Effect of chronic resistive loading on hypoxic ventilatory responsiveness

HARLY E. GREENBERG, RAMMOHAN S. RAO, ANTHONY L. SICA, AND STEVEN M. SCHARF
Division of Pulmonary and Critical Care Medicine, Long Island Jewish Medical Center, Long Island Campus for the Albert Einstein College of Medicine, New Hyde Park, New York 11040

Greenberg, Harly E., Rammohan S. Rao, Anthony L. Sica, and Steven M. Scharf. Effect of chronic resistive loading on hypoxic ventilatory responsiveness. J. Appl. Physiol. 82(2): 500–507, 1997.—Depression of ventilation mediated by endogenous opioids has been observed acutely after resistive airway loading. We evaluated the effects of chronically increased airway resistance on hypoxic ventilatory responsiveness shortly after load imposition and 6 wk later. A circumferential tracheal band was placed in 200-g rats, tripling tracheal resistance. Sham surgery was performed in controls. Ventilation and the ventilatory response to hypoxia were measured by using barometric plethysmography at 2 days and 6 wk postsurgery in unanesthetized rats during exposure to room air and to 12% O₂-5% CO₂-balance N₂. Trials were performed with and without naloxone (1 mg/kg ip). Room air arterial blood gases demonstrated hypercapnia with normoxia in obstructed rats at 2 days and 6 wk postsurgery. During hypoxia, a 30-Torr fall in PO₂ occurred with no change in PCO₂. Hypoxic ventilatory responsiveness was suppressed in obstructed rats at 2 days postloading. Naloxone partially reversed this suppression. However, hypoxic responsiveness at 6 wk was not different from control levels. Naloxone had a small effect on ventilatory pattern at this time with no overall effect on hypoxic responsiveness. This was in contrast to previously demonstrated long-term suppression of CO₂ sensitivity in this model, which was partially reversible by naloxone only during the immediate period after load imposition. Endogenous opioids apparently modulate ventilatory control acutely after load imposition. Their effect wanes with time despite persistence of depressed CO₂ sensitivity.

control of ventilation; ventilatory loads; endogenous opioids; hypoxic response

SUPPRESSION OF VENTILATORY chemosensitivity with concomitant development of hypercapnia may occur in diseases that impose chronic mechanical loads on the ventilatory system (2, 12). Often, the degree of depression of ventilatory chemosensitivity is disproportionately greater than the magnitude of the imposed ventilatory load, implicating depression of ventilatory control (27). One possible explanation for this phenomenon is that chronic loading of the ventilatory system induces an adaptive response that modulates ventilation (12, 26).

Prior studies have demonstrated the presence of such an adaptive response in an animal model that was at least partially mediated by the endogenous opioid system. This mechanism is responsible for decrements in ventilation observed acutely after imposition of a resistive ventilatory load (8, 29). There is now compelling evidence implicating the endogenous opioid system in modulation of ventilatory control (7, 11, 19). For example, these substances and their precursors have been localized in brain stem regions involved in control of ventilation wherein stimulation of µ-opioid receptors has been observed to depress both minute ventilation and CO₂ sensitivity (5). This response to ventilatory loading may be adaptive, because it may delay the onset of ventilatory muscle fatigue by diminishing ventilatory drive and permitting the development of hypercapnia (23).

Although there is evidence for modulation of ventilation by endogenous opioids during the immediate period after load imposition (30), there are few data on the role of endogenous opioids in modulating long-term changes in ventilation. We recently developed a rat model to evaluate the effects of long-term resistive ventilatory loading on ventilatory control. Normoxic chronic hypercapnia developed and persisted for at least 6 mo in these rats after surgical implantation of a load that tripled tracheal resistance. Naloxone administration augmented the ventilatory response to hypercapnia 2 days postload imposition but not after 2–6 mo (8). Naloxone had no effect on ventilation during room air breathing, nor did it have any effect during room air or hypercapnic conditions in control animals. Thus this study demonstrated evidence of endogenous opioid-mediated modulation of the hypercapnic response during the early period after imposition of increased airway resistance (8).

Because there are few data on the effects of chronic resistive loading on hypoxic ventilatory sensitivity, the present investigation was also designed to assess the effect of long-term resistive loading on hypoxic ventilatory sensitivity in our chronic rat model. Prior studies have provided ample evidence implicating the endogenous opioid system in the modulation of hypoxic ventilatory sensitivity. For example, enkephalins have been found in the cat carotid body (9, 16, 38), and infusions of endogenous opioids suppressed hypoxic discharges from carotid sinus nerve afferents in cats (18). Thus endogenous opioids may affect both central and peripheral aspects of ventilatory control. In the present investigation, we tested the hypothesis that the endogenous opioid system results in both acute and chronic depression of hypoxic ventilatory sensitivity after imposition of a ventilatory load.

METHODS

Sexually mature male Sprague-Dawley rats weighing 150-200 g were entered into the protocol. All procedures were
approved by the institutional animal care and use committee and were consistent with National Institutes of Health guidelines. 

Resistive airway loading. In the experimental group, increased tracheal resistance was imposed by using sterile surgical technique for placement of a circumferential tracheal band. Animals were anesthetized with pentobarbital sodium (20–30 mg/kg ip). Atropine (0.4 mg ip) was also given. Local anesthesia was obtained with 2% lidocaine injected subcutaneously in the cervical region. Supplemental oxygen was supplied via a cannula placed in the upper airway to prevent hypoxemia during surgery. A saline-filled catheter was placed in the esophagus and connected to a pressure transducer to measure inspiratory swings in esophageal pressure. A midline ventral cervical incision was made, and the trachea was exposed and dissected so as not to damage adjacent structures. In particular, care was taken so as not to damage the recurrent laryngeal nerve, and surgery did not affect any region adjacent to the carotid bodies. A circumferential plastic band 0.5–0.7 cm long was placed around the trachea. A suture was looped around the band and tightened, thus constricting the trachea so as to increase inspiratory esophageal pressure swings by two- to threefold. The suture was then tied, and the wound was bathed with penicillin G solution; an intraocular injection of penicillin G was also given in the hindlimb. The skin incision was sutured, and animals were returned to their cages for recovery and were given food and water ad libitum.

Sham surgery was performed on the control group and consisted of tracheal dissection without placement of the tracheal band. This was done as a control for the pain and stress of surgery with respect to the effect of blockade of endogenous opioids with naloxone. In addition, because dissection of the trachea was similar in control and obstructed animals, any inadvertent damage to adjacent structures, or postsurgery fibrosis, would be similar in both groups. Animals were examined postmortem, and similar degrees of fibrotic tissue were noted around the trachea in control and obstructed animals. Fibrosis did not visibly extend to any region near the carotid arteries in either group. Rats were subsequently maintained on a 12:12-h light-dark cycle, with all experiments performed in the morning of the daytime or light cycle.

We previously demonstrated that tracheal resistance is approximately three times greater in the obstructed group than in the sham-operated control animals (8).

Barometric plethysmograph measurements. Respiratory variables were measured noninvasively by barometric plethysmography, using a modification of the method of Drorbaugh and Fenn (6). The plethysmograph consisted of two Plexiglas cylinders with a volume of 1.83 liters each. One served as the test chamber in which an awake, unrestrained rat was placed; the other cylinder served as a reference chamber. No leak was present between test and reference chambers. The test and reference chambers were connected by a gas reservoir that permitted the steady inflow of gas. An identical gas outflow port was placed at the opposite side of the chamber. The test and reference chambers were constantly flushed with room air or test gas at 1.5 liters/min each. At this flow rate, 0.8 gas exchanges were provided each minute. This flow of room air was sufficient to maintain the percentage of CO₂ of chamber gas at <1% and the percentage of O₂ at 21% with an animal in the chamber. No measurable increase in chamber pressure was evident with gas flow (<0.1 Torr), with no difference in pressure evident between test and reference chambers. A rapidly responding differential pressure transducer (Validyne, Engineering, Northridge, CA) was connected between test and reference chambers. The signal was amplified and recorded on paper (Gould, Cleveland, OH). The entire plethysmograph, consisting of test and reference chambers, was contained in a lighted, sound-attenuated enclosure to shield the plethysmograph from external disturbances. The amplifier was calibrated with a test animal in the plethysmograph at the beginning of each trial, so that a rapid injection of 1 ml of air into the chamber resulted in a signal deflection of 20 mm on the chart recorder. This volume of injected gas and the resultant calibration were chosen to encompass the maximal range of tidal volume/respiratory effort relevant to this model. The 95% response time of the system was 62 ms. Respiratory activity was recorded from the differential pressure change between test and reference chambers. The amplitude of respiratory deflections observed on the plethysmograph is reported in millimeters of chart deflection (plethysmograph units). Respiratory rate (RR) and inspiratory time (Ti) were also measured for each breath from the plethysmograph tracing.

The validity of the plethysmographic recordings was extensively evaluated and reported previously (8). Results of these prior validation trials are as follows. 1) There is a flat frequency response (no change in amplitude of the plethysmograph signal for a given tidal volume) throughout the range of respiratory frequencies relevant to this protocol (70–200 breaths/min). 2) The signal recorded from the plethysmograph was demonstrated to reflect accurately the timing of the inspiratory and expiratory portions of each breath compared with that obtained from a simultaneously recorded tracing of esophageal pressure swings occurring with ventilation in both control and obstructed animals. In these trials, Ti and expiratory time measurements obtained from the plethysmograph signal were virtually identical to those obtained from the esophageal pressure tracing. This ensures that RR and Ti could be accurately measured from the plethysmograph signal. 3) Comparison of the amplitude of the plethysmograph signal with that of a simultaneously recorded esophageal pressure tracing demonstrated a linear relationship between the peak inspiratory amplitude of these signals in both control and obstructed animals \( r = 0.94; P < 0.0001 \) (8). Therefore, the amplitude of the plethysmograph signal is related to a conventional measure of inspiratory effort that is comparable between obstructed and control rats. 4) Although it has been previously reported that tidal volume can be calculated from plethysmograph pressure tracings (6), we have refrained from such calculations. Prior validation trials with the plethysmograph have demonstrated that a plethysmograph pressure tracing is still generated in association with ventilatory effort even with complete occlusion of the trachea and thus absence of tidal air movement. Thus the plethysmograph signal reflects thoracic and abdominal gas compression and decompression occurring with ventilation as well as warming and humidification of tidal air and should be taken as an index of inspiratory effort rather than tidal volume.

As a result of these findings, the amplitude of the inspiratory portion of the plethysmograph signal was used as an index of inspiratory effort. To quantitate the effort per minute, minute inspiratory effort was calculated as the product of inspiratory effort and RR. Because the amplitude of the signal is highly correlated with a conventional measure of ventilatory effort, inspiratory esophageal pressure, minute inspiratory effort can be considered to be analogous to ventilatory effort per breath \( \times RR \). The hypoxic response was calculated as the percent change in minute inspiratory effort with hypoxic gas exposure compared with room air. For postnalox-
one studies, the hypoxic response was calculated by using the postnaloxone room air value as the baseline. Therefore, the relationship of respiratory effort to the plethysmograph signal may be altered by changes in animal size relative to plethysmograph volume, we have refrained from comparing the absolute value of minute inspiratory effort between the obstructed control groups, as animal weight differed between groups. Similarly, the absolute values of inspiratory effort were not compared within each group over time because animal growth may confound such comparisons. Rather, all between-groups comparisons and evaluations of trends over time are based on the hypoxic response, because this measurement compares animals with themselves under room air and hypoxic conditions with no effect attributable to differences in animal size. Direct comparisons of RR and T\(i\) are not affected by these considerations.

Respiratory measurements. Respiratory testing was performed at 2 days and 6 wk postsurgery. At the start of the protocol, there were 8 animals in the control group and 11 animals in the obstructed group to be used for chronic respiratory measurements over 6 wk. By the end of the study, the sample size was eight in both groups because three obstructed animals died, predominantly in the first postoperative week.

Each animal, unrestrained and unanesthetized, was placed in the plethysmograph and allowed to acclimate to the chamber for at least 10 min. All measurements were taken with the animal awake, as assessed by observation. The plethysmograph signal was then recorded for a 1-min period when the animal was not actively moving within the chamber and grooming activity ceased. Thus motion artifact and exercise activity did not confound our results. Mean data obtained from 20 breaths during each sampling period were recorded. Runs were performed with room air infused into the test and reference chambers at 1.5 liter/min each and after 12% \(\text{O}_2\)-5% \(\text{CO}_2\)-balance \(\text{N}_2\) gas exposure. Because in preliminary studies we observed periodic breathing in both control and obstructed animals without \(\text{CO}_2\) supplementation, 5% \(\text{CO}_2\) was added to the gas mixture to eliminate periodic breathing. The periodic breathing likely resulted from hypocapnia during hypoxic ventilatory stimulation that brought the P\(\text{CO}_2\) below the apneic threshold that induced periodic breathing. Eucapnia was maintained in control animals, and no increase in P\(\text{CO}_2\) was observed in the obstructed rats while breathing this gas mixture (Table 1). Thus the animals did not receive a significant hypercapnic stimulus, and isocapnic hypoxic stimulation was achieved. The animal was then returned to its cage. After a 30-min rest period, naloxone (1 mg/kg) was administered intraperitoneally. Animals were then placed in the plethysmograph, and room air and hypoxic runs were repeated as above. Postnaloxone data were recorded between 10 and 15 min after drug administration, which corresponds to the expected time of maximal response to naloxone administration (29). As a control for naloxone injection, four additional obstructed animals were similarly studied before and after an intraperitoneal injection of saline on the following day. This was to control for the possible effects of intraperitoneal fluid injection. Animals were returned to their cages and given food and water ad libitum between testing days.

Arterial blood gases. In a separate group of control and obstructed animals, arterial blood gases were determined at 2 days and 6 wk postsurgery (n = 8 for obstructed and n = 7 for control rats at each time interval). For this, under local anesthesia, an indwelling catheter was placed in the tail artery for arterial blood sampling. The catheter was heparinized, closed with a stopcock, and tunneled through to the skin surface. Arterial blood samples were drawn from the catheter for blood gas determination while the animals were awake under room air conditions and during exposure to the hypoxic gas mixture in the plethysmograph. The gas exposures before arterial blood gas sampling were of the same duration as in the experimental runs. Because the arterial catheter could not be maintained over the 6-wk course of the protocol, these animals could not be used for ventilatory measurements during the chronic phase of the study; therefore, they were sacrificed after these measurements.

Body temperature. Body temperature was measured with a rectal probe in three control and three obstructed rats during room air and hypoxic gas exposure while the rats were in the plethysmograph for a similar duration as in the experimental runs. These measurements could not be performed during the experimental runs because restraint of the animals was required to maintain the temperature probe in position. The restraint might have affected the ventilatory data.

Data analysis. Data were compiled and expressed as means ± SD for each group at 2 days and 6 wk postsurgery. Respiratory variables were compared between obstructed and control groups at each time. In addition, changes in these variables with naloxone administration were determined. Significance between these variables was analyzed by t-test for independent groups for between-group comparisons and by paired t-test for dependent groups for determination of naloxone effect. The null hypothesis was rejected at the 5% level.

RESULTS

Over the first 2 days postsurgery, obstructed rats lost weight, whereas control animals gained weight. Before surgery, weight in the obstructed group was 160.8 ± 7.2 g, and 2 days postsurgery their weight was 126.9 ± 8.1 g (\(P < 0.001\)). In sham-operated controls, preoperative weight was 159.0 ± 1 g, and 2 days postsurgery weight was 166.8 ± 6.3 g (\(P < 0.05\)). Obstructed animals

Table 1. Arterial blood gases

<table>
<thead>
<tr>
<th>Condition, Time, After Surgery</th>
<th>pH</th>
<th>P(\text{CO}_2), Torr</th>
<th>P(\text{O}_2), Torr</th>
<th>H(\text{CO}_3), meq/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Obstructed</td>
<td>Control</td>
<td>Obstructed</td>
</tr>
<tr>
<td>2 days Room air</td>
<td>7.38 ± 0.04</td>
<td>7.36 ± 0.06</td>
<td>38.8 ± 4.9</td>
<td>60.1 ± 12.3*</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>7.35 ± 0.03</td>
<td>7.35 ± 0.07</td>
<td>39.5 ± 7.6</td>
<td>62.0 ± 13.3*</td>
</tr>
<tr>
<td>6 wk Room air</td>
<td>7.45 ± 0.04</td>
<td>7.34 ± 0.08</td>
<td>35.2 ± 4.5</td>
<td>54.4 ± 13.7*</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>7.45 ± 0.03</td>
<td>7.35 ± 0.06</td>
<td>38.8 ± 2.9</td>
<td>54.6 ± 11.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. Hypoxia, 12% \(\text{O}_2\) - 5% \(\text{CO}_2\)-balance \(\text{N}_2\). *\(P < 0.05\) for obstructed vs. control; †\(P < 0.01\) for obstructed vs. control; ‡\(P < 0.05\) for room air vs. hypoxia.
A decrease in PO2 was noted in obstructed animals with a P O2 of 28.3 torr compared with controls at each time interval (P < 0.05). At 6 wk after surgery, there was no significant difference in room air RR between the two groups at this time.

No change in rectal temperature was observed during room air or hypoxic-stimulation trials in the plethysmograph in either group. Mean rectal temperature was 37.5°C in both groups.

Baseline respiratory variables (prenaloxone). Table 1 illustrates arterial blood-gas values obtained at 2 days and 6 wk after surgery. Under room air conditions, Pco2 was significantly higher in obstructed rats when compared with controls at each time interval (P < 0.05). Although a slight decrease in Pco2 was noted from 2 days to 6 wk postsurgery in the obstructed group, Pco2 remained in the hypercapnic range, and changes over time were not statistically significant. HCO3− was significantly greater in the obstructed compared with the control group at 2 days (P < 0.01) and at 6 wk after surgery (P < 0.05), demonstrating metabolic compensation for chronic respiratory acidosis. P2O was slightly lower in the obstructed animals (P < 0.05).

At 2 days postsurgery, under hypoxic gas conditions (12% O2-5% CO2-balance N2) a 28.3 ± 11.2-Torr decrease in P2O2 was noted in obstructed animals with a 28.3 ± 11.7-Torr decrease in the control group (P = not significant). Thus the hypoxic stimulus was similar in both groups. No significant change in Pco2 or pH was noted in either group under test gas conditions. Thus the test gas produced a similar degree of hypoxic stimulation in both groups and did not provide a significant hypercapnic stimulus in either group. A similar decline in P2O2 with hypoxic gas exposure was observed in both groups at 6 wk postsurgery, again with no significant change in pH or Pco2 under test gas conditions.

Respiratory variables over the course of the protocol are presented in Table 2. At 2 days postsurgery, room air RR of obstructed rats was significantly less than control rats (P < 0.0001). By 6 wk, however, RR significantly increased in the obstructed group (P < 0.001). Consequently, there was no significant difference in room air RR between the two groups at this time.

Comparison of controls, Tı was significantly prolonged in obstructed animals during room air breathing 48 h postsurgery (P < 0.0001). By 6 wk postsurgery, Tı significantly decreased in the obstructed group (P < 0.03) such that there was no significant difference between the groups during room air breathing at this time.

The percent change in ventilatory parameters with hypoxic stimulation is presented in Table 2. Before naloxone administration, at both 2 days and 6 wk postsurgery, obstructed rats failed to increase RR with hypoxic stimulation. This response differed significantly from that observed in controls, i.e., increased RR with hypoxia (P < 0.001 at 2 days and P < 0.04 at 6 wk postsurgery). No significant differences in the percent change in Tı with hypoxic stimulation were observed between groups at either time.

The percent change in minute inspiratory effort with hypoxia (hypoxic response) was significantly depressed in obstructed animals compared with controls at 2 days postsurgery (P < 0.0001). At 6 wk postsurgery, the hypoxic response was no longer suppressed in the obstructed rats, and no significant difference was evident between groups.

Effect of naloxone. Under room air conditions, no change in any ventilatory variable was noted after naloxone administration in the control animals. In the obstructed group, there was no change in inspiratory

Table 2. Ventilatory data

<table>
<thead>
<tr>
<th>Condition</th>
<th>Room air</th>
<th>Hypoxia</th>
<th>%Change</th>
<th>Room air</th>
<th>Hypoxia</th>
<th>%Change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prenaloxone</strong></td>
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</tr>
<tr>
<td>Respiratory frequency, breaths/min</td>
<td>87.7 ± 18.7</td>
<td>82.7 ± 24.4</td>
<td>−6.9 ± 15.3</td>
<td>140.0 ± 31.6*</td>
<td>189.5 ± 20.8</td>
<td>36.7 ± 30.9*</td>
</tr>
<tr>
<td>Tr, s</td>
<td>0.32 ± 0.05</td>
<td>0.31 ± 0.05</td>
<td>−0.3 ± 0.8</td>
<td>0.20 ± 0.02*</td>
<td>0.18 ± 0.01</td>
<td>−0.3 ± 0.9</td>
</tr>
<tr>
<td>Inspiratory effort, arbitrary units</td>
<td>10.3 ± 3.8</td>
<td>14.5 ± 5.6</td>
<td>51.4 ± 40.0</td>
<td>6.4 ± 0.7</td>
<td>12.5 ± 2.4</td>
<td>98.9 ± 42.2*</td>
</tr>
<tr>
<td>Minute inspiratory effort, arbitrary units</td>
<td>876.1 ± 276.3</td>
<td>1,106.2 ± 171.1</td>
<td>37.4 ± 5.1</td>
<td>918.9 ± 256.6</td>
<td>2,378.2 ± 652.0</td>
<td>172.9 ± 10.5*</td>
</tr>
<tr>
<td><strong>Postnaloxone</strong></td>
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<td></td>
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</tr>
<tr>
<td>Respiratory frequency, breaths/min</td>
<td>82.3 ± 9.3</td>
<td>82.7 ± 18.8</td>
<td>−0.4 ± 19.6</td>
<td>116.5 ± 24.3</td>
<td>182.2 ± 16.6</td>
<td>62.4 ± 36.1*</td>
</tr>
<tr>
<td>Tr, s</td>
<td>0.29 ± 0.04</td>
<td>0.26 ± 0.03</td>
<td>−0.2 ± 0.9</td>
<td>0.19 ± 0.02</td>
<td>0.18 ± 0.02</td>
<td>−0.3 ± 0.4</td>
</tr>
<tr>
<td>Inspiratory effort, arbitrary units</td>
<td>9.9 ± 4.5</td>
<td>17.1 ± 10.7</td>
<td>85.8 ± 28.1*</td>
<td>7.9 ± 2.9</td>
<td>13.9 ± 3.2</td>
<td>89.7 ± 57.2*</td>
</tr>
<tr>
<td>Minute inspiratory effort, arbitrary units</td>
<td>752.9 ± 278.4</td>
<td>1,127.9 ± 466.8</td>
<td>67.1 ± 2.8*</td>
<td>926.1 ± 422.9</td>
<td>2,532.5 ± 660.7</td>
<td>204.3 ± 11.8*</td>
</tr>
<tr>
<td><strong>6 wk postsurgery</strong></td>
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<tr>
<td>Prenaloxone</td>
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</tr>
<tr>
<td>Respiratory frequency, breaths/min</td>
<td>134.1 ± 19.1</td>
<td>134.2 ± 25.9</td>
<td>−0.6 ± 16.8</td>
<td>139.3 ± 26.8</td>
<td>165.0 ± 16.8</td>
<td>21.5 ± 19.9*</td>
</tr>
<tr>
<td>Tr, s</td>
<td>0.21 ± 0.02</td>
<td>0.2 ± 0.01</td>
<td>−3.6 ± 9.8</td>
<td>0.22 ± 0.02</td>
<td>0.21 ± 0.01</td>
<td>−6.4 ± 9.9</td>
</tr>
<tr>
<td>Inspiratory effort (arbitrary units)</td>
<td>7.3 ± 1.3</td>
<td>13.9 ± 9.0</td>
<td>93.7 ± 41.5</td>
<td>8.8 ± 1.8</td>
<td>12.8 ± 2.7</td>
<td>48.1 ± 27.5*</td>
</tr>
<tr>
<td>Minute inspiratory effort (arbitrary units)</td>
<td>979.7 ± 204.1</td>
<td>1,868.8 ± 497.8</td>
<td>96.4 ± 59.7</td>
<td>1,216.5 ± 327.1</td>
<td>2,092.9 ± 402.2</td>
<td>78.4 ± 38.9</td>
</tr>
<tr>
<td>Postnaloxone</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory frequency, breaths/min</td>
<td>126.6 ± 20.3</td>
<td>146.0 ± 21.0</td>
<td>15.9 ± 11.1†</td>
<td>135.8 ± 22.1</td>
<td>166.9 ± 22.6</td>
<td>26.3 ± 31.7*</td>
</tr>
<tr>
<td>Tr, s</td>
<td>0.24 ± 0.04</td>
<td>0.19 ± 0.02</td>
<td>−17.6 ± 12.3†</td>
<td>0.23 ± 0.04</td>
<td>0.21 ± 0.02</td>
<td>−5.8 ± 15.4</td>
</tr>
<tr>
<td>Inspiratory effort, arbitrary units</td>
<td>7.6 ± 1.2</td>
<td>14.2 ± 2.3</td>
<td>106.3 ± 40.8</td>
<td>7.9 ± 1.0</td>
<td>12.9 ± 3.3</td>
<td>64.2 ± 42.1</td>
</tr>
<tr>
<td>Minute inspiratory effort, arbitrary units</td>
<td>910.7 ± 249.6</td>
<td>2,131.7 ± 586.1</td>
<td>141.8 ± 68.4</td>
<td>1,070.9 ± 189.9</td>
<td>2,180.1 ± 718.9</td>
<td>115.7 ± 109.0</td>
</tr>
</tbody>
</table>

Tı, inspiratory time. *P < 0.05 control vs. obstructed; †P < 0.05 prenaloxone vs. postnaloxone.
effort, respiratory frequency, or minute inspiratory effort with naloxone. However, a small decrease in \( T_I \) was observed after naloxone in these animals \((P < 0.05)\).

Naloxone administration in obstructed rats at 2 days postsurgery produced a significant increase in the minute inspiratory effort response to hypoxia \((P < 0.04)\). However, the hypoxic response of obstructed rats remained less than that of control animals. No effect of naloxone was noted in the control group. As a control for naloxone injection, a similar volume of saline was injected in obstructed animals. No effect of saline was noted on the minute inspiratory effort response to hypoxia. Saline studies were not performed in the controls because no naloxone effect was noted in these animals.

At 6 wk, administration of naloxone had no effect on the minute inspiratory effort response to hypoxia in either the obstructed or control groups.

The augmentation of the minute inspiratory effort response to hypoxia observed after naloxone in the obstructed group at 2 days was the result of a significant increase in the percent change of inspiratory effort with hypoxic stimulation compared with prenaloxone values \((P < 0.05)\). Naloxone had no effect on the percent change in \( R_R \) or \( T_I \) with hypoxic stimulation at 2 days. At 6 wk, naloxone had no effect on the minute inspiratory effort response to hypoxia. However, there was a change in the pattern of ventilation such that naloxone led to an increase in \( R_R \) and a decrease in \( T_I \) during hypoxia in the obstructed group.

**DISCUSSION**

Within 2 days after imposition of a resistive ventilatory load, obstructed animals developed hypercapnia while remaining normoxic. In conjunction with this, \( R_R \) was decreased and \( T_I \) was prolonged during room air breathing. With similar degrees of hypoxic stimulation, suppression of the hypoxic ventilatory response was noted in the obstructed group compared with controls at 2 days postsurgery. This suppression was partially reversible by naloxone. The effect of naloxone was manifested primarily by increased inspiratory effort per breath without a change in \( R_R \). Both a mechanical load (increased airway resistance) and a chemical ventilatory challenge (hypoxia) were necessary to observe modulation of ventilatory control by endogenous opioids, as naloxone had no effect in control animals under any condition and naloxone only affected the obstructed group during hypoxic stimulation. At 6 wk, postobstruction hypercapnia and normoxia persisted. However, in contrast to previously reported long-term suppression of \( CO_2 \) sensitivity \((8)\), hypoxic ventilatory sensitivity returned to control levels. Naloxone had no effect on the overall minute inspiratory effort response to hypoxia at this time, although an increase in \( R_R \) during hypoxic stimulation was noted with naloxone. Thus endogenous opioid modulation of hypoxic responsiveness appears to be most evident during the early period after load imposition but, although still evident, appears to decline in magnitude by 6 wk afterload imposition.

Several methodological issues merit discussion. Partial reversal of suppression of hypoxic sensitivity observed with naloxone at 2 days postsurgery is not likely to have been caused by nonspecific effects of naloxone, such as reversal of depressant effects of endogenous opioids released in response to the pain and stress of surgery, since naloxone had no effect in sham-operated control animals. In addition, the augmentation of ventilatory effort observed with naloxone is not likely to have been caused by nonspecific central nervous system stimulation, because no effect was observed in the control group. Third, doses of naloxone ranging from 50 to 100 times greater than that utilized in this study have been administered in rats without analeptic effects \((3, 10)\). Thus the observations in this paper are most likely due to antagonism of endogenous opioid effects generated in response to an acute increase in airway resistance in combination with hypoxic stimulation.

The hypoxic stimulus applied resulted in an ~30-Torr fall in \( P_O_2 \) in control and obstructed animals. It resulted in a 205% increase in ventilatory effort in the control group. Thus, although the degree of hypoxemia achieved was not severe, we believe a difference in hypoxic responsiveness between obstructed and control animals was definitively demonstrated. In preliminary studies, a more severe hypoxic stimulus resulted in a high mortality rate in the obstructed rats and was therefore not utilized.

It is evident in Table 1 that the mean \( P_O_2 \) reached during hypoxic gas exposure was somewhat lower in the obstructed than in the control group at both 2 days and 6 wk postsurgery, although these differences did not reach statistical significance. Despite this somewhat greater hypoxic stimulus in the obstructed animals, hypoxic ventilatory responsiveness was less in the obstructed than in the control group at 2 days postsurgery. Thus we do not believe that the different degree of hypoxic stimulation significantly affected this finding. Hypoxic ventilatory responsiveness increased by >2.5-fold in the obstructed group at 6 wk postsurgery. At this time, the mean \( P_O_2 \) achieved during hypoxic gas exposure was somewhat lower than that achieved at 2 days postsurgery in the obstructed group \((49.8 \pm 9.3 \text{ vs. } 54.4 \pm 12.5 \text{ Torr})\). Although this may have contributed to the greater ventilatory response to hypoxia at 6 wk in the obstructed animals, we do not believe that this relatively small difference in extent of hypoxic stimulation can fully account for the >2.5-fold increase in hypoxic ventilatory responsiveness observed in the obstructed animals at this time.

\( CO_2 \) was added to the hypoxic gas mixture to avoid the confounding problem of periodic breathing that we observed in both control and obstructed animals in preliminary studies (not shown) when 12% \( O_2 \) was used alone. Periodic breathing likely occurred during hypoxic ventilatory stimulation when the \( P_CO_2 \) fell below the apneic threshold. This problem was eliminated by addition of 5% \( CO_2 \) to the hypoxic gas, which achieved
isocapnic hypoxia as demonstrated by a stable pH and $P_{\text{CO}_2}$ from room air to hypoxic runs in both groups.

Whereas obstructed rats had an approximately three-fold increase in tracheal resistance (8), we do not believe that the observed suppression of hypoxic ventilatory responsiveness was entirely the result of mechanical limitations. First, augmentation of the ventilatory response to hypoxia and hypercapnia was observed with naloxone, indicating that increases in ventilation could occur in the presence of this resistive ventilatory load. Thus the animals were not breathing at their maximal mechanical limit. Second, we recently demonstrated that suppression of hypercapnic sensitivity persisted in obstructed rats even after elimination of the ventilatory load by tracheostomy (26). Thus mechanical factors cannot fully explain our results, and alteration of ventilatory control in response to airway loading is most likely the primary factor responsible for our findings.

Prior studies have demonstrated augmentation of tidal volume with naloxone administration after acute imposition of a flow-resistive load during room air conditions in a goat model (29). We observed a significant effect of naloxone only during hypoxic stimulation in the obstructed group. Several prior investigations have demonstrated no effect of naloxone on ventilation under unstressed room air conditions in animals models and in humans (13, 39). Thus it has been proposed that a "ventilatory stressor" is necessary to stimulate endogenous opioid modulation of ventilatory control. We believe that the most likely explanation for the discrepancy between our findings and those of the later investigation (29) is related to differences in the magnitude of the imposed resistive load. In our studies, the tracheal band approximately tripled tracheal resistance, whereas flow-resistive loads of 50 and 80 cmH$_2$O·l$^{-1}$·s were applied in the prior goat model (29). The later loads likely represented a much greater increase in airway resistance than that achieved in our protocol. Thus, the "stressor" of loading alone was sufficient for expression of an endogenous opioid effect in the prior studies (29), whereas a concomitant chemical stimulus (i.e., hypoxia) in addition to the resistive load was necessary in our model to observe such an effect.

Although an increase in room air RR was observed in the obstructed group from 2 days to 6 wk postsurgery, we do not believe that this reflected a decrease in tracheal resistance. We previously reported tracheal resistance measurements in this model at 2 days and 147 days after surgery in obstructed and control animals. Specific tracheal conductance (relative to body weight) did not change over this period in the obstructed or control animals (8). Consequently, we feel that changes in RR and ventilatory pattern at this time reflect alterations in ventilatory control over the course of the study.

Obstructed animals lost weight during the first 2 days after surgery, whereas an increase in weight was noted in sham-operated controls. This is consistent with our previously reported data demonstrating a decline in weight and food intake in obstructed rats 2 days postsurgery (8). Because earlier studies have demonstrated depression of hypoxic ventilatory sensitivity in the setting of malnutrition (4), nutritional factors may have affected the hypoxic response shortly after surgery. However, we previously reported no differences between groups in biochemical markers of nutrition such as serum albumin, total protein, cholesterol, blood urea nitrogen, creatinine, or glucose at 2 days after surgery (8). Furthermore, at 6 wk after obstruction, when chronic effects of malnutrition would be expected to be more evident and when body weight was still less in the obstructed group than in controls, the hypoxic response was similar in obstructed and control animals. In addition, at this time after loading and thereafter, no differences in $O_2$ consumption were noted between control and obstructed animals (8). Thus differences in nutrition and metabolic rate cannot fully account for our findings. Nevertheless, multiple factors, including unmeasured nutritional and metabolic factors and impaired growth, as well as other factors in addition to the increase in airway resistance itself, may have contributed to the observed changes in ventilatory control.

The present findings, demonstrating suppression of hypoxic ventilatory sensitivity during the early postloading period, are consistent with our previous data that showed that $CO_2$ sensitivity is also suppressed in response to ventilatory loading at this time (8). The partial reversal of this suppression by naloxone implicates endogenous opioids in modulation ventilatory control in this setting. Endogenous opioid-mediated suppression of hypercapnic ventilatory chemosensitivity likely occurs at central nervous system sites involved in control of ventilation, because $\mu$-opioid agonists directly applied to the ventral medulla have been noted to depress hypercapnic ventilatory sensitivity, tidal volume, and RR (21, 22). Suppression of hypoxic sensitivity observed in the present study is probably also mediated by central effects of endogenous opioids. Support for this contention comes from acute progressive hypoxic stimulation studies in cats. These studies demonstrated an increase in ventilation after naloxone administration at any given level of carotid chemoreceptor activity, suggesting that naloxone increased the central response to carotid chemosensory input (25). In addition, in humans, although ventilation was augmented by naloxone during hypercapnic hypoxia, the ventilatory response to acute withdrawal from hypoxia by $O_2$ administration, which reflects peripheral chemoreceptor activity, was unaffected by naloxone (1). Thus it is not likely that ventilatory modulation by endogenous opioids during hypoxic stimulation occurs at the level of the peripheral chemoreceptors, despite the presence of $\delta$-opioid receptors in these structures (14).

Normalization of hypoxic ventilatory sensitivity was observed in the obstructed animals by 6 wk after load imposition. Although naloxone had no effect on the overall minute inspiratory effort response to hypoxia at this time, it did augment the RR increase seen with hypoxic stimulation. Thus, taken together, our data
suggest that the endogenous opioid system modulates ventilatory control during the early period after imposition of a ventilatory load during both hypoxic and hypercapnic stimulation. However, in this model, the influence of the endogenous opioid system on ventilatory control, although still evident, appears to decline during long-term adaptation to a ventilatory load. Several previous studies have also failed to demonstrate involvement of the endogenous opioid system in long-term adaptation to ventilatory stressors. For example, although naloxone augmented ventilation acutely after hypoxic exposure in rats, it did not have any effect after 24 h of hypoxia (20). Similarly, in cats, although naloxone augmented the ventilatory response to acute hypoxic stimulation, it failed to augment ventilatory sensitivity to hypoxia in chronically hypoxic animals (24, 25). In goats exposed to 14 days of hypoxia, β-endorphin levels did not increase over the observation period, and naloxone did not restore depressed CO2 sensitivity to baseline levels (37). In obese Zucker rats, with chest wall loading due to excess body fat, hypoxic and hypercapnic ventilatory responsiveness is blunted in both young (4- to 6-wk-old) and older (9- to 10-mo-old) rats. Augmentation of the hypoxic ventilatory response was observed with naloxone in the young but not older animals (31). In human chronic mountain sickness, naloxone failed to augment resting ventilation or increase blunted hypoxic ventilatory sensitivity, thus failing to implicate endogenous opioids in long-term adaptation to hypoxia in this setting (34). Thus studies in several species under varying conditions have failed to demonstrate a prolonged effect of endogenous opioids in long-term modulation of ventilatory control.

The decreased influence of endogenous opioids over time may be related to effects of aging or prolonged stress (15). Additionally, chronic opioid stimulation has been shown to result in a decline in opioid receptors and in mRNA coding for voltage-dependent potassium ion channels that produce neuronal hyperpolarization with opioid-receptor stimulation (17). Furthermore, prior studies have implicated regional respiratory muscle lactic acidosis as a putative stimulus for the release of endogenous opioids in the setting of acute flow-resistive loading (23). In conjunction with this, we previously demonstrated an increase in serum lactic acid levels during the early period after load imposition (8) at a time when naloxone augmented both CO2 and hypoxic ventilatory sensitivity. However, serum lactic acid levels normalized during the long-term period after loading (8), corresponding to the time when naloxone had no effect on depressed CO2 sensitivity and when normalization of the hypoxic sensitivity is noted. Thus it is possible that waning of endogenous opioid activity may also be a result of a decline in respiratory muscle lactic acid levels during long-term ventilatory loading. However, we urge caution before equating serum lactic acid levels with regional ventilatory muscle lactic acidosis.

In contrast to these observations, several clinical studies have demonstrated that endogenous opioids modulate ventilatory control in chronic obstructive pulmonary disease (COPD), a disease that also imposes chronic increases in airway resistance. Some reports have demonstrated naloxone-induced modulation of hypercapnic and hypoxic sensitivity as well as flow-resistive load compensation in COPD (28, 35, 36), whereas others were not able to demonstrate such an effect (33). We hesitate to compare the present results with these clinical studies, as our rat model of chronic resistive airway loading was not intended to be a model of COPD, and fundamental physiological differences exist between our model and the ventilatory load imposed by COPD. Furthermore, significant species differences exist in the role of endogenous opioids in control of breathing (32).

In summary, this investigation demonstrates that ventilatory loading results in acute depression of hypoxic sensitivity that is partially reversed by naloxone, thus implicating the endogenous opioid system in short-term modulation of hypoxic ventilatory responsiveness. All animals exhibited a normal response to hypoxic stimulation by 6 wk after load imposition. However, naloxone did have an effect on ventilatory pattern at this time, suggesting some persistence of endogenous opioid effects, although not of sufficient magnitude to alter the overall level of ventilatory effort achieved in response to hypoxia. In contrast, CO2 sensitivity is suppressed during both the acute and long-period periods after loading, and the long-term suppression of CO2 sensitivity is not reversed by naloxone (8). These data support the contention that the endogenous opioid system modulates ventilatory control primarily during the acute period after load imposition and that this effect wanes with time.

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Address for reprint requests: H. E. Greenberg, Division of Pulmonary and Critical Care Medicine, Long Island Jewish Medical Center, 270–05 76th Ave., New Hyde Park, NY 11040.

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