Activity of medullary respiratory neurons during ventilator-induced apnea in sleep and wakefulness

JOHN OREM1 AND EDWARD H. VIDRUK2

1Department of Physiology, School of Medicine, Texas Tech University Health Sciences Center, Lubbock, Texas 79430; and 2Department of Preventive Medicine, University of Wisconsin, Madison, Wisconsin 53705

Orem, John, and Edward H. Vidruk. Activity of medullary respiratory neurons during ventilator-induced apnea in sleep and wakefulness. J. Appl. Physiol. 84(3): 922–932, 1998.—Mechanical ventilation of cats in sleep and wakefulness causes apnea, often within two to three cycles of the ventilator. We recorded 137 medullary respiratory neurons in four adult cats during eupnea and during apnea caused by mechanical ventilation. We hypothesized that the residual activity of respiratory neurons during apnea might reveal its cause(s). The results showed that residual activity depended on 1) the amount of nonrespiratory inputs to the cell (cells with more nonrespiratory inputs had greater amounts of residual activity); 2) the cell type (expiratory cells had more residual activity than inspiratory cells); and 3) the state of consciousness (more residual activity in wakefulness and rapid-eye-movement sleep than in non-rapid-eye-movement sleep). None of the cells showed an activation during ventilation that could explain the apnea. Residual activity of approximately one-half of the cells was modulated in phase with the ventilator. The strength of this modulation was quantified by using an effect-size statistic and was found to be weak. The patterns of modulation did not support the idea that mechanoreceptors excite some respiratory cells that, in turn, inhibit others. Indeed, most cells, inspiratory and expiratory, discharged during the deflation-inflation transition of ventilation. Residual activity failed to reveal the cause of apnea but showed that during apnea respiratory neurons act as if they were disinhibited and disfacilitated.

brain stem; respiratory network; cat; hypocapnea; mechanical ventilation

Mechanical ventilation produces apnea in humans (10) and animals (11). In humans, there is redundancy of the sensory information required to initiate apnea. Hypocapnea and pulmonary vagal, upper airway, and chest wall mechanoreceptor stimulation can each induce apnea independently (13, 22–24). Although we know much about the peripheral mechanisms involved in initiating the apnea in the unanesthetized state, no systematic studies exist of the central neural mechanisms that must ultimately cause it.

Some respiratory muscles and neurons are not active but instead have a residual or tonic discharge during ventilator-induced apnea. Residual activity has been observed during ventilator-induced apnea in the triangularis sterni (expiratory) muscles in dogs (11), in internal intercostal nerves in cats (21), and in expiratory fibers of the recurrent laryngeal nerve in cats (5). Interestingly, it is sometimes reported that the maximal level of this tonic activity is greater than the maximal level during eupnea (5). Tonic activity in inspiratory muscles during apnea has been reported less often than tonic activity in expiratory muscles (Ref. 2, 21; work of Wyss cited in Ref. 7) and may depend on the species studied and on the preparation (1, 2).

Some medullary respiratory neurons are also tonically active during ventilator-induced apnea. Early studies (3, 7, 16) showed examples of both inspiratory and expiratory neurons that were tonically active during this apnea.

Various theories address the source and significance of residual respiratory muscle and neuronal activity during apnea. In early theories, Batsel (3) and Cohen (7) proposed that residual activity of respiratory neurons was the result of inactivity of cells that reciprocally inhibited them. Another theory proposed that tonic activity during hypocapnic apnea was caused by a CO2-dependent drive (2). Bulbospinal expiratory neurons progressed from inactivity to tonic activity, and then rhythmic activity as CO2 tensions increased (2). However, other respiratory neurons were tonically active even at very low CO2 tensions, leading to the theory that residual activity was caused also by non-CO2-dependent drives, including those related to different states (2, 11, 25). Nesland and Plum (16) suggested that residual activity reflected a global excitatory state out of which the rhythm to breathe is generated.

In this study, single medullary respiratory neurons were recorded in intact unanesthetized cats during spontaneous breathing and during ventilator-induced apnea. We wanted to determine whether tonic or residual activity could be the cause of apnea when an animal is hyperventilated. For example, tonic expiratory activity might inhibit inspiratory neurons and lock the oscillator in expiration.

Residual activity during apnea was of interest for another reason. In the intact unanesthetized animal, respiratory neurons vary in “respiratoriness” (19). Some have discharge patterns that correlate highly with the timing of the respiratory cycle; others have patterns that are more weakly related to the cycle. These differences have been quantified (19), and it has been proposed that the poor correlation with the respiratory cycle shown by some respiratory neurons is caused by tonic inputs to them (20). These inputs may be related to activity of the nervous system that depends on state of consciousness (20) or on behavioral control (18). We hypothesized that, if the residual activity of respiratory neurons during apnea was the result of tonic or nonrhythmic inputs to the cells, then there should be a direct relationship between the amount of residual activity during apnea and the amount of nonrhythmic activity of the cell during eupnea.
METHODS

Electrodes for recording electroencephalographic (EEG) and electromyographic (EMG) activity were implanted in four adult cats. In addition, tracheal fistulas were created, and a headcap was attached to the skull.

Surgical Procedures

The animals were anesthetized with acepromazine maleate (2.5 mg) and ketamine (30 mg/kg) and 1–5% halothane in O₂. A midline incision was made from below the cricoid cartilage to just above the suprasternal notch, and the sternohyoid, sternohyoïd, and sternomastoid muscles were retracted to expose the trachea. The trachea was opened longitudinally for a length of five cartilaginous rings. The cut edges of the rings were sewn to the skin on the corresponding side to create a fistula. The animals were placed in a stereotaxic frame, and a midline incision was made to expose the dorsal skull. EEG electrodes and 4–40 stainless steel screws were threaded into the skull and secured with dental cement.

EMG electrodes (Teflon-coated multistranded stainless steel wires; Cooner AS 632) were implanted in the nuchal muscles and in the diaphragm. The animal was placed in a supine position, and an incision was made caudal to the costal margin from the xiphoid process to the midaxillary line. The head and upper body of the cat were elevated to displace the abdominal contents caudally, and the costal margin was elevated to provide access to the diaphragm. Four wires were placed within costal and semitendinous regions of the right diaphragm. The wires were run under the skin to the occipital bone, where they were cemented in place and brought to a connector (Cinch 19 pin). EEG and EMG electrode wires and a prefabricated headcap with a connector and with standoffs for head restraint were fixed to the skull with dental cement. The animals were allowed to recover for at least 1 mo before experimentation. All procedures, preoperative, postoperative, and experimental, were approved by the Institutional Animal Care and Use Committee.

Experimental Procedures

After recovery from surgery, the animals were adapted to the experimental apparatus. For this they were placed in a veterinary catbag, and their heads were restrained by attachment of the headcap to a modified stereotaxic apparatus. The animals could assume either a sphinx position or could be semiprone on their left or right side. Generally, 1 wk or more of daily 2-h adaptation sessions was required before the animals would sleep in the laboratory.

After adaptation and in a second operation under general anesthesia (as above), a small craniotomy (~5 mm diameter) was made in the occipital bone. The craniotomy allowed passage of microelectrodes through the cerebellum into the brain stem.

The animals were sleep deprived mildly before experimental sessions by housing them in a cold (0°C) environment overnight. For recording sessions the trachea was intubated with a shortened endotracheal tube (18-Fr). Needles were inserted into the lumen of the tube to measure intratracheal pressures and to obtain samples of CO₂. The endotracheal tube was connected to a pneumotachograph (Validyne). EEG and EMG activity, instantaneous airflow rates (Validyne CD15 Carver demodulator), intratracheal pressures (Grass PT5A volumetric pressure transducer), and end-tidal CO₂ levels (Beckman LB2 analyzer) were recorded on an analog tape recorder (Hewlett-Packard) and on a chart recorder (Astro-Med MT9500). The EEG was band-pass filtered (1–35 cycles/s) and amplified (Grass wideband alternating current preamplifiers). EMG signals from the diaphragm and nuchal muscles were led to high-impedance probes (Grass HP511) and to amplifiers (Grass PS11) set to pass frequencies from...
300 to 10,000 cycles/s. A solenoid-operated two-position valve was attached to the distal end of the pneumotachograph. With the valve set in one position, the animal breathed room air. In the other position, the animal was connected to a ventilator that delivered 40–50 ml tidal volumes at rates of 30–40 per minute. No attempt was made to match exactly eupneic tidal volumes and frequencies or to maintain normal CO₂ levels. The levels of ventilation used caused hypocapnia. End-tidal CO₂ levels during ventilation typically dropped from 5 to 3.5–2.5%. Tungsten microelectrodes (impedances 1–10 MΩ) were used to record single medullary respiratory neurons. The microelectrodes were mounted to a hydraulic microdrive and driven via the craniotomy through the cerebellum and into the medulla. Signals were led to a high-impedance probe (Grass HIP511) and to a preamplifier (Grass P511) and were recorded on the Astro-Med and analog tape recorders. The recording sessions lasted ~4 h.

Data Analysis

All analyses were performed off-line. Data were played back from the tape recorder into a PS/2 486 computer with a LabWindows data-acquisition system (National Instruments). Cycle-triggered histograms and η² values were determined for each cell before and during ventilator-induced apnea. Procedures for constructing cycle-triggered histograms and calculating the η² value of the activity of a cell have been published (19). The η² values can vary from 0.0 to 1.0 and denote the signal strength and consistency of the respiratory component of the activity of a cell. The latter can vary in different states because of variation in the timing of inspiration and expiration from breath to breath; the η² values used to categorize the cells in this study were obtained from activity occurring during relaxed wakefulness or non-rapid-eye-movement (NREM) sleep. Breathing in these states shows the least variability in the timing of inspiration and expiration from breath to breath. This minimizes smearing of activity across the bins of the cycle-triggered histogram, which will artificially lower η² values. The η² statistic and the analysis of variance on which it is based were also used to detect and quantify phasic modulation by the ventilator. The activity of the neuron was sampled during ~20 cycles of the ventilator. The ventilator cycle was divided into 20 equal parts, beginning with the onset of inflation and ending with the end of deflation. The number of action potentials in each of these 20-iles was tabulated. These values formed a matrix with 20-iles of the ventilator cycle as the columns and ventilator cycles as the rows. This matrix was analyzed with an analysis of variance. A significant F-ratio indicated that the neuron was phasically modulated by the ventilator. The strength of this effect was quantified with the η² statistic. This was the procedure used to determine respiratory modu-

Fig. 3. Locations of scarring produced by microelectrode penetrations into medulla in 4 cats (DX, DL, HR, LN) studied. RB, restiform body; SST, nucleus of spinal tract of the trigeminal; RFN, retrofacial nucleus; FTG, gigantocellular tegmental field; S, tractus solitarii; 12, hypoglossal nucleus; AMB, nucleus ambiguus; DMV, dorsal motor nucleus of vagus; P, pyramidal tract.
lation of the cells during eupnea and to quantify the strength of this modulation, except that the respiratory cycle, rather than the ventilator cycle, was the treatment variable during eupnea.

Mean and maximal discharge rates were determined also during eupnea and during ventilator-induced apnea. For these determinations, cycle-triggered histograms were constructed of activity during eupnea, during the early part of mechanical ventilation (first 18–23 cycles), and during a later part of mechanical ventilation (cycles 19–47). The cycle-triggered histograms were analyzed to obtain the mean and maximal level of activity throughout the cycle as well as the SD of activity across the 20 bins of the histogram. The mean value was calculated as the mean of bins of both the active and inactive phases of the neuron (Figs. 1 and 2). The physiological significance of this mean is unclear, but it is a well-defined value that does not require an arbitrary definition of the active and inactive phases of a cell. This definition becomes increasingly problematic with patterns of activity that augment and/or decrement throughout a major portion of the respiratory cycle. Although problematic in some contexts, the mean value of the activity across the bins of the cycle-triggered histogram characterized well the pattern of activity of many of the neurons during apnea (Figs. 1 and 2). We calculated also the SD of activity from bin to bin in the cycle-triggered histogram and determined the coefficient of variation (SD/mean). The maximal activity in the cycle-triggered histogram was also determined. This was simply the largest bin value in the cycle-triggered histogram. To normalize the amount of residual activity during mechanical ventilation across cells, means, maxima, and coefficients of variation during mechanical ventilation were expressed as fractions of means, maxima, and coefficients of variation during eupnea. For example, if the mean level of activity during eupnea was 50 action potentials/s and the mean level of activity during mechanical ventilation was 25 action potentials/s, then the residual activity based on these means was expressed as the ratio 25/50 or 0.5. Differences in ratios of means and maxima and the coefficients of variation between cell types (inspiratory and expiratory, low and high $\eta^2$ values) were tested by using a two-way analysis of variance with unequal cell frequencies. Ratios based on the coefficients of variation proved misleading because coefficients of variation were often equal when activity of the cell was great and highly modulated and also when activity was minimal, with only occasional action potentials that were unrelated to the respiratory cycle. Therefore, only data from the ratios based on means and maxima are given in RESULTS.

In two figures (see Figs. 8 and 10) in this study, the activity of cells determined from the mean of interspike intervals <300 ms (an arbitrary value chosen to exclude intervals during the inactive phase of the cells) was plotted breath by breath before and during mechanical ventilation to illustrate the effects of this ventilation. These means were used for illustrative purposes only and were not used to calculate the effects of mechanical ventilation.

Histology

After fixation in formalin (10%), frozen sections (40–80 µm in thickness) of the medulla were cut, stained with cresyl violet, and examined for evidence of microelectrode penetrations.

RESULTS

Cell Types and Locations

One hundred thirty-seven neurons were recorded in four cats. Multiple penetrations produced widespread scarring that made exact reconstruction of recording sites impossible. However, histological analysis revealed that recordings were obtained from the ventral column of respiratory neurons extending between the facial nucleus and the upper cervical spinal cord (Fig. 3). There was no scarring in the region of the ventrolateral nucleus of tractus solitarii. Expiratory cells were recorded predominantly at the level of the retrofacial nucleus. Inspiratory cells predominated in penetrations between the obex and the retrofacial nucleus. A frequency histogram of $\eta^2$ values of the population of cells revealed a bimodal distribution with a trough between $\eta^2$ values of 0.3 and 0.5. Accordingly, we divided the cells into low-$\eta^2$-value ($\eta^2 \leq 0.5$) and high-$\eta^2$-value ($\eta^2 > 0.5$) categories.

---

**Fig. 4.** Response of an inspiratory-throughout (I) cell to mechanical ventilation. Note rapid inactivation of cell and brief burst of activity on 5th cycle of ventilator. Ptd, intratracheal pressure, with positive pressures indicated by downward deflections.
Dependence of Residual Activity During Ventilator-Induced Apnea on Cell Type and on $\eta^2$ Value

Respiratory neurons displayed varying amounts of residual activity in response to mechanical ventilation (cf. Figs. 4, 5, 6, 7; see also Fig. 9). The mean and maximal levels of activity were determined from cycle-triggered histograms constructed during eupnea, during the first 18–23 cycles of ventilation and, if activity persisted, during the subsequent 18–23 cycles of ventilation. Residual activity was expressed as the ratios of the values (mean and maxima) during ventilation to those during eupnea. Except for two high-$\eta^2$-value and four low-$\eta^2$-value cells studied in rapid-eye-movement (REM) sleep, these values were determined from periods of ventilation and eupnea occurring during wakefulness and NREM sleep. Analyses of variance ($2 \times 2$ factorial design with unequal cell frequency) showed that low-$\eta^2$-value cells had more residual activity than high-$\eta^2$-value cells and that the amount of residual activity was overall not significantly different during early and late mechanical ventilation (Tables 1 and 2). There was a tendency for high-$\eta^2$-value cells to show lesser mean and maximal residual activity during late ventilation than during early ventilation, but this difference did not reach a statistically significant level. Apnea typically occurred early during mechanical ventilation and often occurred within one or two cycles of the ventilator (Fig. 4). Apnea was evident from diaphragmatic recordings (Fig. 4) or from the smooth profile of the intratracheal pressure trace (Fig. 7).

Inspiratory cells showed less residual activity than expiratory cells (Tables 3 and 4). This difference was observed in both high- and low-$\eta^2$-value categories. It was observed for both mean and maximal value ratios.

In addition to inspiratory and expiratory cells, 21 phase-spanning cells were studied. Only one of these...
cells had an $\eta^2$ value >0.5. The average $\eta^2$ value of these cells during eupnea was 0.20. Data from them are included in Tables 1 and 2. Like other low-$\eta^2$-value cells, they showed large amounts of residual activity during ventilation: mean and maximal ratios during early ventilation were 1.03 and 0.94, respectively. During late ventilation, mean and maximal ratios were 0.918 and 0.967, respectively. Ten of these twenty-one cells showed phasic modulation by the ventilator. The mean $\eta^2$ value of the phasic modulation of these cells was 0.29.

The difference in residual activity between low- and high-$\eta^2$-value cells was not caused by differences in phasic modulation by the ventilator. Forty of seventy-eight (51%) high-$\eta^2$-value cells were modulated by the ventilator, whereas 35 of 59 (59%) low-$\eta^2$-value cells were modulated by the ventilator. This difference was not significant ($\chi^2$ analysis). Similarly, low- and high-$\eta^2$-value cells that were modulated to the ventilator did not differ in the strength of that modulation. The mean $\eta^2$ values of the modulated activities of low- and high-$\eta^2$-value cells were 0.21 and 0.24, respectively.

Fig. 6. Response of an augmenting inspiratory cell to mechanical ventilation. In NREM sleep (A) cell was inactivated rapidly and there was low-rate residual activity. In rapid-eye-movement (REM) sleep (B), residual activity was greater. This greater residual activity was tonic, modulated, and phasic activity. Phasic modulation for this augmenting inspiratory cell, as in most others of this type, resulted in greater activity during deflation-inflation transition. EMG, electromyogram.

Fig. 7. Response of an augmenting expiratory (Eaug) cell to mechanical ventilation. Note sustained activity of cell at onset of ventilation, with a gradual decline in activity with ventilation.
Modulation of high-$\eta^2$-value cells by the ventilator is discussed in greater detail below. Although low-$\eta^2$-value cells, and in particular expiratory cells in this category, had more residual activity than high-$\eta^2$-value cells, none of these cells was intensely activated during mechanical ventilation.

### Responses of High-$\eta^2$-Value Inspiratory and Expiratory Cell Subtypes to Ventilation

Forty-nine high-$\eta^2$-value inspiratory cells could be classified as inspiratory-throughout, decrementing, or augmenting inspiratory cells. Twenty-four high-$\eta^2$-value expiratory cells could be classified as expiratory-throughout, decrementing, or augmenting expiratory cells. Table 5 shows the mean and maximal residual activity during ventilation as a percentage of eupneic levels. It shows also the fraction of each cell type that was modulated to the ventilator and the pattern of that phasic modulation.

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>$\eta^2 \leq 0.5$</th>
<th>$\eta^2 &gt; 0.5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inspiratory</td>
<td>0.66 (26)</td>
<td>0.26 (51)</td>
</tr>
<tr>
<td>Expiratory</td>
<td>1.3 (14)</td>
<td>0.89 (24)</td>
</tr>
</tbody>
</table>

No. in parentheses, nos. of cells. P ($[\eta^2 \leq 0.5] = [\eta^2 > 0.5]$) < 0.05. P (early ventilation vs. late ventilation), not significant.

Residual Activity: State Effects

Residual activity was tonic (e.g., Figs. 1, 2, and 5B), phasic (e.g., Figs. 4 and 6B), modulated by the ventilator (e.g., Fig. 5, small action potentials; Fig. 6), or behavioral (e.g., Fig. 5C). Whatever the form of the residual activity, it showed state dependency in both its form and intensity. Among inspiratory-throughout cells, 36% of those observed during apnea in the REM sleep (n = 11) showed some form of residual activity, whereas only 20% of those observed during apnea in NREM sleep (n = 10) had residual activity. Only two inspiratory-throughout cells were observed in REM sleep, but both had residual activity. Similarly, 67, 20, and 50% of the decrementing inspiratory cells observed during apnea in the REM sleep (n = 12), NREM sleep (n = 15), and REM sleep (n = 4), respectively, had residual activity. A similar pattern showing less residual activity in NREM sleep was shown by augmenting inspiratory cells: 100, 50, and 100% of these observed in the REM sleep (n = 11), NREM sleep (n = 12) and REM sleep (n = 3), respectively, had residual activity.

### Table 3. Mean residual activity (fraction of mean eupneic activity) during ventilator-induced apnea

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>$\eta^2 \leq 0.5$</th>
<th>$\eta^2 &gt; 0.5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inspiratory</td>
<td>0.62 (26)</td>
<td>0.20 (51)</td>
</tr>
<tr>
<td>Expiratory</td>
<td>1.20 (14)</td>
<td>0.59 (24)</td>
</tr>
</tbody>
</table>

No. in parentheses, nos. of cells. P ($[\eta^2 \leq 0.5] = [\eta^2 > 0.5]$) < 0.05. P (inspiratory = expiratory) < 0.05.

The percentage of cells having residual activity was higher for expiratory than for inspiratory cells. Of augmenting expiratory cells observed during apnea in the REM sleep (n = 8), NREM sleep (n = 7), and REM sleep (n = 2), 100, 57 and 100%, respectively, had residual activity. This was similar to the pattern shown by inspiratory cells. However, of decrementing expiratory cells observed during apnea in the REM sleep (n = 4), NREM sleep (n = 4), and REM (n = 3) sleep, 100, 100, and 33%, respectively, had residual activity. This differed from the pattern shown by inspiratory and augmenting expiratory cells, not only in the persistence of residual activity during REM sleep but also in the...
reduction in that activity in REM sleep. Only one expiratory-throughout cell was observed in REM sleep, but it too showed less residual activity in that state (Figs. 9 and 10). Expiratory-throughout cells observed during apnea in wakefulness (n = 5) and NREM sleep (n = 4) all showed some residual activity.

Phasic Modulation of High-\(\eta^2\)-Value Cells by the Ventilator

Phasic modulation by the ventilator was evident in 38 of the 73 (52%) high-\(\eta^2\)-value cells studied. Phasic modulation was more common for expiratory cells (79% modulated) than for inspiratory cells (29% modulated). This difference was significant (P < 0.05; \(\chi^2\) analysis). A higher percentage of augmenting expiratory cells was modulated (92%) than expiratory-throughout or decrementing expiratory cells (67 and 60%, respectively). Of the inspiratory cells, only the augmenting inspiratory cells tended to be modulated (75%). Phasic modulation produced \(\eta^2\) values that were maximally \(\sim\)0.5 and averaged, across expiratory and inspiratory cells, 0.23 and 0.25, respectively. Phasic modulation did not result in an orderly patterning among the different cell types (Table 5): Indeed, inspiratory, particularly augmenting inspiratory, and expiratory cells both tended to be modulated during the deflation-inflation transition (Fig. 11).

DISCUSSION

Central respiratory neurons were eventually neither excited (with the exception of the expiratory cells discussed below) nor inhibited during ventilator-induced apnea. They displayed varying amounts of residual activity that depended on the \(\eta^2\) values of their activities and on the state of consciousness. The activity, whether tonic and/or modulated by the ventilator, was often nonreciprocal in neurons that discharge reciprocally during eupnea. Inspiratory neurons were, in general, more active than inspiratory neurons during apnea, but their activity levels were intermediate between their most active and their most inactive levels during eupnea. Thus mechanical ventilation creates a state in which respiratory neurons are clearly excitatory and are often active but in which the patternings among them is lost.

Significance of Residual Activity

Residual activity during apnea depended on 1) the \(\eta^2\) value of the cell, 2) the cell type (inspiratory vs. expiratory), and 3) the state of consciousness.

The \(\eta^2\) value. Low-\(\eta^2\)-value cells, whether inspiratory or expiratory, had more residual activity during apnea than high-\(\eta^2\)-value cells. With the assumption that mechanical ventilation eliminates the respiratory component in the activity of a cell and reveals components other than those related to rhythm generation, this finding supports the interpretation that the \(\eta^2\) statistic (19) quantifies the amount of nonrespiratory input to a cell (17, 20). The greater amount of residual activity in low-\(\eta^2\)-value cells was not caused by a greater percentage of modulated cells in that category because there was no difference in the percentages of high- and low-\(\eta^2\)-value cells that were modulated by the pump or in the strength of that phasic modulation.

Cell type. Expiratory cells had more residual activity than inspiratory cells. This may indicate that expira-

<p>| Table 5. Responses of high-(\eta^2)-value cell types to mechanical ventilation |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Mean Residual Activity</th>
<th>Maximal Residual Activity</th>
<th>Fraction Modulated</th>
<th>Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inspiratory cells (n = 17)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>0.19</td>
<td>0.17</td>
<td>3/17</td>
<td>I (1), DI (2)</td>
</tr>
<tr>
<td>Late</td>
<td>0.18</td>
<td>0.14</td>
<td>2/17</td>
<td></td>
</tr>
<tr>
<td>Decrementing inspiratory cells (n = 16)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>0.21</td>
<td>0.15</td>
<td>4/16</td>
<td>DI (2), I (1)</td>
</tr>
<tr>
<td>Late</td>
<td>0.12</td>
<td>0.08</td>
<td>0/15</td>
<td>ID (1)</td>
</tr>
<tr>
<td>Augmenting inspiratory cells (n = 16)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>0.42</td>
<td>0.30</td>
<td>12/16</td>
<td>DI (6), ID (2),</td>
</tr>
<tr>
<td>Late</td>
<td>0.32</td>
<td>0.23</td>
<td>9/15</td>
<td>I (3), D (1)</td>
</tr>
<tr>
<td>Augmenting expiratory cells (n = 13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>0.64</td>
<td>0.43</td>
<td>12/13</td>
<td>DI (8), D (2),</td>
</tr>
<tr>
<td>Late</td>
<td>0.30</td>
<td>0.25</td>
<td>5/12</td>
<td>ID (2)</td>
</tr>
<tr>
<td>Decrementing expiratory cells (n = 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>0.96</td>
<td>0.65</td>
<td>3/5</td>
<td>ID (2), DI (1)</td>
</tr>
<tr>
<td>Late</td>
<td>0.45</td>
<td>0.32</td>
<td>2/4</td>
<td></td>
</tr>
<tr>
<td>Expiratory cells (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>1.35</td>
<td>0.91</td>
<td>4/6</td>
<td>DI (1), ID (1),</td>
</tr>
<tr>
<td>Late</td>
<td>1.12</td>
<td>0.80</td>
<td>2/4</td>
<td>D (2)</td>
</tr>
</tbody>
</table>

Values are ratios of eupneic means and maxima. Nos. in parentheses, nos. of cells; early and late, first 18–23 cycles of ventilation and second 18–23 cycles of ventilation, respectively. Under Pattern: I, inflation peak; D, deflation peak; ID, peak at inflation-deflation transition; DI, peak at deflation-inflation transition.
tory cells receive more nonrespiratory inputs than inspiratory cells, but this is not reflected in the distribution of $\eta^2$ values of inspiratory and expiratory cells. Alternatively, apnea may create a state in which expiratory cells are relatively more depolarized than inspiratory cells. We do not know by what mechanism or for what purpose this might be the case.

State of consciousness. There was the least amount of residual activity in NREM sleep. This is consistent with the idea that tonic or nonrespiratory inputs are

---

Fig. 9. Response of an expiratory (E) cell to mechanical ventilation in NREM and REM sleep. In NREM sleep, ventilation caused activation of cell that was sustained (A). In REM sleep, eupneic activity of cell decreased and only occasional action potentials were observed (B). Ventilation in REM sleep activated cell, but this activation was weak relative to activation in NREM sleep and it was intermittent (C).
least in that state (11, 20). In wakefulness, both tonic activity, activity related to behaviors such as sniffing and swallowing, and behavioral breathing often occurred. In REM sleep, residual activity also occurred. The patterns of this REM-related activity were not recognizable. The activity appeared as erratic bursts perhaps related to the fractionated breathing that occurs in REM sleep in eupnea.

Mechanisms of Apnea

One purpose of this study was to understand the mechanism of apnea by observing the behavior of respiratory neurons. One possible outcome was to observe activation of some respiratory cell type that might cause apnea by inhibiting other cell types. Some expiratory cells showed a tonic activation during mechanical ventilation. This activation was intense and sustained in wakefulness and NREM sleep, but not in REM sleep. Therefore, they cannot account for the apnea in REM sleep. They may be motoneurons. Their behavior is similar to that of the triangularis sterni (expiratory) muscles during ventilator-induced apnea during sleep and wakefulness (11) and to expiratory fibers of the recurrent laryngeal nerve (5). Furthermore, their inactivation during REM sleep suggests that they are motoneurons and are inhibited/disfacilitated in that state like many other motoneurons. The source of the excitatory drive to these cells is not known. Our results and those of others (1, 3, 7, 16) show that it does not come from a central excitatory state of expiratory neurons.

As another possibility, apnea may result from mechanoreceptor reflexes. There is elegant work by Hayashi et al. (12), Feldman and Cohen (9), and Manabe and Ezure (14) showing that decrementing expiratory cells are activated by pulmonary stretch receptors and that
they, in turn, inhibit inspiratory cells. It seemed likely that mechanical ventilation would produce excitation of decrementing expiratory cells with each inflation and that this would inhibit inspiratory cells to cause apnea. Our results do not support this idea. Inspiratory cells are not inactivated during the apnea, and decrementing expiratory cells that were modulated in phase with the ventilator discharged at either the transition from inflation to deflation or at that from deflation to inflation rather than throughout inflation, as might be expected from the work of Hayashi and others (12). The strength of that modulation had an $h^2$ value of 0.26 averaged across the three decrementing expiratory cells studied. This is a relatively weak effect. It is comparable to the strength of modulation of other respiratory cells—more than one-half of which had peak discharges during the deflation-inflation transition of the ventilator.

Another mechanism that must be implicated in the apnea is unloading of the central chemoreceptors. Hypocapnea develops slowly with ventilation (4) because of the buffering capacity of the blood, and there is a delay before central chemoreceptors sense a change in CO$_2$ levels. Changes in CO$_2$ are sensed by peripheral chemoreceptors within 3–5 s and by central chemoreceptors within ~25 s in awake lambs (6). Apnea sometimes occurred too rapidly in our study to be attributable to chemoreceptor unloading, but the latter must eventually play a role. It is not known how low CO$_2$ concentrations affect respiratory cells to cause apnea. Some cells show a progressive decline in neuronal activity during ventilation that parallels decreasing CO$_2$ levels. The best examples of this were obtained from augmenting expiratory cells.

Eventually, respiratory cells, with the exception of the expiratory cells that may be motoneurons, appear to be in a disinhibited and disfacilitated state during apnea. The mechanisms causing this state are not known.

The authors acknowledge Becky Tilton, Jonathan Rude, and Carrie Hines for technical assistance. Dr. Cary Anderson Culbertson assisted with some of the recordings. Dr. Thomas Dick provided important interpretative insights and helped develop the themes of the manuscript.

This work was supported by National Heart, Lung, and Blood Institute Grant HL-21257 (to J. Orem) and Specialized Center of Research Grant (to E. H. Vidruk).

Address for reprint requests: J. Orem, Physiology Dept., Texas Tech Univ. HSC, Lubbock, TX 79430 (E-mail phyjmo@ttuhsc.edu). Received 27 June 1997; accepted in final form 18 November 1997.

REFERENCES