Medullary raphe neuron activity is altered during fictive cough in the decerebrate cat

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Baekey, David M., Kendall F. Morris, Sarah C. Nuding, Lauren S. Segers, Bruce G. Lindsey, and Roger Shannon. Medullary raphe neuron activity is altered during fictive cough in the decerebrate cat. J Appl Physiol 94: 93–100, 2003; 10.1152/japplphysiol.00341.2002.—Chemical lesions in the medullary raphe nuclei region influence cough. This study examined whether firing patterns of caudal medullary midline neurons were altered during cough. Extracellular neuron activity was recorded with microelectrode arrays in decerebrated, neuromuscular-blocked, ventilated cats. Cough-like motor patterns (fictive cough) in phrenic and lumbar nerves were elicited by mechanical stimulation of the intrathoracic trachea. Discharge patterns of respiratory and nonrespiratory-modulated neurons were altered during cough cycles (58/133); 45 increased and 13 decreased activity. Fourteen cells changed firing rate during the inspiratory and/or expiratory phases of cough. Altered patterns in 43 cells were associated with the duration of, or extended beyond, the cough episodes. The different response categories suggest that multiple factors influence the discharge patterns during coughing; e.g., respiratory-modulated and tonic inputs and intrinsic connections. These results suggest involvement of midline neurons (i.e., raphe nuclei) in the cough reflex.

Cough is elicited by stimulation of central airway receptors that project to the nucleus tractus solitarii (6, 17). Airway receptor second-order neurons in the nucleus tractus solitarii project to areas of the brain stem known to contain populations of neurons involved in respiratory control (9). Our laboratory has previously reported evidence supporting a model for the participation of ventrolateral medullary respiratory neurons (i.e., Bötzinger/pre-Bötzinger/ventral respiratory group) in the generation of the cough motor patterns of respiratory pump and laryngeal muscles (1, 30, 31).

There is a substantial body of work supporting involvement of medullary midline raphe neurons in the modulation of breathing. We have described respiratory-related neuronal assemblies in the midline of the medulla that have reciprocal interactions with the ventrolateral medullary respiratory network (19, 20, 22, 24, 26). Caudal raphe neurons are influenced by carotid body chemoreceptors (24, 26) and arterial baroreceptors (19, 20, 28), both of which alter breathing. Raphe neurons may also function as central chemoreceptors (4).

One report suggested that raphe neurons also influence the cough motor pattern. Kainic acid lesions in the medullary midline region (i.e., raphe nuclei and adjoining reticular formation) eliminated cough patterns in phrenic and lumbar nerve neurograms (14). As a next step in understanding the possible role of raphe neurons in the expression of the cough motor pattern, we examined discharge patterns during fictive cough. Preliminary accounts of the results have been reported (3, 32).

METHODS

General methods. Most of the methods have been described in detail elsewhere (30, 31). Experiments were performed under protocols approved by the University of South Florida’s Animal Care and Use Committee. Data were obtained from 16 adult cats (2.5–4.1 kg) of either sex. Animals were initially anesthetized with intravenous sodium thiopental (22.0 mg/kg) and later decerebrated by use of the technique of Kirsten and St. John (15).

Before surgery, atropine (0.5 mg/kg im) was administered to reduce mucus secretion in the airways, and dexamethasone (2.0 mg/kg iv) was given to help prevent hypotension and minimize brain stem swelling. Femoral arteries and veins were catheterized for monitoring of arterial blood pressure, arterial blood samples were periodically analyzed for PO2, P CO2, pH, and HCO3− concentration; these parameters were maintained within normal limits. Solutions of 5% dextrose in 0.45% NaCl, 5% dextran, or lactated Ringer solution were administered intravenously as needed to maintain a mean blood pressure of at least 100 mmHg. Until decerebration was completed (to render the animal insentient), the level of anesthesia was assessed periodically by toe pinch. If the withdrawal reflex occurred or there was an increase in blood pressure or respiration, additional anesthesia was given until the response was absent.

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For decerebration, the external carotid arteries were ligated rostral to the lingual arteries bilaterally. A craniotomy was performed in the parietal bones. The brain stem was transected at the midcollicular level, and nervous tissue rostral to the transection was aspirated. During the decerebration and thereafter, animals were continuously infused intravenously with gallamine triehiodide (4.0 mg·kg$^{-1}$·h$^{-1}$) and artificially ventilated through a tracheal cannula with a phrenic-driven respirator. End-tidal CO$_2$ was maintained at 4.0–5.0%. A bilateral thoracotomy was performed to minimize brain stem movement that could result from changes in thoracic pressure during positive-pressure ventilation. The animals were ventilated with 100% O$_2$ to prevent hypoxemia that often occurs during long-term experiments because of ventilation-perfusion mismatching resulting from the open chest. The functional residual capacity of the lungs in thoracotomized animals was maintained within a normal range by adjustment of end-expiratory pressure. Periodically, the trachea was suctioned and the lungs were hyperinflated. Fictive coughs were used so that pulmonary stretch receptor activity matched the central inspiratory and expiratory phases of the respiratory cycle. This synchronization was necessary for reproducible expressions of the cough motor pattern (7, 11, 29), as well as determination of neuron discharge patterns with cycle-triggered-histograms.

Data acquisition, entry, and preprocessing. During the experiments, signals were monitored on oscilloscopes, a polygraph, and audio monitors and were recorded on magnetic tape for off-line analysis. These data included signals from the microelectrode arrays, efferent nerve activities, arterial blood pressure, tracheal pressure, and stimulus-timing signals. Multifiber efferent phrenic and lumbar activities were integrated to obtain a moving time average of activity in the nerves. These analog signals, together with arterial blood pressure, tracheal pressure, stimulus-timing signals, and signals from each microelectrode, were digitized and stored for subsequent analysis. Time stamps were derived from the integrated phrenic signal to indicate the onset of each inspiratory and expiratory phase. Action potentials of single neurons were converted to times of occurrence with spike-sorting software (Datawave Tech). Data files were transferred to Hewlett-Packard workstations for subsequent processing and analysis. The signals of efferent multiunit nerve activities were high-pass filtered (40 Hz, 3-dB cutoff) and, along with the common synchronization-timing pulses, were stored for subsequent merging with the spike times data files.

Spike train analysis methods and classification criteria. The following measures were computed from a 5-min control period that preceded the beginning of the cough trials. Spike trains were subjected to two statistical evaluations of respiratory modulation, and a measure of the degree of respiratory modulation, $\tau^2$, was calculated (24, 27). Cycle-triggered histograms were used to classify cells with statistically significant respiratory modulation according to the phase [inspiratory (I) or expiratory (E)] in which they were more active. Neurons with peak firing rates in the first half of the phase were classified as decrementing (Dec), whereas those cells with peak firing rates in the second half of the phase were denoted as augmenting (Aug). Cells with peak activity at the transition between the inspiratory and expiratory phase were labeled I/E. Neurons, generally with low firing rates, that could not be placed in one of these major classifications were denoted as I-Other or E-Other. Cells that were silent during control cycles but were evoked or recruited during cough were labeled as Recruit (e.g., peak activity during the expiratory phase of cough, E-Recruit). Neurons with no preferred phase of maximum activity were classified as nonrespiratory modulated. It should be noted that no attempt was made to classify individual neurons as serotonergic on the basis of the analysis of data in this study.

Autocorrelograms were computed for each spike train to ensure that it represented the activity of one neuron. A recording with action potentials from two or more neurons would include short intervals not constrained by refractoriness. Spike trains were also evaluated for changes in peak discharge rate during cough. The peak rate during the first cough cycle, averaged over five trials, had to be significantly different from the mean of five control cycles preceding the coughs ($P < 0.05$, Student’s $t$-test).

RESULTS

The spike trains of 133 neurons were recorded along the midline of the medulla 1.3–6.3 mm rostral to the obex and 1.9–3.7 mm below the dorsal surface. This
region included the raphe obscurus and the caudal portion of the raphe magnus.

Most neurons discharged tonically, i.e., had firing probabilities greater than zero in all phases of the respiratory cycle. Other characteristics of the neurons are listed in Table 1. Thirty-six neurons (27%) were respiratory modulated; 21 showed peak firing rates during the expiratory phase, 11 during the inspiratory phase, and 4 at the inspiratory-expiratory phase transition. Values for the measure of respiratory modulation ($\eta^2$) were generally low (mean $\pm$ SD, range): inspiratory (0.13 $\pm$ 0.14; 0.02–

Fig. 1. Firing rate histograms of expiratory-modulated neurons and integrated activity of phrenic and lumbar nerves during control and cough cycles. A–D: examples from different animals. Cough cycles are indicated by a large increase in phrenic nerve discharge immediately followed, or partially overlapped, by a large increase in lumbar nerve activity. Neuron discharge patterns: E, expiratory; Aug, augmenting; Dec, decrementing; Other, uncharacterized; Recruit, activity evoked during cough. $I_{PHR}$, integrated phrenic nerve; $I_{LUM}$, integrated lumbar nerve; S, duration of stimulus. * Increased firing rate associated with the expiratory phase of the cough cycles. Firing rates (in spikes/s) are shown on the right and refer to the largest bin in the corresponding plot. Number to the left under each histogram identifies the neurons for comparison with cycle-triggered histograms.
0.43), expiratory (0.03 ± 0.02; 0.02–0.12), and inspiratory-expiratory phase spanning (0.05 ± 0.03; 0.02–0.1).

The discharge patterns of 58 of 133 cells (44%) were altered during cough cycles; of these, 26 (44%) were respiratory modulated and 32 (56%) were nonrespiratory modulated (Table 1). The mean and range of neuron firing rates during control and cough cycles are shown in Table 2.

Figure 1 shows firing rate histograms of five expiratory-modulated neurons and integrated activity of phrenic and lumbar nerves during control and cough cycles. A cough cycle is indicated by a large increase in phrenic nerve discharge immediately followed, or partially overlapped, by a large increase in lumbar nerve activity. Figure 2 displays control cycle-triggered histograms (CTH) for four of the five neurons illustrated in Fig. 1.

Nineteen neurons active during control cycles were expiratory modulated. This sample included one decrementing (E-Dec) and one augmenting (E-Aug) neuron and 17 classified as E-Other. Nine of these expiratory neurons showed elevated firing rates during cough. Two neurons responded with increased peak firing rates primarily during the expiratory phase (e.g., E-Dec neuron in Fig. 1A). Three cells displayed both elevated tonic and peak expiratory activity for the duration of the cough episodes; e.g., see E-Aug neuron discharge pattern in Fig. 1A (asterisks indicate increased activity associated with the expiratory phase). Four expiratory neurons showed increased tonic discharge rates extending beyond cessations.

| Table 1. Neuron discharge patterns and changes in peak firing rate during fictive cough |
|---------------------------------|-----|-----|-----|-----|
|       | n   | ↑   | ↓   | →   |
| E     | 21  | 11  | 3   | 7   |
| I     | 11  | 4   | 4   | 3   |
| I/E   | 4   | 4   | 0   | 0   |
| NRM   | 97  | 26  | 6   | 65  |
| Total | 133 | 45  | 13  | 75  |

Neurons were classified as expiratory (E), inspiratory (I), inspiratory/expiratory phase spanning (I/E), and nonrespiratory modulated (NRM). Direction of change in peak firing rate: ↑, increase; ↓, decrease; →, no change. The medullary domain in which recordings were made is fully defined in the text.

| Table 2. Neuron peak firing rates during control and fictive cough |
|---------------------------------|-----|-----|-----|
|       | c   | ↑ cg| ↓ cg|
| E     | 16.0| 25.1| 11.6| 6.4 |
|       | 6.0–40.7| 7.4–46.3| 6.8–14.8| 0–9.8 |
| I     | 8.2| 15.3| 25.5| 19.6 |
|       | 6.7–10.2| 14.0–15.6| 18.7–32.6| 7.6–30.9 |
| I/E   | 36.2| 60.7| 32.1–40.3| 43.8–73.6 |
| NRM   | 12.4| 27.5| 18.2| 11.0 |
|       | 5.2–23.3| 11.1–75.8| 6.0–36.4| 2.8–25.0 |

The numbers in each block are the mean and range (spikes/s). Arrows indicate direction of change in firing rates. c, Control. cg, cough.

Fig. 2. Cycle-triggered histograms for neurons 73, 58, 76, and 72 in Fig. 1. Number of cycles averaged 261, 122, 261, and 193 for A, B, C, and D, respectively. \( \eta^2 \), Measure of the degree of respiratory modulation.
tion of the cough episodes (e.g., Fig. 1B). Three expiratory cells ceased firing during cough episodes for periods of one to multiple cycles (e.g., Fig. 1C). Two additional neurons that were silent during control cycles were recruited primarily during the expiratory phase of cough (e.g., Fig. 1D).

Nine neurons had an inspiratory modulation during control cycles and were classified as I-Other. Increases in both tonic and inspiratory phase firing rates were observed in two cells during cough (e.g., Fig. 3B). Four inspiratory neurons responded during cough with decreased activity (e.g., Fig. 3C). Two other silent neurons were recruited primarily during the inspiratory phase of cough (e.g., Fig. 3A).

Four neurons with peak firing rates at the transition from the inspiratory to expiratory phase during control cycles showed increased activity in both phases during cough with peak activity persisting at the phase transition (e.g., Fig. 4).

Of 97 neurons with no respiratory modulation (NRM) during control cycles, 26 increased discharge rate after the cough stimulus. The response patterns

Fig. 3. Responses of inspiratory (I) neurons during cough (A–C). Number cycles for cycle-triggered histograms (CTH) averaged 170 (D) and 156 (E).
varied from brief bursts to elevated activity that extended beyond the cough period (e.g., neurons 62, 75, and 77, Fig. 5). Six nonrespiratory-modulated cells showed a decline in discharge rate during and after the cough periods (e.g., neuron 85, Fig. 5).

Cells that changed activity during cough episodes were examined to rule out alterations associated with systemic arterial blood pressure (19, 20, 28, 35). Changes in blood pressure were observed occasionally during cough episodes; there was a reduction with a subsequent rebound above control levels. The reduction resulted from hyperinflation of the lungs, reduced pulmonary blood flow, and cardiac output. The rebound occurred as lung volume decreased after hyperinflation. The hyperinflation was due to a prolonged duration of inspiratory airflow from the ventilator because of increased duration and amplitude of phrenic activity. Only one of the neurons with altered activity during cough was also affected by the change in blood pressure.

DISCUSSION

The results of this study establish that elements of the caudal medullary raphe (magnus, obscurus) neuronal network are altered during fictive cough. There were enhanced and attenuated inspiratory, expiratory, and tonic changes in neuron discharge. The predominant response was an increase in nonrespiratory re-
lated (tonic) activity lasting longer than the cough episodes. The different response categories of the neurons suggest that multiple factors influence the discharge patterns during cough; e.g., respiratory-modulated and tonic inputs and intrinsic raphe connectivity.

Twenty-three percent of the raphe cells changed activity during the inspiratory and/or expiratory phases of cough. One likely source of the altered respiratory activity during cough is the medullary ventilatory respiratory group (including the Bötzinger and pre-Bötzinger complexes), which is involved in producing eupneic and cough motor patterns (30, 31). Inferences from cross-correlation of simultaneously recorded spike trains suggested that midline neurons receive respiratory drive from ventral respiratory group neurons (22). Other sources of brain stem respiratory-modulated inputs to consider include lung receptor relay neurons in the nucleus tractus solitarii, the dorsal respiratory group, and the pontine respiratory group. Pulmonary stretch receptors appear not to be responsible for the altered respiratory modulation during cough. Recent results of our laboratory showed that the strength of the respiratory modulation of midline raphe neurons was attenuated by pulmonary stretch receptors during eupnea, in a manner similar to the effect on pontine respiratory group neurons (25). The dorsal respiratory group is not a likely source of respiratory inputs; it contains inspiratory neurons that do not project to the raphe nuclei (8). Neurons of the pontine respiratory group ("pneumotaxic center") project to the raphe nuclei (10) and are a possible source of altered respiratory modulation during cough. A preliminary study of our laboratory identified changes in pontine respiratory group neuron activity during fictive cough (2).

Peripheral chemoreceptor and baroreceptor afferents have a respiratory modulation, and the information is transmitted presumably via central connections to raphe neurons (28, 34). However, these receptor reflexes are unlikely sources of altered inputs during cough. The animals were ventilated with 100% O₂ and arterial CO₂ and pH were maintained in the normal range for cats. Under these conditions, the respiratory-related oscillation in peripheral chemoreceptor discharge is near zero (5). During most cough trials, there was no significant change in blood pressure and thus baroreceptor activity. In those cough episodes with alterations in blood pressure, only one neuron changed firing rate in synchrony with changes in blood pressure; this cell also responded to cough.

Most of the neurons with altered firing rates during cough (77%) displayed changes in nonrespiratory-related (tonic) activity associated with, or extending beyond, the duration of the cough episodes. Changes in tonic activity did not appear to be associated with the duration of the stimulus. The complex, prolonged changes in activity were due most likely to the effects of recurrent interactions with other brain stem sites as well as within the raphe network (21, 22). Possible sources of input include the nucleus tractus solitarii and pontine respiratory group. The nucleus tractus solitarii contains airway cough receptor second-order neurons that relay information to the brain stem network (6, 9, 17). If this cough afferent information is relayed to the brain stem network, it is most likely through unknown polysynaptic pathways. Anatomical tracing studies suggest there are no direct connections from nucleus tractus solitarii to the raphe network (36). Most pontine respiratory group neurons have tonic discharge patterns with superimposed respiratory modulation, and there are changes in both patterns during fictive cough (2). Hence the pontine respiratory group could be a source of both phasic and tonic activity to raphe neurons during cough as well as during eupnea.

When considered together with previous reports, the changes in neuron activity in this study support a role for the raphe network in the modulation of the cough motor pattern. There is substantial evidence for action of raphe neurons on the medullary dorsal and ventral respiratory groups (18, 22, 24, 33) and phrenic motor neurons (12, 13). Furthermore, kainic acid destruction of neurons in the region of the raphe nuclei eliminated cough patterns in phrenic and lumbar neurograms, presumably because of a loss of facilitatory input to brain stem circuits and/or spinal respiratory motor neurons that mediate the cough reflexes (14). These studies are consistent with the emerging picture of raphe neurons as integrators of afferent input and modulators through efferent actions on brain stem motor and autonomic control systems (20, 22, 23).

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