, and, on the
basis of animal studies, it is unlikely to have blocked the pulmonary stretch receptors, which are located more deeply and require a greater inhaled concentration of anesthesia (Cross et al. 1976; Fahim and Jain 1979). Because it is not practical in human subjects to block all the afferents from the intrapulmonary receptors (both superficial and deep), we have examined the short-latency reflex responses to a brief airway occlusion in subjects with bilateral pulmonary denervation as a result of recent pulmonary transplantation.

METHODS

Noninvasive experiments were performed on five subjects who had recently received either double lung or heart and lung transplants (10–50 days previously) as treatment for a variety of cardiopulmonary disorders (see Table 1). The subjects were all clinically well at the time of the study but were on a regimen of immunosuppression and antimicrobial medications. For the heart and lung recipients (n = 2), the trachea was divided 1 cm above the carina. For the bilateral lung recipients (n = 3), the main bronchi were sectioned just proximal to their bifurcation. Thus, after these surgical interventions, all intrathoracic afferents below the transthoracic and all intrapulmonary afferents were disconnected from the CNS and could not contribute to reflex responses. In the manuscript we refer to this as pulmonary denervation. Results were compared with those from control subjects with a similar range of ages and heights (22–42 yr and 165–175 cm, respectively; n = 5). The control subjects were studied with the use of identical procedures. All subjects were seated comfortably for measurements of inspiratory muscle reflexes. All procedures were approved by the appropriate institutional ethics committee and informed written consent was obtained.

Measurements of lung function

Standard tests of lung function were performed on subjects seated in a pressure-compensated flow body plethysmograph. Measurements included total lung capacity (TLC), functional residual capacity, vital capacity, forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), and forced expiratory ratio (FVC/FEV1). Individual results for each transplant subject are shown in percentage of the prestimulus EMG level. Although the main aim of the study was to determine the presence or absence of the initial IR to occlusion in subjects deprived of pulmonary afferent input, measurements were also made of the latency to the peak of the excitatory peaks in the EMG records was introduced by Newsom and the edge of the sternum with the other electrode on the sternum. Surface electrodes over the anterolateral chest wall did not provide measurements of the latency to the peak of the excitatory response measured at the intrapulmonary receptors (both super®cial and deep), which are practical in human subjects to block all the afferents from the intrapulmonary receptors (both superficial and deep), we have examined the short-latency reflex responses to a brief airway occlusion in subjects with bilateral pulmonary denervation as a result of recent pulmonary transplantation.

Inspiratory muscle reflexes to occlusion

Subjects breathed through a low-resistance airway at a target inspiratory flow of ~0.5 l/s (achieved with the use of visual feedback displayed on a computer monitor). Inspiratory flow was measured by a pneumotachometer and lung volume was obtained by integration. During random breaths a silent balloon valve (Hans Rudolph, No. 9300, Kansas City, MO) was inflated during inspiration and the airway was occluded for 250 ms. This occlusion produced a small negative change in mouth pressure (measured proximal to the occlusion valve) of 2–8 cmH2O within 10 ms (Fig. 1B). This occlusion halts inspiratory flow and therefore the shortening of the inspiratory muscles, and is thus equivalent to a muscle stretch. Subjects were instructed to “breathe through” the occlusion with a constant inspiratory effort. This is equivalent to the “do not intervene” task commonly used in the studies on limb muscles (e.g., Doemges and Rack 1992).

Surface EMG recordings were made from the scalene muscles bilaterally and from the parasternal intercostal muscles on the right side (Fig. 1A). For the scalene a surface electrode (1 cm diam) was placed in the posterior triangle of the neck at the level of the cricoid cartilage with the other electrode placed 4 cm inferiorly. The sternocleidomastoid muscle is electrically silent during quiet breathing in the seated posture (De Troyer et al. 1994), even in subjects with hyperinflation and increased inspiratory drive (Gandevia et al. 1996). For the parasternal intercostal muscles, one electrode was placed over the third intercostal space 2 cm from the edge of the sternum with the other electrode on the sternum. Surface electrodes over the anterolateral chest wall did not provide records of diaphragmatic EMG during experimental trials of sufficient signal-to-noise ratio for analysis. EMG signals were sampled at 2 kHz, filtered (53 Hz to 1.0 kHz), and stored on computer via a CED 1401 interface (Cambridge Electronic Design, Cambridge, UK). Trials contaminated by electrocardiogram artifact were rejected on-line.

Subjects performed several experimental runs, each consisting of 30 occluded trials. For each trial, EMG, mouth pressure, and lung volume were recorded for 500 ms, including ~125 ms before the occlusion (Fig. 1). Measurements of the onset and peak of the IR (IR onset and IR peak) were made with the use of cursors from the averages of 30 single trials of rectified EMG (see Fig. 1C). The amplitude of the response measured at IR peak was expressed as a percentage of the prestimulus EMG level. Although the main aim of the study was to determine the presence or absence of the initial IR to occlusion in subjects deprived of pulmonary afferent input, measurements were also made of the latency to the peak of the subsequent excitatory response (ER) and its amplitude (ER peak). The terminology IR and ER used to label putative inhibitory and excitatory peaks in the EMG records was introduced by Newsom Davis and Sears (1970). It is used here to permit comparison between the two subject groups rather than to imply the exact

Table 1. Subject information and lung function data

<table>
<thead>
<tr>
<th>Subject</th>
<th>Transplant</th>
<th>Time Since Operation</th>
<th>Prior Disease</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>TLC, l</th>
<th>FRC, l</th>
<th>VC, l</th>
<th>FEV1, l</th>
<th>FVC, l</th>
<th>FER, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1F</td>
<td>Heart/lungs</td>
<td>12 days</td>
<td>Idiopathic pulmonary hypertension</td>
<td>25</td>
<td>168</td>
<td>4.39 (83)</td>
<td>2.74 (98)</td>
<td>3.05 (77)</td>
<td>2.05 (60)</td>
<td>2.61 (67)</td>
<td>78.5</td>
</tr>
<tr>
<td>2F</td>
<td>Heart/lungs</td>
<td>30 days</td>
<td>Eisenmenger's syndrome</td>
<td>31</td>
<td>153</td>
<td>3.15 (73)</td>
<td>2.18 (89)</td>
<td>1.95 (63)</td>
<td>1.37 (51)</td>
<td>1.95 (63)</td>
<td>70.3</td>
</tr>
<tr>
<td>3F</td>
<td>Lungs</td>
<td>50 days</td>
<td>Emphysema</td>
<td>46</td>
<td>154</td>
<td>5.44 (124)</td>
<td>4.14 (166)</td>
<td>2.70 (97)</td>
<td>2.54 (109)</td>
<td>2.67 (98)</td>
<td>95.1</td>
</tr>
<tr>
<td>1M</td>
<td>Lungs</td>
<td>10 days</td>
<td>Emphysema</td>
<td>58</td>
<td>176</td>
<td>7.00 (100)</td>
<td>4.29 (121)</td>
<td>3.83 (90)</td>
<td>3.11 (92)</td>
<td>3.83 (90)</td>
<td>81.2</td>
</tr>
<tr>
<td>2M</td>
<td>Lungs</td>
<td>21 days</td>
<td>Cystic fibrosis</td>
<td>35 ± 7</td>
<td>163 ± 4</td>
<td>5.50 (90)</td>
<td>3.95 (135)</td>
<td>3.29 (67)</td>
<td>2.09 (51)</td>
<td>3.28 (70)</td>
<td>63.7</td>
</tr>
</tbody>
</table>

Values in bottom row are means ± SE for age, height, and predicted lung function values. Values in parentheses are percent of predicted values. Data are shown for each transplant subject including sex (M, male; F, female), organs transplanted, total lung capacity (TLC), functional residual capacity (FRC), vital capacity (VC), forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), and forced expiratory ratio (FER).
FIG. 1. Experimental setup, single trials, and averaged responses to airway occlusion in a heart-lung transplant subject. 

A: experimental setup showing electrode placement over scalenes and parasternal intercostal muscles and occlusion valve setup. B: average traces of mouth pressure (top) and lung volume (bottom) from 1 experimental run (30 trials) in 1 transplant subject (2F). Volume is expressed relative to end-tidal volume. Onset of occlusion is represented by vertical dashed line and is measured from point where inspiratory flow is halted and respiratory integrator resets volume signal to 0. C: 5 single trials of raw electromyogram (EMG, top traces) and average rectified EMG of 30 trials (bottom) from same subject. Points where latencies and amplitudes of responses were measured are indicated.

Unloading responses

The inspiratory muscles that were loaded during airway occlusion were also investigated during unloading. Unloading accompanied by sudden muscle shortening occurs at the end of the airway occlusion as the airway reopens and inspiratory flow recommences. Single trials were aligned with the end of the airway occlusion and the EMG responses were rectified and averaged. These short-latency responses to unloading have been previously observed in control subjects (Newsom Davis and Sears 1970; J. Butler, D. McKenzie, and S. Gandevia, unpublished data). In this study the unloading responses were recorded in the two transplant subjects (subjects 1F and 2M) who were able consistently to maintain an inspiratory effort before and after the occlusion.

Statistics

Results from the transplant subjects were compared with the results from the matched control subjects with the use of a two-way analysis of variance. Post hoc tests (Student-Newman-Kuels) were used to identify differences between the two groups for the responses in the scalene and parasternal muscles. Significance levels were set at $P < 0.05$.

RESULTS

In every transplant subject, despite recent pulmonary denervation, short-latency IRs to brief airway occlusion were observed in single trials and in averages of rectified EMG from both the scalene and parasternal intercostal muscles (Figs. 1C and 2A). All transplant subjects were able to maintain the target inspiratory effort at the start of the occlusion. However, three patients had difficulty sustaining the effort for the duration of the occlusion (250 ms). The reason for this is unclear, but it did not relate to any discomfort...
associated with production of the target inspiratory flow (0.5 l/s). Results for loading at the start of the occlusion are presented for scalenes and parasternal intercostal muscles separately. A brief description of the responses to unloading in two subjects follows.

**Loading responses**

In the five transplant subjects, the IR for the scalenes ($IR_{onset}$) began at 27 ± 2 (SE) ms. The peak of the inhibition ($IR_{peak}$) occurred at 62 ± 9 ms and at this time the preocclusion level of EMG activity was reduced by 50 ± 10%. The subsequent ER was observed for scalenes in all subjects, and the mean latency and amplitude were not significantly different from those in the control subjects (see Table 2 for details).

For the parasternal intercostal muscles, the initial IR was observed in four of the transplant subjects (no responses were observed in 1 subject). $IR_{onset}$ occurred at 29 ± 4 ms. $IR_{peak}$ occurred at 65 ± 11 ms and reduced the preocclusion level of EMG activity by 36 ± 3%. The subsequent ER was also present in all subjects in the parasternal intercostal muscles, but its amplitude varied between subjects, presumably because of the exact task performed by the subject. When there is increased drive to the muscle, the amplitude of the $ER_{peak}$ is
TABLE 2. Individual subject and average data from the scalenes

<table>
<thead>
<tr>
<th>Subject</th>
<th>$IR_{onset}$ Latency, ms</th>
<th>$IR_{peak}$ Latency, ms</th>
<th>$IR_{peak}$ Amplitude, %</th>
<th>$ER_{onset}$ Latency, ms</th>
<th>$ER_{peak}$ Amplitude, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1F</td>
<td>23 ± 3</td>
<td>71 ± 2</td>
<td>-67 ± 4</td>
<td>137 ± 3</td>
<td>34 ± 16</td>
</tr>
<tr>
<td>2F</td>
<td>25 ± 0</td>
<td>84 ± 2</td>
<td>-80 ± 1</td>
<td>184 ± 6</td>
<td>10 ± 7</td>
</tr>
<tr>
<td>3F</td>
<td>24 ± 1</td>
<td>34 ± 0</td>
<td>-28 ± 3</td>
<td>48 ± 1</td>
<td>33 ± 8</td>
</tr>
<tr>
<td>1M</td>
<td>36 ± 3</td>
<td>73 ± 3</td>
<td>-31 ± 2</td>
<td>106 ± 2</td>
<td>35 ± 5</td>
</tr>
<tr>
<td>2M</td>
<td>27 ± 1</td>
<td>46 ± 1</td>
<td>-45 ± 5</td>
<td>98 ± 1</td>
<td>78 ± 10</td>
</tr>
<tr>
<td>Average</td>
<td>27 ± 2</td>
<td>62 ± 9</td>
<td>-50 ± 10</td>
<td>114 ± 22</td>
<td>38 ± 11</td>
</tr>
<tr>
<td>Control average</td>
<td>35 ± 5</td>
<td>64 ± 7</td>
<td>-41 ± 7</td>
<td>110 ± 10</td>
<td>47 ± 10</td>
</tr>
</tbody>
</table>

Values are means ± SE of data for responses to airway occlusion for each subject in the scalenes. Peak inhibitory response ($IR_{peak}$) amplitude is measured relative to the prestimulus level of electromyographic (EMG) activity in the muscle. Group averages are shown for the transplant and control subjects in the bottom 2 rows. $IR_{onset}$, onset of inhibitory response; $ER_{peak}$, peak excitatory response.

Unloading responses

Responses to unloading at the end of the airway occlusion were observed in the recordings from scalenes in the two transplant subjects who maintained inspiratory effort throughout the occlusion (Fig. 2). The average onset latency for the initial reduction of EMG activity was 15 ms in one subject and 18 ms in the other. In the matched control subjects, the responses to unloading occurred at the same time (mean 19 ms). The initial unloading responses were of a similar magnitude in transplant and control subjects.

Discussion

In the current study, we have demonstrated that the initial reduction in inspiratory muscle EMG to a brief airway occlusion is not critically mediated by receptors in the lungs because the same EMG reduction was observed in all subjects who had had the pulmonary branches of the vagus nerves cut bilaterally during transplantation surgery. Given the short time interval between the surgical section of the pulmonary vagni and the testing (median 21 days), it is unlikely that any reinnervation had occurred. For similar patients studied 11 mo after surgery, there was no evidence for a return of the vagally mediated Hering-Breuer reflex (Seals et al. 1993).

Previous studies of the short-latency inhibition of human inspiratory muscles produced by sudden inspiratory loading have provided indirect evidence that intramuscular receptors generate the response (Butler et al. 1995; Newsum Davis and Sears 1970; Plassman et al. 1987). The reflex responses to sudden loading remained intact after the surface of the upper airway was blocked by topical anesthesia (Butler et al. 1995; Newsum Davis and Sears 1970). Moreover, the reflex was not abolished in subjects who were intubated with auffed endotracheal tube while awake (Butler et al. 1995).

The upper airway was completely bypassed in these subjects, so that the occlusion was delivered only to structures below the level of the midtrachea, providing conclusive evidence that upper airway and proximal tracheal receptors are not necessary to mediate the short-latency inhibition. Inhalation of nebulized lignocaine to anesthetize the rapidly adapting control subjects. To illustrate the similarity in the responses of the transplant subjects and control subjects, the averages of rectified trials from representative matched control subjects for subjects 1F and 2M (from Table 1) are shown in Fig. 2 for both scalenes and parasternal intercostal muscles.
cles during voluntary efforts (Gandevia et al. 1990; Macefield et al. 1993). Overall, the simplest explanation for the short-latency silent period following unloading at the end of the airway occlusion is that the inspiratory muscle contractions are accompanied by effective fusimotor drive to muscle spindles. Concordant with this interpretation, the loss of pulmonary afferents had no effect on the unloading response.

One possibility for the difference between the reflex responses to loading of limb and inspiratory muscles is that the initial response in inspiratory muscles is mediated by Golgi tendon organ afferents. The occlusion stimulus during active muscle contraction would cause not only muscle spindle afferents but also tendon organ afferents to discharge (e.g., Stephens et al. 1975). For the scalene and intercostal muscles, there is a relatively short conduction distance to the spinal cord and therefore there may be less dispersion of the Ia and Ib afferent volleys than for those from more “distal” limb muscles. As first postulated by Newsom Davis and Sears (1970), this difference in the timing of the afferent volleys may contribute to the initial short-latency inhibition observed in inspiratory muscles and the lack of an initial short-latency excitation. In addition, on the basis of tendon jerk latencies for the human intercostal muscles (12–13 ms) (Macefield and Gandevia 1992), and the latency of the initial inhibition, there is sufficient time (~15 ms central delay) for a supraspinal contribution to the initial inhibition following loading. Indeed, there is some evidence in the cat for oligosynaptic inhibition of inspiratory muscles by tendon organ afferents. Both extracellular and intracellular recordings of the discharge of medullary inspiratory neurons in the cat have revealed inhibition following selective activation of tendon organ afferents innervating intercostal muscles (Bolser and Remmers 1989; Shannon et al. 1988). At least some of the inhibited inspiratory neurons had axons that projected into the spinal cord (Bolser and Remmers 1989).

Studies in animals have shown that there may be complex interactions between vagal and proprioceptive reflex effects on different pools of inspiratory motoneurons. For example, on the basis of responses to occlusion for the duration of a single “breath” in anesthetized dogs, vagal afferents have been deduced to provide significant facilitation to parasomatic intercostal motoneurons during eupnea (De Troyer 1991). Similarly, vagal afferents facilitate phrenic motoneurons during eupnea in the dog (Bartoli et al. 1975). However, such facilitation is less obvious for the motoneuron pools of the levator costae and external intercostal muscles. These muscles are facilitated during single breath occlusions, an effect probably mediated by muscle spindle afferents (De Troyer 1991) in contrast to the disfacilitation of the parasomatic intercostals. This facilitation of particular motoneuron pools appears to parallel the density of homonymous muscle spindle afferents. In the present study in human subjects, both the scalenes and parasomatic intercostal muscles showed the same IR to transient airway occlusion after the onset of inspiratory flow despite their apparent differences in muscle spindle density (Duron et al. 1978; Voss 1971). The difference between the studies involving occlusion for the duration of a single breath described above and the present study may be in part due to the effect of general anesthesia as well as the rapidity of the onset and timing of the load.

The present study of human subjects provides definitive evidence that intrapulmonary receptors are not necessary for the usual pattern of reflex responses to loading or unloading of human inspiratory muscles. Although the conclusion is strictly applicable only to patients without pulmonary afferent input, the data critically strengthen the previously indirect argument that inspiratory muscle afferents subserve these reflexes.

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