Pattern and mechanism of airway response to hypocapnia in normal subjects

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O’CAIN, CHARLES F., MICHAEL J. HENSLEY, E. R. McFADDEN, JR., AND R. H. INGRAM, JR. Pattern and mechanism of airway response to hypocapnia in normal subjects. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 47(1): 8-12, 1979.—We examined the bronchoconstriction produced by airway hypocapnia in normal subjects. Maximal expiratory flow at 25% vital capacity on partial expiratory flow-volume (PEFV) curves fell during hypocapnia both on air and on an 80% helium-20% oxygen mixture. Density dependence also fell, suggesting predominantly small airway constriction. The changes seen on PEFV curves were not found on maximal expiratory flow-volume curves, indicating that inhalation to total lung capacity substantially reversed the constriction. Pretreatment with a β-sympathomimetic agent blocked the response, whereas atropine pretreatment did not, suggesting that hypocapnia affects airway smooth muscle directly, not via cholinergic efferents.

Previous studies in man have shown that airway hypocapnia produces bronchoconstriction. Using voluntary hyperventilation as the means to produce hypocapnia, Newhouse et al. (17) and Sterling (20) noted increases in flow resistance, whereas Nielsen and Pedersen (18) recorded decreases in maximal expiratory flow rates. Temporary unilateral pulmonary artery occlusion results in a more profound fall in local PCO₂, and investigators employing this technique have found ventilation to shift from the occluded lung in association with a rise in resistance and a fall in compliance both in animals (12, 19) and in man (8, 21). Although a fall in compliance suggests that small airways may have been constricted, there is little direct evidence relative to the site of bronchoconstriction in man. In addition, there have been conflicting interpretations of the role of cholinergic efferents in the airway response to hypocapnia. Both Newhouse et al. (17) and Sterling (20), studying normal human subjects, have suggested that the bronchoconstrictor response to hypocapnia is considerably reduced by prior administration of atropine. Studies in dogs, however, using unilateral pulmonary artery occlusion, have shown that vagotomY does not affect the increase in airway resistance produced by hypocapnia (12, 19).

In the present study we have assessed the effects of volume history and gas density on maximal expiratory flow rates as a means for determining the site and magnitude of acute airway responses during airway hypocapnia and the effects of either anticholinergic or β-adrenergic pretreatment on these responses in normal subjects.

Methods

Five male subjects between the ages of 26 and 32 yr were studied after informed consent was obtained. Each subject was experienced in respiratory maneuvers and had normal base-line pulmonary function. Although one was a cigarette smoker (subj 1), all denied respiratory symptoms and none had experienced an upper respiratory tract infection during the preceding 2 mo.

In each subject, partial and maximal expiratory flow-volume curves (PEFV and MEFV, respectively) were obtained both on air and on an 80% helium-20% oxygen mixture (HeO₂) under each of three conditions: 1) during normal resting ventilation (control); 2) during voluntary hyperventilation in which the end-tidal PCO₂ (PETCO₂) fell to approximately 20 Torr (hypocapnia); and 3) during voluntary hyperventilation with CO₂ added to the inspired gas mixture in sufficient quantities to maintain PETCO₂ at control levels (isocapnia).

The studies were performed with the subjects seated in an integrated-flow pressure-corrected body plethysmograph (Fig. 1) (14). The volume signal was statically calibrated before each set of measurements, and the signal was pressure corrected by the method outlined by Leith and Mead (14). Flow at the mouth was measured with a Fleisch no. 4 pneumotachograph, linearized in the expiratory direction (9), and calibrated by passing air and HeO₂ through it at various known flow rates. A rolling-seal spirometer was placed in series with the mouth pneumotachograph to measure expired volume. Respirated nitrogen and CO₂ concentrations were monitored using a Med-Science 505 Nitralyzer, and a Godart Capnograph, respectively. All signals were recorded with a 7706 Hewlett-Packard direct-writing oscillographic recorder. Plethysmographic volume and mouth flow signals were displayed as x-y coordinates on a storage oscilloscope and photographed.

The following experimental protocol was performed: the subject breathed spontaneously for several minutes before being asked to slowly inspire approximately 0.5 liter from functional residual capacity (FRC). This inspiration was stopped with a shutter, and thoracic gas volume (TGV) was determined by the Boyle’s law tech-
AIRWAY RESPONSE TO HYPOCAPNIA

FIG. 1. Schematic drawing of plethysmograph-spirometer circuit. See text for description.

nique (6). The pneumotachograph circuit was then selected and PEFV and MEFV curves were recorded in succession. Four to six of these TGV-PEFV-MEFV sets were recorded, both on air and on HeO2. The HeO2 measurements were not made until end-tidal nitrogen was less than 5%, and subsequent air measurements were not made until end-tidal nitrogen was greater than 75%.

The PEFV and MEFV curves were matched at residual volume. Because it was possible that volumes measured by box flow integration had artifactual variations due to integrator drift and also due to nonlinearity of the box pneumotachograph at very high flow rates, separate recordings of expired volumes during the maneuvers were made with the rolling-seal spirometer, and any sets of results in which the residual volumes of the PEFV and MEFV curves failed to match on this record were discarded. Expiratory flows were compared at 25% vital capacity (VC) above residual volume on the PEFV curves (Vmax 25% VC PEFV) and at 25 and 50% vital capacity above residual volume on the MEFV curves. Density dependence of maximal expiratory flow (Vmax) was expressed as the ratio of Vmax on HeO2 to Vmax on air at the volumes chosen.

In three of the subjects, the hypocapnia protocol was repeated following pretreatment with atropine sulfate, and again after a β-adrenergic agonist, isothiourine. These drugs were administered as aerosols in doses previously shown to produce comparable degrees of overall bronchodilatation for the duration of the study (11). Specific airway conductance, measured by the technique of DuBois et al. (7) in a constant-volume-pressure plethysmograph was used as the index of bronchodilatation.

Data were analyzed by a paired t test or a one-way analysis of variance.

RESULTS

Hyperventilation produced a substantial fall in PETCO2 on both air and HeO2 in each subject. During isocapnic hyperventilation PETCO2 was similar to control, and for each experimental condition, there was no significant difference in PETCO2 between air and HeO2 studies (Table 1). Minute ventilation during hypocapnia was similar to that during isocapnia for both air and HeO2 (Table 1).

Static lung volumes did not change from control values during either hypocapnia or isocapnia, thus assuring that isovolume points could be chosen for comparison of maximal expiratory flow (Table 1).

At 25 and 50% VC, on the MEFV curves, hypocapnia did not result in significant changes from control in either maximal flow or density dependence (Table 2). When hypocapnia was compared to isocapnia, the changes on MEFV curves were in some instances statistically significant, though small (Table 2). When maximal flow on the PEFV curves was examined, however, Vmax at 25% VC on air exhibited greater falls with hypocapnia than seen on the MEFV curves in each subject (Fig. 2, Table 2). When HeO2 PEFV curves were compared, the fall was yet more pronounced (Fig. 2, Table 2) and as a result, density dependence at this lung volume fell substantially in each subject (Fig. 3, Table 2).

In the three subjects who repeated the hypocapnia

### Table 1. PETCO2, minute ventilation, and vital capacity in subjects breathing air and HeO2

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Hypocapnia</th>
<th>Iso capnia</th>
</tr>
</thead>
<tbody>
<tr>
<td>PETCO2, mmHg</td>
<td>37.0</td>
<td>34.6</td>
<td>20.5</td>
</tr>
<tr>
<td>Ttot, l/min</td>
<td>±2.1</td>
<td>±1.4</td>
<td>±0.8</td>
</tr>
<tr>
<td>Ve, l</td>
<td>14</td>
<td>13</td>
<td>47</td>
</tr>
<tr>
<td>I/min</td>
<td>±3</td>
<td>±3</td>
<td>±5</td>
</tr>
<tr>
<td>VC, liters</td>
<td>5.51</td>
<td>5.54</td>
<td>5.50</td>
</tr>
<tr>
<td>Density</td>
<td>±0.53</td>
<td>±0.52</td>
<td>±0.53</td>
</tr>
</tbody>
</table>
| Values are means ± SE. Statistical comparisons were assessed using paired t tests.

### Table 2. Maximal expiratory flows from MEFV and PEFV maneuvers while breathing air and HeO2

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Hypocapnia</th>
<th>Iso capnia</th>
<th>Between-Group Comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>4.43</td>
<td>4.51</td>
<td>4.56</td>
<td>CH NS NS NS</td>
</tr>
<tr>
<td>Vmax 50% VC</td>
<td>±0.56</td>
<td>±0.54</td>
<td>±0.51</td>
<td>NS NS NS NS</td>
</tr>
<tr>
<td>MEFV, l/s</td>
<td>1.75</td>
<td>1.73</td>
<td>1.80</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Vmax 25% VC</td>
<td>±0.26</td>
<td>±0.27</td>
<td>±0.28</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MEFV, l/s</td>
<td>±0.54</td>
<td>±0.53</td>
<td>±0.53</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HeO2</td>
<td>6.93</td>
<td>6.77</td>
<td>7.07</td>
<td>NS NS &lt;0.02</td>
</tr>
<tr>
<td>Vmax 50% VC</td>
<td>±1.02</td>
<td>±1.02</td>
<td>±1.00</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MEFV, l/s</td>
<td>±2.61</td>
<td>2.48</td>
<td>2.66</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Vmax 25% VC</td>
<td>±0.51</td>
<td>±0.49</td>
<td>±0.49</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>PEFV, l/s</td>
<td>±0.36</td>
<td>±0.31</td>
<td>±0.36</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Density</td>
<td>1.55</td>
<td>1.48</td>
<td>1.53</td>
<td>NS NS &lt;0.02</td>
</tr>
<tr>
<td>dependence</td>
<td>±0.06</td>
<td>±0.05</td>
<td>±0.05</td>
<td>NS NS &lt;0.02</td>
</tr>
<tr>
<td>25% VC MEFV</td>
<td>1.43</td>
<td>1.40</td>
<td>1.46</td>
<td>NS NS NS NS</td>
</tr>
<tr>
<td>Vmax 50% VC</td>
<td>±0.08</td>
<td>±0.06</td>
<td>±0.06</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>25% VC PEFV</td>
<td>1.50</td>
<td>1.33</td>
<td>1.54</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>±0.04</td>
<td>±0.04</td>
<td>±0.04</td>
<td>±0.04</td>
<td>NS ns &lt;0.005</td>
</tr>
</tbody>
</table>

Values are means ± SE. Statistical comparisons were assessed using paired t tests. Vmax 50% VC MEFV and Vmax 25% VC MEFV are maximal flow at 50% and 25% of the vital capacity, respectively, on maximal expiratory maneuver. Vmax 25% VC PEFV is maximal flow at 25% vital capacity on partial expiratory maneuver. CH, control; H, hypocapnia; I, isocapnia. NS, not significant (P > 0.05).
studies following drug pretreatments, atropine and isootharine produced comparable changes in specific airway conductance (mean increase over control 89% for atropine, 87% for isootharine). Airflow rates increased following each drug (Fig. 4); however, as previously described (13) density dependence fell following atropine and increased after isootharine (Fig. 5). With hypocapnia, \( V_{\text{max}} \) 25% VC PEFV and density dependence fell despite atropine pretreatment. In contrast, following isootharine there was no fall in \( V_{\text{max}} \) 25% VC or density dependence with hypocapnia (Figs. 4 and 5).

**DISCUSSION**

From the present data and from previous studies, it appears possible to detect the predominant site of airway responses by measuring maximal expiratory flow rates with gases of different density and by assessing the effect of volume history on the magnitude of change in airflow rates and on the degree of density dependence. As concerns the interpretation of density dependence, Despas, Leroux, and Macklem (4) utilized the equal pressure point concept of Mead et al. (15). If equal pressure points are located in large airways with a small total cross-sectional area and large Reynolds's numbers, the density-dependent flow regimes of convective acceleration and turbulence predominate and there is a greater relative contribution of these large airways to flow limitation. Localization of equal pressure points in smaller airways with a large total cross-sectional area and small Reynolds's numbers results in more nearly laminar flow that is independent of gas density. Thus a lower than normal degree of density dependence indicates a relatively larger contribution of small airways to upstream resistance at flow limitation. We previously applied these ideas to the assessment of acute airway responses to histamine and found that volume history profoundly affected the pattern of density dependence in such a manner that small airway obstruction was apparent only on PEFV maneuvers (3). In that study, cigarette smokers had combined volume-history and density-dependent effects of small airway obstruction before challenge, but responded to aerosol histamine with predominant large airway effects whereas nonsmokers responded to histamine with changes of small airway obstruction. It was thought that the previously demonstrated differences in aerosol distribution between smokers and nonsmokers (5) could account for the differences observed. Hence we used airway hypocapnia as a stimulus that was not dependent
on aerosol deposition both to understand better the localization of airway constriction and to gain some insight into the site and mechanism of the hypocapnic response.

The observation that volume history is important in modifying both normal airways and their response to challenge is not new. Inhalation to total lung capacity reduces normal airway tone as shown by Green and Mead (10) and Vincent et al. (22). Such a volume history also reduces the effects of induced bronchoconstriction as shown by Nadel and Tierney (16) and by Bouhuys et al. (1). It was the latter authors who proposed the use of PEFV curves as a more sensitive indicator of acute bronchoconstriction. Brown et al. (3) found that by combining volume history and density-dependent data means of localizing acute responses was possible. Our findings that the reduction in both air and HeO2 flow rates and the fall in density dependence with hypocapnia were seen more clearly on the PEFV curves emphasize that definite responses in small airways are quite sensitive to volume history. Thus, in contrast to the histamine study where changes in airflow rates were quite apparent (though less in magnitude) after a TLC volume history, the present study demonstrates, independent of aerosol deposition, that subtle small airway changes may be missed by a measurement technique involving or applied after a full inflation of the lung.

In general our results are consistent with other studies on hypocapnic airway constriction. Nielsen and Pedersen (18) demonstrated a small but consistent diminution in maximal flow rates at 60% of TLC in normal subjects when PCO2 was below 35 Torr and attributed this small change to constriction of peripheral airways. Although in a similar manner we found a small change in airflow rates on MEFV curves when isocapnic hyperventilation values were used as controls, our data on density dependence with partial curves are more striking while being consonant with their conclusion. Sterling (20) found a significant decrease in specific conductance with hypocapnia as opposed to isocapnic hyperventilation and observed a significant, though smaller decrease after atropine. Sterling did not address himself to site of constriction, but concluded that cholinergic mechanisms were important in mediating the constriction. Newhouse et al. (17) presented similar data using changes in resistance and found that atropine failed to abolish the response, but that combinations of isoproterenol and atropine completely blocked changes seen with hypocapnia. Measurements of resistance and conductance, however, may reflect changes both in peripheral and in central, even upper, airways. Our own data after atropine was used indicate that the small airway component, at least, of the hypocapnic response is not mediated via cholinergic pathways, in as much as the effect was clearly seen after pretreatment with atropine. There could well have occurred changes in central and upper airways in our subjects that would have been missed by our measure of maximal expiratory flow rates to assess the response to hypocapnia.

The opposite changes seen in density dependence after anticholinergic versus β-adrenergic bronchodilatation, despite similar degrees of overall bronchodilation before the hypocapnic stimulus, are identical to those previously reported (13). Increasing density dependence after β-adrenergic stimulation was interpreted to indicate a relatively greater small airway dilatation; the result was an increase in the relative contribution of large airways to upstream resistance at flow limitation. Decreased density dependence after anticholinergic agents was taken to mean relatively greater large airway dilatation with a relatively greater contribution of small airways to upstream resistance under conditions of flow limitation. Analysis of isovolume pressure-flow curves supported these interpretations. In a subsequent study, utilizing techniques not involving maximal expiratory flow maneuvers, data also indicated that the predominant effect of β-adrenergic agents was on small airways and that the major effect of anticholinergic agents was on large air-
ways (11). In the present study, the failure of atropine to abolish the small airway effect and the complete inhibition of this response by isoetharine are consistent with a predominant large airway effect of the former agent and a predominant small airway effect of the latter one. Furthermore, the hypocapnic response of small airways appears to result from a direct action on smooth muscle and is not mediated by cholinergic efferents.

There are two factors that must be considered before accepting our interpretation of the density-dependence data to indicate small airway constriction with hypocapnia. First, a precise interpretation of density-dependence data requires the assumption that EPP do not differ in normal subjects, more mouthward while EPP on HeO2 must remain stationary or move peripherally. We know of no theoretical considerations and no data that suggest that such different directional movements would occur. Thus it seems much more likely that predominant constriction of small airways accounts for our findings, according to the previous reasoning of Despas et al. (4).

Second, changes in lung recoil pressure could have affected our results. Although recoil pressure was not measured in this study, previous investigations have shown no significant change with hypocapnic hyperventilation in normal subjects (2, 17). Other studies, employing temporary unilateral pulmonary artery occlusion, have reported a fall in compliance (8, 12), but at levels of hypocapnia more profound than those seen in the present study. This fall in compliance is probably due to widespread constriction of small airways and, conceivably, alveolar ducts. Thus it seems likely that lung recoil would increase if it were to change at all with the level of hypocapnia used in the present study. The effect of an increase in recoil pressure would be to increase flow rates and/or lengthen the upstream segment, both of which would tend to increase density dependence. Therefore, our observed fall in density dependence would perhaps represent an underestimate of the changes in small airways.

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