Effects of abdominal or cardiopulmonary sympathetic afferents on upper cervical inspiratory neurons

Yuan, Y., M. J. Chandler, R. D. Foreman, and J. P. Farber. Effects of abdominal or cardiopulmonary sympathetic afferents on upper cervical inspiratory neurons. Am J Physiol Regulatory Integrative Comp Physiol 278: R1289–R1295, 2000.—Responses of upper cervical inspiratory neurons (UCINs) to abdominal visceral or cardiopulmonary sympathetic stimulation were studied using extracellular recordings from 213 UCINs in 54 pentobarbital sodium-anesthetized and paralyzed rats. Phrenic nerve activity was used to assess inspiration. The UCINs discharging during inspiration only were mainly in the C1 segment, whereas phase-spanning UCINs were mostly in the C2 segment. Phase-spanning activity was typically retained after overventilation or vagotomy. When greater splanchnic nerve (GSN) or cardiopulmonary sympathetic afferent (CPSA) fibers were electrically stimulated, augmented UCIN activity was observed in 65% of cells responding to CPSA stimulation but in only 17% of cells responding to GSN. Response latencies were 10.7 ± 0.5 and 20.6 ± 1.5 (SE) ms, respectively. Many augmented responses to CPSA stimulation (64%) and all augmented responses to GSN stimulation were followed by suppression of UCIN discharge (biphasic response). Phrenic nerve activity was suppressed by both GSN and CPSA stimulation, but with shorter latency for the latter (29 ± 0.7 vs. 14.0 ± 0.7 ms). Excitation of UCINs using CPSA stimulation occurs more often and by a more direct pathway than for GSN input.

A BILATERAL COLUMN of upper cervical inspiratory neurons (UCINs) is found in the vicinity of the border of the intermediate gray matter and lateral funiculus. This pool of neurons extends from the caudal end of the nucleus retroambigualis into the rostral half of C3 segment in cats or the middle of the C2 segment in rats (6, 27, 28). It was initially suggested that this prominent group of neurons was part of a propriospinal circuit that organized respiratory output because of its possible analogy with the propriospinal C3–C4 system regulating forelimb movement (14, 27, 28, 29). Both electrophysiological and histological studies proved the existence of collateral arborizations of the descending axons of these neurons in the region of phrenic and other respiratory motor neurons, but a minority of these axons have been demonstrated to have direct monosynaptic connections with respiratory motor neurons (7, 14, 21, 27, 28, 32, 40). Probable monosynaptic inputs onto UCINs from medullary respiratory neurons have been demonstrated (19, 20, 30, 41), and there may be input from the nucleus raphe magnus as well (5). These relays from respiratory regions of the brain are not, however, required for transmitting drive to spinal cord respiratory neurons (31, 41). Although the functional significance of UCINs remains elusive, the possible involvement of UCINs in generation of respiratory activity in spinalized animals (8, 10) and in ventilatory behaviors of respiratory motoneurons (15, 31, 34) are attractive but currently unproven possibilities.

Other possible functions of UCINs are suggested by the fact that the upper cervical cord helps regulate sensory integration of more caudal regions of the spinal cord (36). Dorsal horn neurons of the upper cervical cord may participate in such integration (16), and these neurons receive both visceral and somatic input (16, 42). UCINs also are known to receive visceral information from the vagus nerve (12, 15). On the other hand, the extent of sympathetic input to UCINs is unknown as are UCIN responses to specific visceral stimuli.

The objective of this study was to explore possible functional roles of UCINs by determining their responses to sympathetic cardiopulmonary and abdominal visceral stimulation. We also reexamined some electrophysiological characteristics of this group of neurons with respect to their discharge patterns (5, 6, 27, 28, 34).

MATERIALS AND METHODS

Animal Preparation

Experiments were performed on 54 adult male Sprague-Dawley rats weighing between 300 and 500 g. Anesthesia was induced with pentobarbital sodium (60 mg/kg ip). Catheters were placed in the right carotid artery and external jugular vein to record blood pressure and to administer drugs, respectively. Pentobarbital sodium was infused (20–40 mg kg\(^{-1}\)·h\(^{-1}\) iv) to maintain anesthesia, and pancuronium bromide (0.4 mg/kg iv) was administered before neuronal recordings to produce muscle relaxation. The level of anesthesia was regulated by observing blood pressure stability and pupil diameter. Animals were ventilated artificially via a tracheotomy using a small animal respirator supplying 50–65 breaths/min at a 5- to 6-ml tidal volume. To suppress central respiratory drive temporarily by overventilation, respiratory frequency was increased to 70–110 breaths/min. A thermostatically controlled heating pad and overhead infra-
red lamp were used to maintain rectal temperature at \approx 36.5^\circ\text{C}.

Stimulation and recording. Forty-four rats were used for visceral stimulation protocols. Ten additional rats were used only for sorting UCIN discharge patterns. One group of animals (group I, \( n = 22 \)) was prepared for cardiopulmonary sympathetic afferent (CPSA) stimulation, and another group (group II, \( n = 22 \)) was used for abdominal visceral stimulation. For group I, the left stellate ganglion was exposed through a thoracotomy. One hook of a bipolar stimulating electrode was placed around the sympathetic chain just caudal to the stellate ganglion, and the other hook was placed around the ventral ansa subclavia (42). For group II, bipolar electrodes were mounted around the greater splanchnic nerve (GSN) proximal to its junction with the left adrenal nerve (1, 37). All nerves remained intact. Manually triggered trains of electrical stimuli ranged from 2 to 20 spikes (2–17 V, 0.2 ms) for the CPSA and from 1 to 4 spikes (5–30 V, 0.2–0.3 ms) for the CPSA. Frequency of stimulation was set at 333 or 500 Hz. Colorectal and small intestinal distensions (SID) were used to mechanically stimulate abdominal visceral a in 14 of the above rats. Colorectal distension (CRD) was produced by inserting a 4- to 5-cm-long flexible latex balloon intranally into the descending colon and rectum (33). The balloon was connected by polyethylene tubing to a sphygmomanometer for inflating the balloon with air and for monitoring pressure. SID was produced by opening the wall of the proximal jejunum and inserting a latex balloon into the lumen (13). Saline was used to distend the small intestinal balloon, and the degree of distension was quantified by the volume of saline injected.

Rats were placed in a stereotaxic frame and suspended by thoracic vertebrae and ischial clamps. Either the left or right phrenic nerve was exposed in the neck region, and a bipolar hook electrode was placed around it to determine inspiratory drive from the brain stem. A laminectomy was performed to expose the C1–C2 segments of the spinal cord. Extracellular recordings were made with carbon-filament glass microelectrodes from 213 UCINs in the area of C1–C2. All of the signals were monitored by the Spike-2 computer-based system (Cambridge Electronic Design), and selected signals were saved for later analysis. Peristimulus histograms were constructed using the saved raw data. Bin width was 1 or 2 ms, and the number of sweeps ranged between 20 and 30. To identify a response on a histogram, the following criteria were used for an augmented response: the sum of augmented activity in one to three adjacent bins was at least two times that of any equal number of adjacent bins with the highest activity in the control period before the stimulus. The number of bins was chosen based on abruptness and duration of response with respect to the bin width used. For a suppressed response, the sum of activity in 10 adjacent bins was one-half or less than that of 10 adjacent bins in the control period before the stimulus. The latencies of responses were determined by measuring the time, on the histogram, from the beginning of the stimulating artifacts to the beginning of the bins where the responses fell into the above criteria. Student's t-test (paired or unpaired) was used for comparing the latencies of responses between groups. Statistical significance was established as \( P < 0.05 \).

**RESULTS**

Discharge Patterns of UCINs

A total of 213 UCINs were recorded extracellularly in the C1–C2 cervical cord of the 54 rats. Recording sites approximated those used by Lipski et al. (28), which were in the area 1–8 mm caudal to the obex, 0.5–2.1 mm below the spinal cord surface, and 0.8–2.0 mm lateral to the midline. As defined by the activity of the phrenic nerve, the discharge patterns of these recorded UCINs could be classified into two major types and were further subdivided based on similar criteria as for medullary neurons (Fig. 1 and Table 1; see Ref. 38).

Phasic UCINs. UCINs (144 of 213; 68%) fired only during inspiration, or, in a few cases (11 neurons), some irregular discharges between inspiratory bursts were observed. The latter activity was changeable during recording and disappeared in four of four neurons tested after vagotomy. All of the neurons in this group discharged throughout inspiration (Fig. 1, A1), except for three neurons that fired only in the late inspiratory (Fig. 1, A2) phase.

Phase-spanning UCINs. UCINs (69 of 213; 32%) discharged beyond the inspiratory phase. These included 63 phase-spanning expiratory-inspiratory (Fig. 1, B1) UCINs and 6 phase-spanning inspiratory-expiratory (Fig. 1, B2) UCINs. The phase-spanning expiratory-inspiratory UCINs showed a preinspiratory onset of discharge during the stage 2 expiratory phase followed by a further increase in activity with inspiration. The phase-spanning inspiratory-expiratory UCINs had a postinspiratory (stage 1 expiratory phase) discharge of lesser frequency after the higher-frequency inspiratory discharge. These pre- and postinspiratory activities could sometimes quantitatively vary over time (observed in 18 UCINs) but did not disappear and were not eliminated by vagotomy in six neurons recorded before and after vagotomy. Additionally, 20 other phase-spanning UCINs were recorded in five animals after bilateral vagotomy.

Table 1 also shows the distribution of UCINs between C1 and C2 segments. Among phasic UCINs, 92% were in the C1 region, and the others were in C2 (rostral portion, based on surface position). For the phase-spanning UCINs, all six phase-spanning inspiratory-expiratory UCINs were located in the C1 segment. On the other hand, 68% of phase-spanning expiratory-inspiratory UCINs were found in the C2 segment (rostral portion), and the remaining 32% extended to C1 (caudal portion). Among the 158 UCINs found in the C1 segment, 138 (87%) were phasic UCINs and phase-spanning inspiratory-expiratory UCINs, and 20 (13%) were phase-spanning expiratory-inspiratory UCINs. Conversely, 43 out of 55 UCINs (78%) found in the C2 segment had the phase-spanning expiratory-inspiratory discharge pattern, and the remaining 12 UCINs (22%) in this region were the phasic (throughout-inspiration) type. In accordance with a previous report (Lipski et al., 28), it was found that the density of UCINs in the C2 segment was much less than in C1, and relatively few neurons were found in the caudal half of C2. There were no significant differences in the distribution of UCINs in the left or right side of the upper cervical spinal cord.

Changes in the discharge pattern of 19 UCINs (12 with bilateral vagotomy) were examined while the central respiratory drive (monitored by observing the activity of the phrenic nerve) was suppressed by over-
It took 1–5 min, sometimes with successive increases in respiratory rate, to completely eliminate phrenic nerve activity. Five of six phase-spanning expiratory-inspiratory UCINs retained a low frequency of tonic activity during the period of phrenic suppression. Twelve phasic UCINs were suppressed at the same time as phrenic nerve activity during overventilation and recovered simultaneously with phrenic nerve activity after overventilation was stopped. One phasic UCIN was suppressed just after the phrenic nerve was suppressed and recovered earlier than the phrenic nerve.

Abdominal visceral stimulation. A total of 81 UCINs in 22 rats were tested for responses to multishock stimuli applied to the GSN. Except for six neurons in three animals, where technical issues prevented observation of phrenic nerve activity, inspiratory phrenic discharges were suppressed by GSN stimulation. Two major types of responses were observed in 69 UCINs. 1) The first was suppression of discharge (n = 57, 83% of responding cells). The spontaneous discharge of UCINs in the inspiratory phase was suppressed together with phrenic nerve output (Fig. 2, A1 and A2). Discharge during the expiratory phase of phase-spanning UCINs was also suppressed by GSN stimulation. The post-stimulus delay was 30.2 ± 0.7 (SE) ms in UCINs (Fig. 2, A3) and 29 ± 0.7 ms in the phrenic nerve (Fig. 2, A4). The duration of phrenic inhibition ranged from 10 ms to shutting off the inspiratory burst for the remainder of the cycle. 2) The second was a biphasic response (n = 12, 17% of responding cells). A short activation was followed by suppression of UCIN discharge, whereas the phrenic activity was simply inhibited (Fig. 2B). The latency to augmentation ranged between 15 and 30 ms (20.6 ± 1.5 ms). The augmented neuronal activity could consist of single or multiple discharges that began immediately before phrenic activity shut off, sometimes even extending to the period when phrenic activity was suppressed. The augmented responses of neurons also existed in the expiratory phase. In a few instances, phrenic discharges were suppressed by GSN input while the UCIN firing continued. This occurred intermittently in 6 of the 69 responding UCINs. Thirty-three of the preceding neurons were obtained after the animal was vagotomized. There was no apparent difference in response between vagotomized and intact preparations. During the time interval used for recording GSN stimulations, there were no consistent changes in blood pressure or heart rate.

Table 1. Number and distribution of UCINs

<table>
<thead>
<tr>
<th></th>
<th>Ph UCINs</th>
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<tr>
<td></td>
<td>T-I</td>
<td>L-I</td>
</tr>
<tr>
<td>C1</td>
<td>129</td>
<td>3</td>
</tr>
<tr>
<td>C2</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>141</td>
<td>3</td>
</tr>
<tr>
<td>% of sum</td>
<td>66.2</td>
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UCINs, upper cervical inspiratory neurons; Ph, phasic; PS, phase spanning; T-I, throughout inspiration; L-I, late inspiration; E-I, expiratory-inspiratory; I-E, inspiratory-expiratory.
Little response to CRD and SID was observed in UCINs and the phrenic nerve, especially during moderate CRD (<80 mmHg) and SID (<2 ml). Of the 44 UCINs tested for CRD and SID, 16 neurons showed a small decrease in firing rate under extremely high distension pressures (>100 mmHg for CRD and >2 ml saline for SID). There was no obvious change in discharge patterns of these UCINs. In most situations, phrenic nerve activity responded to CRD and SID in accordance with UCINs, except in three CRD-stimulated animals where rate and intensity of phrenic discharge were reduced while UCIN discharge was generally maintained.

CPSA Stimulation

In 22 rats, 102 UCINs were tested for responses to electrical CPSA stimulation. Brief trains of stimuli to CPSA suppressed inspiratory phrenic discharge with a latency of 14.1 ± 0.7 ms (n = 79; Fig. 3, A5, B5, and C5). The remaining 23 cells had no accompanying phrenic suppression because technical issues prevented observation of phrenic nerve activity. Three different responses in 82 (80%) of 102 UCINs (42% of responsive cells; Fig. 3A). The latency to suppression of UCIN firing varied between 14 and 40 ms (26.8 ± 2.3 ms). The augmented response also was found in the expiratory phase with similar latency.

Augmented response. Augmented discharge, not followed by suppression of cell activity, was observed in 19 UCINs (23% of responsive cells; Fig. 3B). The latency to this augmentation of UCIN discharge (11.5 ± 0.8 ms) was similar to that of the biphasic response.

Suppression. Both inspiratory and phase-spanning expiratory activities were suppressed in 29 UCINs (35% of responsive cells; Fig. 3C). The latency of this suppression was 22.0 ± 1.6 ms, which was similar to the latency to suppress UCIN activities in the biphasic response group.

Latencies to responses of UCINs and phrenic nerves are compared in Fig. 4. We found a significantly shorter latency to neuronal augmentation than the latency to phrenic suppression (paired t-test: P < 0.01). There was also a significantly longer latency to neuronal suppression than the latency to phrenic suppression (paired t-test: P < 0.01).

DISCUSSION

Discharge Pattern

Based on their discharge patterns in relation to efferent phrenic nerve activity, the UCINs were classified into two major types: phasic UCINs (throughout- and late-inspiratory UCINs) and phase-spanning UCINs (phase-spanning expiratory-inspiratory and inspiratory-expiratory UCINs). The majority of UCINs were throughout-inspiratory neurons, which were mainly located in the C1 region. Phase-spanning neu-
rons, on the other hand, were more likely to be found in C2. Nonaka and Miller (34) found in the cat that most incidences of UCIN discharge between inspirations could be categorized as changeable. It has also been reported that the discharge of UCINs during the noninspiratory portion of the respiratory cycle could be stopped by vagotomy (or by increasing end-tidal CO2; see Refs. 27 and 28). We saw a similar effect in some of our UCINs. As described in RESULTS, 29 UCINs revealed some changeable discharges during the expiratory phase; this was a lower incidence than noted for the cat (34). These discharges could be divided into two types. One type (found in 11 UCINs) showed some irregular and scattered discharge between the inspiratory ramps. This discharge could be stopped by vagotomy (tested in 4 cells), and all of these neurons were considered as phasic UCINs in this study. Sometimes the relatively tonic or slowly ramping activity recorded from phase-spanning neurons during expiration quantitatively varied over the course of the recording period (18 UCINs). However, this type of activity could not be

Fig. 3. Responses of UCINs and phrenic nerve to CPSA stimulations. A1, B1, C1, A2, B2, and C2 are the compacted (A1, B1, and C1) and expanded (A2, B2, and C2) traces of phrenic and UCIN responses to CPSA stimulations in inspiratory phase, respectively. Traces in A1, B1, C1, A2, B2, and C2 are labeled as in Fig. 1. Histograms in A3, B3, and C3 are from UCINs during inspiration. Histograms in A4, B4, and C4 are from UCINs during expiration, whereas histograms in A5, B5, and C5 are from phrenic nerve. A1–A5: biphasic (augmented-suppressed) response of UCIN and simple suppression of phrenic activity. Augmented response of UCIN occurred in both inspiratory and expiratory phases. A1–A5: biphasic response of UCIN in both inspiratory phase and expiratory phase and suppressed phrenic activity. C1–C5: suppression of both UCIN and phrenic activities. Expiratory activities of phase-spanning UCINs were also suppressed. Arrows mark CPSA stimulation. *Point at which augmented responses of UCIN occurred; °onset of UCIN and phrenic nerve suppression on raw traces and histograms.

Fig. 4. Comparison of the latencies for both augmentation (AUG) and suppression (SUP) of UCIN and phrenic activities in response to CPSA stimulations. UCIN AUG/PHRENIC SUP is based on 46 paired responses, whereas UCIN SUP/PHRENIC SUP is based on 62 paired responses. Responses that could not be paired were not used for this comparison. Latencies to UCIN AUG include results from biphasic and augmented groups; latencies to UCIN SUP include results from biphasic and suppressed groups. Error bars represent SE. **P < 0.01, paired t-test.
stopped by vagotomy and could be recorded postvagotomy. The reason for the variability in expiratory discharge was not clear. It might be a comprehensive effect of several factors, such as changes of anesthetic status and end-tidal CO₂. Nonaka and Miller (34) indicated that there was not a simple relationship between the existence of tonic firing and CO₂ levels. In our experiments, expiration-phased activity usually became tonic when apnea was produced by lowering CO₂ levels using overventilation. Thus the different discharge patterns and behavior of UCINs, as well as their different distributions, might imply a complexity of function and integration of this group of neurons.

GSN Stimulation

UCINs responded to GSN stimulation in the following two different ways: suppression and biphasic (augmentation-suppression). We could detect no difference between the responses to ipsilateral and contralateral GSN stimulation or phase-spanning vs. phasic UCINs. Phrenic activity was mainly suppressed by GSN stimulation. Albano and Garnier (3) reported that GSN stimulation induced a long inhibition of bulbar inspiratory neurons and activation-inhibition of phrenic activity in cat. They found that the latency to bulbar inhibition was consistently less than the latency to phrenic inhibition, and this latency difference matched the conduction time between bulbar and phrenic events. Nevertheless, GSN effects on phrenic nerves persisted in spinalized cats. These observations suggest that both bulbospinal and intraspinal circuits might be involved in the respiratory response to GSN stimulation. In the present study, the majority of UCINs were suppressed along with the phrenic nerve, but occasional dissociation of UCIN and phrenic suppression could be observed. A minority of UCINs revealed short activation before inhibition, whereas the phrenic nerve was simply inhibited. There was no evidence that the responses of UCINs or phrenic nerves to GSN stimulation were affected by each other. The reason for the different phrenic nerve responses (no augmented activity was found in the present study) to GSN stimulation in our study and in the study of Albano and Garnier (3) might be related to species or stimulus mode (17, 18, 37). Responses of UCIN and phrenic nerve to GSN stimulation did not require vagal innervation. The GSN in the cat enters the spinal cord primarily at the T₆-T₁₀ segments (26), and ~5–6% of fibers entering this region are visceral afferents (9). In rats, cats, and monkeys, the latencies of spinal neuron responses were consistent with Aδ- and C-fiber activation (1, 2, 4). The relatively long latency of both UCIN and phrenic responses in the present study was most likely the result of a spinal-bulbospinal pathway. Compared with results in the cat (35), we observed little response of phrenic activity, as well as UCINs, in rats to small intestinal distension and CRD.

CPSA Stimulation

The white rami of the high thoracic cord have been shown to contain both cardiac and pulmonary sympathetic afferent fibers (18, 22, 23), and the stellate ganglia/ansa subclavia have been used as the source of CPSA input in rats (42). The responses of UCINs and phrenic activities to CPSA stimulation in the present study differed from GSN stimulation. For UCINs, a purely augmenting response occurred in some cases in addition to biphasic responses and suppression, and the latencies to augmentation were shorter. Latencies to suppression of phrenic discharge were less than for GSN stimulation. These responses occurred in vagotomized and intact animals. Kostreva et al. (24) demonstrated in the vagotomized dog and monkey that stimulation of CPSA resulted in an immediate suppression of phrenic efferent nerve activity, diaphragmatic electromyogram (EMG), or external intercostal EMG. These results were consistent with our findings because the phrenic activity was suppressed with a short latency by CPSA stimulation. The latency to UCIN augmentation, which occurred in more than one-half of the tested cells, was on average shorter than that to phrenic suppression. By calculating conduction velocities, Kostreva and co-workers (24) concluded that the afferent fibers conveying the respiratory inhibition were the Aδ-fiber type. We suggest that the relatively short-latency CPSA-induced suppression of phrenic discharge and augmentation of UCIN firing are consistent with oligosynaptic pathways, which could involve supraspinal mechanisms, or may be entirely spinal. Our finding that UCIN stimulation occurs ~2 ms before phrenic suppression suggests an additional synaptic event for the latter. Whether UCINs could be involved in phrenic suppression remains to be determined. The persistent inhibitory effect of cardiopulmonary sympathetic stimulation on respiratory output (11, 24) is consistent with a bulbospinal effect. The pathway of the relatively longer latency suppression of UCINs is most likely to be spino-bulbospinal in origin.

Perspectives

Most investigators currently accept the hypothesis that breathing rhythm and motor outflows result in part from network integration (36, 39). Although such output appears to be unaffected by blockade of UCINs (31), Aoki et al. (8) and Coglianese et al. (10) have reported rhythmic respiratory activity in spinalized animals. However, the potential role of UCINs in spinal cord respiratory rhythm and their relationship to brainstem circuitry, which generates respiratory rhythm, remains speculative. Whatever the functions of UCINs are, it does seem clear that they are not simply relaying respiratory drive from the medulla. This investigation and previous studies using vagal stimulation (12, 15, 28) show that visceral input, apparently dominated by heart and lung for sympathetic afferents, is integrated by this pool of cells. Furthermore, the expiration phase activity exhibited by many of these neurons does not require phasic output by brainstem respiratory controllers. If these inputs do not change respiratory motor outflow, another possibility may be that they are capable of influencing perception of respiratory effort through effects on interneurons.
This work was supported by National Institute of Neurological and Communicative Disorders and Stroke Grant NS-35471. Address for reprint requests and other correspondence: J. P. Farber, Dept. of Physiology, Univ. of Oklahoma Health Sciences Center, P.O. Box 26901, Oklahoma City, OK 73190 (E-mail: Jay-Farber@ouhsc.edu). Received 8 October 1999; accepted in final form 9 December 1999.

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