Medullary lateral tegmental field: control of respiratory rate and vagal lung inflation afferent influences on sympathetic nerve discharge

Shaun W. Phillips, Gerard L. Gebber, and Susan M. Barman

Department of Pharmacology and Toxicology, Michigan State University, East Lansing, Michigan

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Medullary lateral tegmental field: control of respiratory rate and vagal lung inflation afferent influences on sympathetic nerve discharge. Am J Physiol Regul Integr Comp Physiol 288: R1396–R1410, 2005. First published December 16, 2004; doi:10.1152/ajpregu.00632.2004.—We used spectral analysis and event-triggered averaging to determine the effects of chemical inactivation of the medullary lateral tegmental field (LTF) on 1) the relationship of intratracheal pressure (ITP, an index of vagal lung inflation afferent activity) to sympathetic nerve discharge (SND) and phrenic nerve activity (PNA) and 2) central respiratory rate in paralyzed, artificially ventilated dial-urethane-anesthetized cats. ITP–SND coherence value at the frequency of artificial ventilation was significantly ($P < 0.05; n = 18$) reduced from 0.73 ± 0.04 (mean ± SE) to 0.24 ± 0.04 after bilateral microinjection of muscimol into the LTF. Central respiratory rate was unexpectedly increased in 12 of these experiments (0.28 ± 0.03 vs. 0.95 ± 0.25 Hz). The ITP–PNA coherence value was variably affected by chemical inactivation of the LTF. It was unchanged when central respiratory rate was also not altered, decreased when respiratory rate was increased above the rate of artificial ventilation, and increased when respiratory rate was raised from a value below the rate of artificial ventilation to the same frequency as the ventilator. Chemical inactivation of the LTF increased central respiratory rate in four of six vagotomized cats but did not significantly affect the PNA–SND coherence value. These data demonstrate that the LTF 1) plays a critical role in mediating the effects of vagal lung inflation afferents on SND but not PNA, 2) helps maintain central respiratory rate in the physiological range, but 3) is not involved in the coupling of central respiratory and sympathetic circuits.

Cardiorespiratory synchronization; dorsal respiratory group; Hering-Breuer reflex; phrenic nerve activity; respiratory-related rhythm; ventral respiratory group

Address for reprint requests and other correspondence: S. M. Barman, Dept. of Pharmacology and Toxicology, Michigan State Univ., East Lansing, MI 48824 (E-mail: barman@msu.edu).

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It has long been recognized that neural mechanisms controlling the respiratory and cardiovascular systems are tightly linked (25, 34, 38, 49, 55). This interaction facilitates the complementary functions of the two systems: respiration maintains appropriate levels of arterial blood gases, while the cardiovascular system transports these gases to and from tissues. One indication of this cardiorespiratory coordination was noted by Adrian et al. (1) in 1932 when they made the first recordings of sympathetic nerve discharge (SND). They found that the amplitude of the cardiac-related bursts of SND waxed and waned on the time scale of the respiratory cycle. There are at least two sources of respiratory modulation of SND: input from vagal lung inflation afferents and an influence of the respiratory rhythm generator on central sympathetic neurons (4–6, 21, 25, 33–35, 59). Although some investigators (38, 49) have proposed that there is a common network controlling both respiratory and cardiovascular functions, others have suggested that cardiorespiratory synchronization reflects both the direct coupling of two distinct central networks and shared input from peripheral afferents (4, 6, 21, 59).

Activation of slowly adapting pulmonary stretch receptors (myelinated vagal afferents) during lung inflation shortens the inspiratory phase of phrenic nerve activity (PNA); this inspiratory cut-off mechanism is called the Hering-Breuer reflex (20, 25, 43, 58). In addition to controlling respiration, activation of pulmonary stretch receptors has been shown to inhibit or excite SND, depending on the volume of lung inflation (4, 21, 59). Anatomic and electrophysiological studies (25, 27, 58) show that pulmonary vagal afferents terminate in the nucleus of the tractus solitarius (NTS). However, the neuronal connections beyond this point that are responsible for changes in SND have not been identified. A recent study from this laboratory (46) showed that the sympathoexcitatory and sympathoinhibitory effects of electrical stimulation of cervical vagal afferents were prevented by microinjection of the GABA agonist muscimol into the medullary lateral tegmental field (LTF) in cats. The LTF includes portions of nucleus reticularis parvocellularis and nucleus reticularis ventralis. Several laboratories have shown that this region of the cat brain stem contains neurons with activity correlated to SND (7–9, 12, 18, 30, 56), and chemical activation of the LTF can alter blood pressure (17, 26, 32, 41, 54). Importantly, there is a growing body of evidence supporting the view that, at least in the cat, the LTF plays a crucial role not only in setting the basal level of SND (9, 10, 45) but also in mediating responses to activation of baroreceptor and chemoreceptor afferents (45, 46). Specifically, blockade of non-N-methyl-d-aspartate (non-NMDA) receptors in the LTF reduced the level of basal SND and the sympathoexcitatory effect of arterial chemoreceptor activation (10, 45, 46), whereas blockade of NMDA receptors attenuated baroreflex-mediated sympathoinhibition (45). These observations prompted us in the current study to test the hypothesis that the LTF is involved in mediating the effects of vagal lung inflation afferents on SND and PNA.

To account for the respiratory modulation of SND noted in vagotomized animals, Guyenet et al. (33) suggested that the rostral tip of the ventrolateral medulla represents the critical link between the central respiratory rhythm generator and the sympathetic network. They reported cases in which microinjection of kynurenate into this region eliminated respiratory modulation of lumbar SND without markedly affecting PNA. Moreover, sympathetic premotor neurons in the rostral ventrolateral medulla (RVLM) have respiratory-related activity in

vagomized rats (35). However, the LTF also contains neurons with activity correlated to both SND and PNA in vagotomized cats (7). Because LTF neurons are likely antecedent to RVLM neurons in sympathoexcitatory pathways at least in the cat (8, 30), it is possible that central respiratory and sympathetic circuits interact at a level antecedent to RVLM-spinal neurons. Thus a second objective of the current study was to test the hypothesis that LTF neurons are involved in central respiratory modulation of SND in vagotomized cats.

During the course of these experiments, we unexpectedly found that central respiratory rate was often dramatically increased after microinjection of muscimol into the LTF in vagus-intact and vagotomized cats. Thus a third objective of the study was to characterize the effects of chemical inactivation of the LTF on the following parameters of PNA: the interval between the onsets of consecutive inspiratory bursts (I-I interval), durations of inspiration and expiration (I- and E-duration), I:E ratio, inspiratory burst amplitude, and slope of the inspiratory phase of PNA.

METHODS

General Procedures

The protocols used in these studies on 38 adult cats (1.8–4.0 kg) were approved by the All-University Committee on Animal Use and Care of Michigan State University. Cats were anesthetized with an intraperitoneal injection of a mixture of sodium diatrizoburate (60 mg/kg), urethane (240 mg/kg), and monoethytylurea (240 mg/kg). The femoral artery and veins were cannulated to measure arterial pressure and to administer drugs, respectively. Most (n = 34) cats were paralyzed (gallamine triethiodide, 4 mg/kg iv, initial dose), pneumothoracotomized, and artificially ventilated with room air; bilateral cervical vagotomy was performed in eight of these cats. Four cats with intact vagus nerves were not paralyzed and were allowed to breathe spontaneously throughout the experiment; intratracheal pressure (ITP) was used as an index of central respiratory rate in these cats. ITP was monitored by using a Grass Instruments volumetric pressure transducer (model PT5). Upward deflections in the records of ITP denote the inflation phase of the artificial ventilation cycle in paralyzed cats and the inspiratory phase of the central respiratory cycle in spontaneously breathing cats.

The parameters of artificial ventilation (36 ± 2 ml and 24 ± 2 cycles/min) were within the physiological range (24) so that end-tidal CO₂ was held near 4.6% (Traverse Medical Monitors Capnometer, model 2200). Rectal temperature was kept near 38°C with a heat lamp. Before neuromuscular blockade, the adequacy of anesthesia was indicated by the absence of a palpebral reflex. When cats were paralyzed, an adequate level of anesthesia was indicated by the inability of noxious stimuli (pinch, heat, surgery) to increase blood pressure and desynchronize the frontal-parietal electroencephalogram (see Ref. 7 for recording methods). Cats were placed in a stereotaxic apparatus and spinal investigation unit.

Mean blood pressure was maintained at the same level throughout the analysis period (117 ± 5 vs. 110 ± 5 mmHg before and after microinjection of muscimol, respectively) by adjusting the rate of an intravenous infusion of a mixture of dextran and saline. This was done to maintain a reasonably stable level of baroreceptor afferent nerve activity.

Sympathetic and Phrenic Nerve Recordings

The inferior cardiac and vertebral branches of the left stellate ganglia were exposed retroperitoneally by removing the head of the 1st rib (31, 46). These nerves project to the heart and vasculature of the forelimb, respectively. The right phrenic nerve was isolated in the neck (6, 59). The nerves were covered with silicone release agent (Dow Corning 7 compound). Potentials were recorded monophonically from the central ends of the cut nerves on platinum bipolar electrodes. The capacity-coupled preamplifier bandpass was set at 30 Hz to 3 kHz for SND and 10 Hz to 1 kHz for PNA. The signals were then passed through a 50/60 Hz noise eliminator (Hum Quest Scientific) and a moving average (CWE, model MA-821RSP) with a 50-ms (SND) or 100-ms (PNA) time constant. The moving average performs a full-wave rectification and uses a third-order Paynter filter (36), thereby reconstituting the low-frequency components in SND and PNA. An advantage of this method is that it provides a high degree of smoothing and a better dynamic response than simpler first-order filters (integrators).

Microinjections

Muscimol (Sigma, St. Louis, MO) was diluted in 0.9% saline, and the solution (0.5 mM) was adjusted to a pH of 6–8 (litmus paper test) to assure solubility. This GABA agonist depresses neuronal activity by acting on cell bodies (42). Muscimol was microinjected through a glass micropipette (~40-μm tip diameter) that was glued (cyanoacrylate) to the needle of a 5-μl Hamilton syringe. The syringe and micropipette were mounted on a microinjection unit (Kopf Instruments, model 5000). A 50-nl (n = 34) or 100-nl (n = 7) injection of muscimol was made slowly (10 s) by turning the calibrated micrometer on the microinjection unit.

The dorsal surface of the brain stem was exposed by removing portions of the occipital bone and cerebellum. The midline, obex, and dorsal medullary surface were used as landmarks for placement of the micropipette. Microinjections were made at two sites in the LTF on each side of the medulla (total of 100–200 nl/side) over a period of ~3 min. The micropipette was positioned in tracks located ~2 and 3 mm rostral to the obex and 2.5–2.8 mm lateral to the midline; microinjections were made bilaterally at a depth of 3.5–3.8 mm from the dorsal surface. Multiple injections were made so that the drug would spread through the portion of the LTF that contains neurons with activity correlated to SND, ranging from 1–4 mm rostral to the obex, 2–3 mm lateral to the midline, and from 2.25 to 4.75 mm below the dorsal surface. The most rostral injection site in the LTF is ~2 mm caudal, 1 mm medial, and ~1 mm dorsal to the region of the RVLM that contains neurons with activity correlated to SND (8, 30). The most caudal injection site in the LTF is 1–1.5 mm medial and ~1 mm dorsal to the most rostral portion of the caudal ventrolateral medulla (CVLM) that contains neurons with activity correlated to SND (11).

In five cats we microinjected muscimol bilaterally into the vicinity of the portion of the ventral respiratory group (VRG) adjacent to the LTF. Specifically, the micropipette was positioned 3.8 mm lateral to the midline at a depth of 3.8 mm below the dorsal surface in tracks located ~2 and 3 mm rostral to the obex (same stereotaxic planes as LTF injection sites). Others (23, 29, 52) have shown that this portion of the VRG contains neurons with inspiratory-related activity, including some that project to the spinal cord.

In six cats we microinjected muscimol bilaterally into the vicinity of the dorsal respiratory group (DRG) within the ventrolateral NTS. This included three cats in which we had previously (30–60 min earlier) injected muscimol into the vicinity of the VRG. The DRG contains inspiratory premotor neurons as well as neurons that are in close association with terminals of glossopharyngeal and vagal lung inflation afferents (14, 20, 22, 27, 43, 58). For DRG injections, the micropipette was positioned 2.4 mm lateral to the midline, 1.7 mm below the dorsal surface in tracks located 0.5 and 1.5 mm rostral to the obex.

In two cats, saline was injected into LTF (same sites as muscimol) and in one cat muscimol was microinjected into the inferior olive (ventral to the LTF) bilaterally. None of these injections affected SND or PNA or the relationship of these signals to ITP.
The brain stem was removed and fixed in 10% buffered formalin. Frontal sections were cut and stained with cresyl violet to locate levels of microinjection with reference to the stereotaxic planes of Berman (15). Figure 1, right (top to bottom) contains cross-sections of the medulla showing a track through the LTF at ~3 mm rostral to the obex, through the VRG at ~2 mm rostral to the obex, and in the DRG at ~1.5 and 0.5 mm rostral to the obex. The pipette is initially placed ~0.5 mm deeper than the intended injection site and then retracted upward. This is done to avoid compression of the brain stem with the pipette. As a result, the bottom of the track is actually deeper than the injection site. The dots in Fig. 1, left, show the target sites of the injections into the LTF and the vicinity of the VRG and DRG.

The protocol used in these studies was as follows. Control data blocks (6-min duration) were collected; the micropipette was then positioned into the LTF, and a complete set of injections of muscimol was made on the left and right sides of the medulla. In most experiments, data were collected continuously during the injection and for 36 min after the injection. Data were again collected in 15- to 30-min intervals for up to 2 h after injection of muscimol. Although changes in SND and PNA were noted during or soon after microinjection of muscimol into the LTF (see Figs. 2, 6, and 8), the data block beginning 10 min after microinjection was used to quantify the effects of chemical inactivation of the LTF on the interrelationships of ITP, SND, and PNA. This time point was chosen because our past work showed that the maximum changes in basal SND had occurred at this time, and SND had reached a new steady-state level (9, 10, 45).

Data Processing

Data (1-ms resolution) were acquired by using a Digidata1322A digitizer (Axon Instruments; Union City, CA) and were stored on a DAT Data Recorder (model RD-145T; TEAC America, Montebello, CA). Fast Fourier transform was performed on 6-min data blocks (20-ms sampling interval; 35 20-s windows with 50% overlap) to construct autospectra of SND, PNA, and ITP. Coherence functions relating pairs of these signals were also computed. The moving averaged records of SND and PNA were used for spectral analysis. The autospectrum of a signal shows how much power (voltage squared) is present at each frequency. Autospectra before and during microinjection of muscimol are plotted on the same power scale. The coherence function (normalized cross-spectrum) is a measure of the strength of linear correlation of two signals at each frequency. The squared coherence value (referred to as coherence value) is one in the case of an ideal linear relationship and zero if two signals are unrelated. A coherence value ≥0.1 was considered to reflect a statistically significant relationship when 35 windows were averaged (13). Spectral analysis was done over a frequency band of 0 to 20 Hz with a resolution of 0.05 Hz/bin. The figures in this report show only the 0- to 1-Hz frequency band that contained the ITP-related power in SND and PNA.

We used Datapac software (Run Technologies; Mission Viejo, CA) to measure the I-I interval, I- and E-durations, I:E ratio, trough-to-peak inspiratory burst amplitude, and slope of the inspiratory phase of PNA. These measurements were made from the average of 10–20 cycles of moving averaged PNA triggered by a reference event coinciding with the onset of the inspiratory phase of PNA. In some cases, cycle-by-cycle measurements of the I-I interval (or ITP-ITP interval) were depicted in the form of a time series using software written in our laboratory (31).

Statistical Analysis

Data are expressed as means ± SE. P ≤ 0.05 indicated statistical significance. Except where noted, paired t-tests were used to compare the following parameters before and after microinjection of muscimol: the ITP-SND and ITP-PNA coherence values at the frequency of artificial ventilation, PNA-SND coherence value at the central respiratory rate (peak frequency in the PNA autospectrum) in vagotomized cats, ITP-related power in SND and PNA as a percentage of the total power in the 0- to 1-Hz band, PNA-related power in SND, central respiratory rate, and the above listed parameters of PNA. Coherence values were subjected to z-transformation before statistical analysis. ITP-related power refers to power in the SND (PNA) autospectrum in a frequency band (as defined from the autospectrum of ITP) whose peak was at the rate of artificial ventilation. A macro written in Microsoft Excel was used to measure ITP-related power. A line is fitted to connect the left and right limits of this frequency band of SND; ITP-related power is calculated as the area above this line. The
0- to 1-Hz total power is calculated by arithmetically summing the values for the bins in this frequency range. ITP-related power divided by 0- to 1-Hz total power is multiplied by 100 to determine the percentage of the total power that was ITP related. We expressed ITP-related power as a percentage of the total power because chemical inactivation of the LTF reduces the absolute power in SND (9, 46). Also, we measured PNA-related power in SND in a similar manner (range of power determined by the peak in the autospectrum of PNA) in vagotomized cats.

**RESULTS**

Effects of Chemical Inactivation of LTF on the Relationship of ITP to SND and PNA and on Central Respiratory Rate in Vagus-Intact Cats

We studied the effects of chemical inactivation of the LTF on the relationship of ITP to SND and PNA in 18 paralyzed, artificially ventilated cats with intact vagus nerves. The results from these experiments were divided into two groups based on the relationship between ITP and PNA in control. In the 1st group (\(n = 11\)), central respiratory rate (as reflected by PNA) was locked in a 1:1 relationship to the artificial ventilation cycle (as reflected by ITP; \(0.29 \pm 0.01\) Hz), indicating strong entrainment of PNA to lung inflation. In the 2nd group (\(n = 7\)), the frequency of the central respiratory rhythm was lower than that of artificial ventilation (\(0.26 \pm 0.05\) vs. \(0.57 \pm 0.07\) Hz).

*Results from 1st group of cats.* Following chemical inactivation of the LTF, the central respiratory rhythm either remained locked in a 1:1 relationship to ITP (\(n = 5\)) or increased to a rate higher than that of artificial ventilation (\(n = 6\)). In the experiment illustrated in Fig. 2, A and B, 1:1 locking was maintained. Figure 2, A and B, shows recordings of arterial pressure, PNA, ITP, and inferior cardiac SND before and 5 min after bilateral microinjection of muscimol into the LTF, respectively. As is typically the case in paralyzed, artificially ventilated cats with intact vagus nerves (21, 59), ITP and PNA were out of phase, and cardiac-related bursts of SND were largest near maximal deflation of the lungs (Fig. 2A). In contrast, 5 min after chemical inactivation of the LTF, PNA but not SND remained synchronized to ITP (Fig. 2B).

We used spectral analysis to quantify the effects of chemical inactivation of the LTF on the relationship of ITP to SND and PNA. In the example shown in Fig. 3A, the control (before microinjection of muscimol) autospectra of SND (Fig. 3A1) and PNA (Fig. 3A3) contained a prominent peak at the frequency of artificial ventilation (marked with an “X”) which...
was 0.25 Hz in this experiment (ITP autospectrum, not shown). Most of the power in the 0- to 1-Hz band of SND (56%) and PNA (62%) was within this peak. Both SND and PNA were strongly correlated to ITP as evidenced by high ITP-SND (Fig. 3A2) and ITP-PNA (Fig. 3A4) coherence values (0.81 and 0.98, respectively) at the frequency of artificial ventilation. ITP-related power in SND was virtually eliminated 10 min after microinjection of muscimol (Fig. 3A1), and the ITP-SND coherence value was reduced to 0.25 at this time (Fig. 3A2). However, under the conditions of maintained locking of the central respiratory cycle to the artificial ventilation cycle, ITP-related power in PNA (Fig. 3A3) and the ITP-PNA coherence value were essentially unchanged after chemical inactivation of the LTF (Fig. 3A4).
In the experiments illustrated in Fig. 2, C and D, and Fig. 3B, 1:1 locking of the central respiratory cycle to the artificial ventilation cycle was disrupted by chemical inactivation of the LTF. In the case shown in Fig. 2, C and D, central respiratory rate increased to four times that of artificial ventilation 6 min after bilateral microinjection of muscimol into the LTF. At this time, modulation of SND burst amplitude on the time scale of ITP was essentially eliminated. Figure 3B shows the results obtained with spectral analysis in another cat. Central respiratory rate increased from 0.35 to 0.55 Hz as reflected by the shift in the primary peak in the autospectrum of PNA (Fig. 3B3). ITP-related power in PNA was nearly abolished. Consequently, the ITP-PNA coherence value at the frequency of artificial ventilation was markedly reduced from 0.91 to 0.23 (Fig. 3B4) 10 min after microinjection of muscimol. As usual, the coherence between ITP and SND was markedly reduced (Fig. 3B2), and the peak in the SND autospectrum was shifted to the frequency of the central respiratory cycle (Fig. 3B1).

Figure 4A summarizes the results from the 1st group of 11 cats in which the central respiratory cycle was locked in a 1:1 relationship to the artificial ventilation cycle in control. Both inferior cardiac and vertebral SND were recorded in three cats. The data from both sympathetic nerves were similar and thus were pooled. Independent of whether central respiratory rate was increased (Fig. 4A5), the ITP-SND coherence value at the frequency of artificial ventilation (0.73 ± 0.05 vs. 0.19 ± 0.04; P < 0.0001) and ITP-related power in SND (39 ± 7 vs. 9 ± 3% of total power; P = 0.0004) were routinely reduced after bilateral microinjection of muscimol into the LTF (Fig. 4, A1 and A2). In contrast, the ITP-PNA coherence value and ITP-related power in PNA were variably affected depending on whether central respiratory rate was increased after chemical inactivation of the LTF (Fig. 4, A3 and A4). Note that the ITP-PNA coherence value and ITP-related power in PNA usually decreased considerably in those cats in which central respiratory rate increased (asterisks in Fig. 4, A3 and A4) but was little changed when PNA remained locked in a 1:1 relationship to ITP. Central respiratory rate was significantly (P = 0.0313) increased from 0.29 ± 0.01 to 0.69 ± 0.26 Hz 10 min after microinjection of muscimol into the LTF of these cats. On a group basis, the ITP-PNA coherence value (0.94 ± 0.03 vs. 0.73 ± 0.07; P = 0.0091) and ITP-related power in PNA (68 ± 4 vs. 46 ± 10% of total power; P = 0.0382) were modestly (albeit significantly) decreased.

Results from 2nd group of cats. Figure 2, E and F, shows data from one of the seven cats in the 2nd group in which the central respiratory rate was the same as the rate of artificial ventilation during control (Cont). Graphs 1-5: effects of chemical inactivation of the LTF on the ITP-SND coherence values, ITP-related power expressed as a percent of the total power in the 0- to 1.0-Hz frequency band of SND, ITP-PNA coherence values, ITP-related power as a percent of total power in PNA, and central respiratory rate, respectively. Results from inferior cardiac and vertebral SND are pooled in A and B. Asterisks in A3 and A4 mark data from cats in which central respiratory rate was increased by microinjection of muscimol. In A5, n = 5 denotes that central respiratory rate was 0.25 Hz both before and after microinjection of muscimol in 5 cats.
artificial ventilation but increased to the rate of artificial ventilation after microinjection of muscimol. Before chemical inactivation of the LTF, there was minimal power in the autospectrum of PNA at the frequency of ITP (indicated by the "X") and the ITP-PNA coherence value at this frequency was not significantly different from zero (Fig. 3, C3 and C4). The significant ITP-PNA coherence value (0.28) at the frequency of the central respiratory cycle (0.30 Hz) presumably reflects the fact that this frequency was a subharmonic of the rate of artificial ventilation (0.60 Hz). The autospectrum of SND contained two peaks, one at the frequency of the central respiratory cycle and an even larger one at the frequency of artificial ventilation (Fig. 3C1). The ITP-SND coherence values were statistically significant at both frequencies (Fig. 3C2). After bilateral microinjection of muscimol into the LTF, the peak in the autospectrum of SND shifted to a higher frequency that now coincided with the rate of artificial ventilation. The shift to the higher frequency was accompanied by an increase in the ITP-PNA coherence value at the frequency of artificial ventilation from 0.08 to 0.96 and an increase in ITP-related power in PNA from 1 to 64% of the total power. In contrast, the corresponding values for SND were markedly reduced. Specifically, ITP-SND coherence was reduced from 0.84 to 0.02 and ITP-related power in SND was reduced from 31 to 1% of the total power.

Figure 4B summarizes the data from the seven cats in the 2nd group, including four in which both inferior cardiac and vertebral SND were recorded (data pooled). In all cases, chemical inactivation of the LTF was accompanied by a reduction in the ITP-SND coherence value at the frequency of artificial ventilation (0.74 ± 0.06 vs. 0.31 ± 0.07; P < 0.0001; Fig. 4B1) and in the ITP-related power in SND (25 ± 3 vs. 8 ± 2% of total power; P < 0.0001; Fig. 4B2). However, in marked contrast to the results noted for the 1st group of cats, Fig. 4, B3 and B4, shows that the relationship between ITP and PNA was markedly strengthened after chemical inactivation of the LTF in five of the seven cats in the 2nd group. On a group basis, the ITP-PNA coherence value (0.36 ± 0.11 vs. 0.77 ± 0.06; P = 0.0206) and ITP-related power in PNA (4 ± 2 vs. 24 ± 4% of total power; P < 0.0097) were significantly increased after microinjection of muscimol into the LTF. In one of the two experiments in which the coherence between ITP and PNA was not increased, muscimol microinjection into the LTF did not increase central respiratory rate. In the other case, the peak in the autospectrum of PNA was shifted from 0.30 to 1.30 Hz (i.e., to a value higher than the rate of artificial ventilation); thus the respiratory oscillator was apparently outside of the range of entrainment by vagal lung inflation afferents both before and after chemical inactivation of the LTF. A comparison of central respiratory rate before and after chemical inactivation of the LTF in the 2nd group of cats is shown in Fig. 4B5. On a group basis, respiratory rate was significantly (P = 0.0313) increased from 0.26 ± 0.05 to 0.81 ± 0.26 Hz 10 min after microinjection of muscimol into the LTF.

Effects of microinjection of muscimol into LTF on the correlation of SND to the arterial pulse and total power in the 0- to 5-Hz band of SND. In agreement with our past work (46), the coherence value relating inferior cardiac SND to the arterial pulse at the frequency of the heart beat was significantly (P < 0.0001; n = 21) reduced from 0.86 ± 0.03 to 0.34 ± 0.07 by chemical inactivation of the LTF. Also in agreement with past studies (9, 46), the total power in the 0- to 5-Hz band of inferior cardiac SND was significantly (P = 0.0025) reduced to 75 ± 7% of control after microinjection of muscimol. Similar results were noted for vertebral SND. In three cats, during control, SND was not correlated to the arterial pulse (AP-SND coherence value was <0.1); these cats are referred to as “functionally baroreceptor denervated.” Nonetheless, the ITP-SND coherence value was high in these cats before microinjection of muscimol into the LTF.

Recovery from effects of microinjection of muscimol into the LTF. In 9 of the 14 cats in which we continued to monitor recordings for up to 2 h after microinjection of muscimol into the LTF, there was partial recovery in the ITP-SND coherence value at the frequency of artificial ventilation. Specifically, the coherence value initially decreased from 0.63 ± 0.06 to 0.12 ± 0.04 and recovered to 0.47 ± 0.07. There was also partial recovery in central respiratory rate in seven cats. Specifically, respiratory rate was increased from 0.24 ± 0.04 to 0.68 ± 0.15 Hz by chemical inactivation of the LTF and was then reduced to 0.32 ± 0.07 Hz over the next 30–100 min in these cats.

Effects of chemical inactivation of the LTF on the pattern of PNA in paralyzed, artificially ventilated cats. We determined the effects of chemical inactivation of the LTF on the following parameters measured from event-triggered averages of PNA (reference event coinciding with the onset of inspiration): the I-I interval, I- and E-durations, I:E ratio, inspiratory burst amplitude, and slope of the inspiratory phase of PNA. Measurements were made from recordings taken before microinjection of muscimol as well as at a time postinjection when central respiratory rate had reached a maximum. Figure 5A shows event-triggered averages (each based on 15 respiratory cycles) of PNA during control (unfilled line plot) and after chemical inactivation of the LTF (gray filled area) in one of the experiments in which central respiratory rate was increased. The horizontal dashed lines show measurements of I- and E-durations and I-I interval for the control event-triggered average of PNA; the vertical line shows the inspiratory burst amplitude.

Figure 5B summarizes the data from the 12 vagus-intact cats in which central respiratory rate increased after microinjection of muscimol into the LTF. On a group basis, there were significant (P < 0.0001) reductions in the I-I interval (from 4.08 ± 0.75 to 1.47 ± 0.31 s), I-duration (from 1.58 ± 0.27 to 0.64 ± 0.14 s), and E-duration (from 2.49 ± 0.51 s to 0.82 ± 0.17 s). There was not a significant change in the I:E ratio (0.71 ± 0.07 vs. 0.76 ± 0.08). Chemical inactivation of the LTF was accompanied by a significant (P = 0.0021) decrease in the inspiratory burst amplitude from 2.44 ± 0.18 to 1.64 ± 0.25 V.

We also measured the slope of the rising phase of inspiratory activity. In 9 of 12 cats, there was an initial rapid rate of rise and then a “ramplike” increase in inspiratory activity during control. In five cats, the two-phase pattern of incrementing inspiratory activity persisted after bilateral microinjection of muscimol into the LTF. In the other four cases, the initial rapid rate of rise of PNA was no longer evident, and only the ramplike increase in inspiratory activity persisted after chemical inactivation of the LTF. In three cats, only the ramplike increment in PNA appeared during both control and after
one of the objectives of this study was to determine if the LTF was involved in the coupling of central sympathetic and respiratory circuits that is commonly seen in vagotomized cats (5, 6, 34, 35). These experiments also allowed us to determine if central respiratory rate is increased by chemical inactivation of the LTF in vagotomized cats. Recordings were made in six vagotomized cats in which the ITP-SND and ITP-PNA coherence values at the frequency of artificial ventilation were not significantly different from zero during control. Figure 6, A and B, shows arterial pressure, PNA, ITP, and SND before and 7 min after bilateral microinjection of muscimol into the LTF, respectively, of one of these cats. The shifting phase relations between ITP and PNA in Fig. 6A indicate that the two signals were not correlated, as expected after vagotomy. Note also that the amplitude of bursts of SND waxed and waned on the time scale of PNA rather than ITP, with peak SND occurring in the mid- to late-inspiratory phase of PNA (Fig. 6A). In this experiment, there was a marked increase in central respiratory rate after chemical inactivation of the LTF (Fig. 6A); nonetheless, peak SND was still reached in the inspiratory phase of each cycle of PNA. Figure 6C shows the time course of change in the I-I interval in this cat. The arrow marks the completion of the last of four injections of muscimol into the LTF. Note that the I-I interval began to decrease before the last injection was made and continued to further decrease over the next 10–15 min.

Figure 7A shows the results of spectral analysis for this experiment. Despite the marked increase in central respiratory rate from 0.55 to 1.35 Hz (Fig. 7A2). PNA-related power in SND was similar (41 vs. 46% of the total power in the 0- to 1.5-Hz band; Fig. 7A1) before and after chemical inactivation of the LTF, respectively. Likewise, the PNA-SND coherence value at the frequency of the peak in the PNA autospectrum was similar before and after (0.73 and 0.87, respectively) microinjection of muscimol (Fig. 7A3).

Figure 7B summarizes the data from the six vagotomized cats, including three in which we recorded both inferior cardiac and vertebral SND (data pooled). On a group basis, neither the PNA-SND coherence value at the frequency of the central respiratory cycle (0.86 ± 0.04 vs. 0.79 ± 0.04; Fig. 7B1) nor PNA-related power in SND (44 ± 4 vs. 37 ± 3% of total power; Fig. 7B2) were significantly affected by chemical inactivation of the LTF. Although central respiratory rate was increased in four of six cats, on a group basis, it was not significantly affected (0.29 ± 0.07 vs. 0.45 ± 0.18 Hz; Fig. 7B3). The asterisks in Fig. 5B show the changes in the I-I interval, I- and E-durations, and inspiratory burst amplitude for the four vagotomized cats in which central respiratory rate was increased after chemical inactivation of the LTF.
Effects of Chemical Inactivation of the LTF in Spontaneously Breathing Cats with Intact Vagus Nerves

Muscimol was microinjected bilaterally into the LTF of four spontaneously breathing, vagus-intact cats in which ITP was used as an index of the central respiratory cycle. Central respiratory rate was significantly \( (P = 0.0382) \) increased from \( 0.45 \pm 0.05 \) to \( 0.88 \pm 0.06 \) Hz after chemical inactivation of the LTF in these cats. Figure 8, A and B, shows recordings of arterial pressure and ITP before and 7 min after bilateral microinjection of muscimol into the LTF, respectively, in one of these cats. Figure 8C shows the time course of change in ITP-ITP interval during and after microinjection of muscimol into the LTF of this cat. Note that central respiratory rate began to increase before completion of the full set of injections into the LTF (arrow marks the end of the injections) and continued to further increase over the next 8 min. End-tidal CO\(_2\) was unchanged at a time when respiratory rate was increased in three of the four spontaneously breathing cats. In the fourth cat, end-tidal CO\(_2\) increased from 5.4 to 6.2% after microinjection of muscimol into the LTF.

Effects of Microinjection of Muscimol into the Vicinity of the VRG Adjacent to the LTF or the DRG on Respiratory Rate and the Relationship of ITP to SND and PNA

We determined if the increases in central respiratory rate produced by microinjection of muscimol into the LTF could be explained by spread of the injectate to adjacent medullary regions with known respiratory functions, i.e., the VRG and DRG (16, 19, 28, 29, 47, 48). We also determined the effects of chemical inactivation of these regions on the relationship of ITP to SND and PNA in these cats.

VRG injections of muscimol. We microinjected muscimol into the vicinity of a restricted portion of the VRG (adjacent to the LTF injection sites) of five paralyzed, artificially ventilated cats with intact vagus nerves. Central respiratory rate was essentially the same before \( (0.30 \pm 0.03 \text{ Hz}) \) and 10 min after \( (0.28 \pm 0.04 \text{ Hz}) \) microinjection of muscimol into the vicinity of the portion of the VRG adjacent to the LTF. As shown by the composite data in Fig. 9B, neither the I-I interval \( (3.44 \pm 0.29 \text{ s} \) vs. \( 4.04 \pm 0.83 \text{ s}) \), I-duration \( (1.39 \pm 0.17 \text{ s} \) vs. \( 1.53 \pm 0.23 \text{ s}) \), nor E-duration \( (2.04 \pm 0.14 \text{ s} \) vs. \( 2.53 \pm 0.62 \text{ s}) \) were significantly affected by bilateral microinjection of muscimol. Moreover, neither the I:E ratio nor the slopes of the inspiratory phase of PNA were significantly changed. Although there was a tendency for the inspiratory burst amplitude to decrease \( (2.65 \pm 0.43 \text{ V} \) vs. \( 1.79 \pm 0.43 \text{ V}) \), this change was not statistically significant. In the example shown in Fig. 9A, except for the modest decrease in burst amplitude, PNA was essentially unchanged after muscimol microinjection into the vicinity of the VRG. The 1:1 relationship between ITP and SND was essentially unchanged after muscimol microinjection into the vicinity of the VRG.

We determined if the increases in central respiratory rate produced by microinjection of muscimol into the LTF could be explained by spread of the injectate to adjacent medullary regions with known respiratory functions, i.e., the VRG and DRG (16, 19, 28, 29, 47, 48). We also determined the effects of chemical inactivation of these regions on the relationship of ITP to SND and PNA in these cats.
(0.67 ± 0.04 vs. 0.70 ± 0.11) and ITP-PNA coherence values (0.90 ± 0.04 vs. 0.87 ± 0.10) at the frequency of artificial ventilation as well as the ITP-related power as a percentage of the total power in the 0- to 1-Hz band of SND (30 ± 11 vs. 35 ± 11%) and PNA (61 ± 9 vs. 67 ± 9%) were similar before and after chemical inactivation of this region.

**DRG injections of muscimol.** We microinjected muscimol into the vicinity of the DRG of six paralyzed, artificially ventilated cats with intact vagus nerves, including three cats in which muscimol had been injected into the VRG 1 h earlier. Central respiratory rate was significantly (P = 0.0100) decreased from 0.33 ± 0.02 Hz to 0.18 ± 0.02 Hz 10 min after bilateral microinjection of muscimol into the vicinity of the DRG in these six cats. As shown in Fig. 10B, both the I-I interval (3.10 ± 0.13 vs. 7.37 ± 1.96 s; P = 0.0022) and I-duration (1.21 ± 0.14 vs. 4.84 ± 2.23 s; P = 0.0260) were significantly increased after chemical inactivation of the DRG. Because the variances of the control and post-muscimol data sets were markedly different in these cases (Kolmogorov-Smirnov test), a nonparametric test (Wilcoxon matched pairs test) was used to evaluate the effects of microinjection of muscimol into the DRG. In contrast to the changes in I-I interval and I-duration, E-duration (1.90 ± 0.15 vs. 2.50 ± 0.37 s) was not significantly changed. As a consequence, there was a significant (P = 0.0313) increase in the I:E ratio from 0.68 ± 0.12 to 3.58 ± 2.61. The slope of the initial rapid rise in inspiratory activity (181 ± 82% of control) was not significantly changed, but the slope of the ramplike increment in PNA was significantly (P = 0.0195) reduced to 63 ± 11% of control. Inspiratory burst amplitude (1.89 ± 0.40 vs. 2.40 ± 0.30 V) was not significantly changed. Figure 10A shows event-triggered averages of PNA before and after microinjection of muscimol in one of these cats. Note the marked increase in the I-I interval and I-duration 10 min after chemical inactivation of the DRG. In this case, the I:1 locking of PNA to ITP was disrupted as indicated by a reduction in the ITP-PNA coherence value from 0.97 to 0.18.

We also microinjected muscimol into the vicinity of the DRG of two vagotomized cats. Results were similar to those seen in vagus-intact cats. Specifically, the I-I interval increased in both cats, primarily due to an increase in I-duration; neither E-duration nor inspiratory burst amplitude were markedly changed.

We tested the effects of chemical inactivation of the DRG on the relationship of ITP to SND and PNA in the six vagus-intact cats. There were significant reductions in both the ITP-SND...
Effects of bilateral microinjection of muscimol into the vicinity of the VRG at sites adjacent to the LTF on various parameters of PNA. A: format same as in Fig. 5A, each average based on 15 respiratory cycles. B: summary of changes in various parameters of PNA in 5 vagus-intact cats. The arrow marks the completion of the last in a set of four injections.

DISCUSSION

There are two major novel findings of this study. First, our data demonstrate for the first time that the medullary LTF functions to maintain central respiratory rate in the physiological range in artificially ventilated and spontaneously breathing, anesthetized cats. Regarding this point, bilateral microinjection of muscimol into the LTF often more than doubled central respiratory rate. Second, the LTF plays a critical role in mediating the effects of vagal lung inflation afferents on SND. This is indicated by the marked reductions in the ITP-SND coherence value at the frequency of artificial ventilation and ITP-related power in SND after bilateral microinjection of muscimol into the LTF. Our data also demonstrate that the LTF is not critical for coupling of central respiratory and sympathetic networks in vagotomized cats because the PNA-SND coherence value at the frequency of the central respiratory cycle and PNA-related power in SND were not affected by chemical inactivation of the LTF.

LTF and Control of Central Respiratory Rate

Chemical inactivation of the LTF decreased the I-I interval by reducing both I- and E-durations without changing the I:E ratio. Moreover, the ramplike increase in inspiratory activity persisted. These observations suggest that the increase in central respiratory rate was due to the release of inhibition of the rhythm generator responsible for eupnea rather than conversion to gasping. In gasping, instead of increasing in a ramplike fashion, peak inspiratory activity is reached almost instantaneously (51). Central respiratory rate also increased in all four spontaneously breathing cats after chemical inactivation of the LTF. The increase in central respiratory rate after chemical inactivation of the LTF cannot be attributed to an increase in end-tidal CO2 or body temperature as these parameters were essentially unchanged in all except one of the experiments on a spontaneously breathing cat.

It has long been known that electrical stimulation of an extensive region of the medullary reticular formation, including the LTF, can alter respiration (3). St.-John et al. (53) reported that after kainic acid-induced lesions of the LTF, cats maintained a eupneic pattern of breathing, but gasping could no longer be produced by cooling the brain stem at the...
pontomedullary junction. Thus they proposed that the LTF is involved in generating gasping but not eupnea. St.-John and colleagues did not report an increase in central respiratory rate after chemical lesion of the LTF. Nonetheless, as was the case in their experiments, a eupneic pattern of respiration persisted in our cats after chemical inactivation of this medullary region.

What caused the marked increase in central respiratory rate when muscimol was microinjected into the LTF? Although the LTF contains neurons with respiratory-related activity (37, 57), there are no data to suggest that these neurons are involved in respiratory rhythmogenesis. Thus it seems more probable that muscimol silenced a group of tonically active LTF neurons that normally inhibit the respiratory rhythm generator. There is anatomic evidence for projections from the LTF to respiratory regions of the medulla including the DRG, VRG, Bötzinger complex, and pre-Bötzinger complex (50). The pre-Bötzinger complex, in particular, has been implicated in respiratory rhythmogenesis (16, 23, 28, 47, 48).

Chemical inactivation of the LTF also reduced inspiratory burst amplitude. This effect of muscimol was not simply secondary to the increase in respiratory rate as it also occurred in cats in which there was no change in the I-I interval. Perhaps in addition to the neurons that inhibit elements of the respiratory rhythm generator, the LTF contains neurons that provide excitatory drive to inspiratory premotor neurons and thus influence the depth of respiration.

**LTF and Vagal Lung Inflation Afferents**

ITP-related power in SND expressed as a percentage of the total power in the 0- to 1.0-Hz frequency band was reduced after chemical inactivation of the LTF. Thus the reduction in ITP-related power cannot be explained simply by a generalized reduction in background power in SND (9, 46).

Whereas many studies have dealt with the pathway and neuronal types that mediate the influence of vagal lung inflation afferents on respiration (20, 22, 25, 27), to our knowledge, this is the first study to identify a medullary region other than the NTS that is involved in mediating the effects of vagal lung inflation afferents on SND. In the current study, chemical inactivation of the LTF reduced total power in the 0- to 5-Hz band of SND to 75% of control. Moreover, our past work (10, 45) showed that SND was reduced to ~50% of control after blockade of non-NMDA receptor-mediated transmission in the LTF of baroreceptor-innervated and -denervated cats. These observations support the view that the LTF plays an important role in setting the level of basal SND, at least in the cat. Indeed, we have identified LTF neurons with activity correlated to SND, that are inhibited by baroreflex activation, and whose axons project to the RVLM (8). Future studies should be directed at determining if inhibition of these putative sympathoexcitatory LTF neurons accounts for the effects of activation of vagal lung inflation afferents on SND.

In contrast to the marked reductions in the ITP-SND coherence value and ITP-related power in SND after chemical inactivation of the LTF, the corresponding parameters reflecting the influence of vagal lung inflation afferents on PNA were more variably affected, with ITP-PNA coherence values and ITP-related power in PNA not changing, decreasing, or increasing in individual experiments. When the central respiratory cycle remained locked in a 1:1 relationship to the artificial ventilator cycle, the ITP-PNA coherence value did not change. However, when chemical inactivation of the LTF led to an increase in central respiratory rate above that of the frequency of artificial ventilation, the ITP-PNA coherence value and the ITP-related power in PNA were reduced. We interpret the reductions in the ITP-PNA coherence value and ITP-related power in PNA under these conditions to reflect the fact that the central respiratory rate increased to a point beyond which vagal lung inflation afferent activity could entrain PNA in a 1:1 relationship to ITP. In other cases in which the central respiratory rate was initially below the rate of artificial ventilation, chemical inactivation of the LTF increased it to the frequency of artificial ventilation. Under these conditions, both the ITP-PNA coherence value and the ITP-related power in PNA were increased, presumably because central respiratory rate was moved into the range over which PNA could be locked in a 1:1 relationship to ITP. In combination, these results lead us to conclude that the LTF is not involved in mediating the effects of vagal lung inflation afferents on the respiratory rhythm generator.

Bachoo and Polosa (4) reported that SND is increased by activation of low threshold vagal inflation afferents and inhibited by activation of higher threshold vagal inflation afferents. The low threshold afferents are known to mediate the Hering-Breuer reflex (25, 28). We typically found that SND was decreased rather than increased during lung inflation (see Fig. 2), therefore implying that both the low and higher threshold
inflation afferents were activated under the conditions of our experiments, and the effects on SND of the latter group predominated. The extent to which each type of vagal inflation afferent participated in the synchronization of SND to ITP and the entrainment of PNA to lung inflation remains unclear. Nonetheless, whether the afferents are the same or different, our results provide new information on the question of whether vagal lung inflation afferent activity alters SND not only indirectly via their actions on respiratory networks but also via a more direct pathway to sympathetic neurons (4, 39).

The fact that chemical inactivation of the LTF could disrupt the relationship between ITP and SND when that between ITP and PNA was maintained supports the view that the effects of vagal lung inflation afferents on sympathetic circuits are mediated, in part, over a pathway that bypasses the respiratory oscillator. This view is also supported by a study from this laboratory in which we (59) used partial coherence analysis to mathematically remove the portions of the ITP and SND signals attributable or common to PNA. We reasoned that the ITP-SND coherence value at the frequency of breathing should remain significant after partialization with PNA if the effects of lung inflation on SND were mediated, in part, via a pathway that bypasses the respiratory oscillator. This was found to be the case in paralyzed, artificially ventilated cats when ITP and PNA were strongly coupled in a 1:1 relationship and in spontaneously breathing cats.

Häbler et al. (34) and Kunitake and Kannan (40) suggested that vagal lung inflation afferents do not directly affect SND; rather, the inhibition of SND accompanying lung inflation was considered merely to reflect changes in baroreceptor nerve activity due to inflation-induced changes in arterial pressure. If such is the case, one could suggest that our data merely confirm our past work showing a role of the LTF in mediating the baroreflex in the cat (45). However, this explanation is not tenable for several reasons. First, in vagotomized cats in which the baroreflex remained functional (high coherence between the arterial pulse and SND), the ITP-SND coherence value was not significantly different from zero during control. Second, in some cats with intact vagus nerves, SND did not cohere to the arterial pulse (“functionally baroreceptor denervated”), yet there was a strong correlation of SND to ITP. Third, pneumothoracotomy was routinely used to minimize ventilator pump-related changes in arterial pressure. These data support the view that vagal lung inflation afferents affect SND independently of the baroreflex.

The selective interruption of the influence of vagal lung inflation afferents on SND after chemical inactivation of the LTF also is at odds with the theory that there is a common central cardiorespiratory network (38, 49). In the models proposed by Koepchen et al. (38) and Richter and Spyer (49), separate control of the cardiovascular and respiratory systems is manifested only at the level of bulbospinal and spinal neurons. In contrast to their models, our data support the view that selective control of SND is evident at the level of LTF neurons which are antecedent to RVLM-spinal sympathoexcitatory neurons (8, 30).

Synchronization of Central Sympathetic and Respiratory Circuits

Because at least some LTF neurons with activity correlated to SND also have activity correlated to PNA in vagotomized cats (7), we determined if an interaction of central respiratory and sympathetic circuits within the LTF could explain the respiratory-related rhythm commonly seen in SND of vagotomized animals (5, 6, 34, 35). If such were the case, one would have expected the PNA-related power in SND and the PNA-SND coherence value at the frequency of the central respiratory rhythm to be reduced by bilateral microinjection of muscimol into the LTF. This did not occur in vagotomized cats; thus our data indicate that the LTF is not critical for the direct coupling of central respiratory and sympathetic networks. Guyenet et al. (33) proposed that the rostral tip of the ventrolateral medulla represents the critical link between the respiratory rhythm generator and the sympathetic network. Koshiya and Guyenet (39) have also suggested that ventrolateral pontine neurons play a role in respiratory modulation of SND.

Did Muscimol Act Within the LTF or Spread to Other Medullary Sites to Alter PNA and Vagal-Mediated Reflex Control of SND?

According to investigators who have calculated the spread of injection of a bolus of fluid in the brain (44), the 50-nl volume of muscimol used in most of our experiments is not expected to spread beyond a radius of ~0.5 mm. The LTF injection sites were considerably more than 0.5 mm from medullary regions known to be involved in control of PNA (DRG, VRG, Botzinger complex; Refs. 2, 14, 20, 23, 49, 58) and SND (NTS, CVLM, and RVLM; Refs. 7–12, 30, 43, 45, 46). Moreover, data obtained in the current study can be used to rule out spread of the injectate from the LTF to these other sites or to the dendrites of neurons in these regions that might extend toward the LTF.

Specifically, in contrast to the effects produced by chemical inactivation of the LTF, microinjection of muscimol into the vicinity of the VRG adjacent to the LTF did not significantly affect central respiratory rate. It may seem surprising that chemical inactivation of this region caused only a modest reduction in the inspiratory burst amplitude since this portion of the VRG contains inspiratory premotor neurons (23, 29). However, it should be noted that we injected muscimol into only a small portion of the VRG which, in its entirety, extends from 10 mm from the spinomedullary border to the retrofacial nucleus (29). Also, our data corroborate the findings of Anderson and Speck (2) who showed that blockade of excitatory amino acid receptors in the same region of the VRG did not markedly affect PNA. These investigators also identified neurons with respiratory-related activity in this portion of the VRG.

In contrast to the effects produced by chemical inactivation of the LTF, microinjection of muscimol into the vicinity of the DRG decreased central respiratory rate, increased I-duration, and reduced both ITP-PNA and ITP-SND coherence values at the frequency of artificial ventilation. These effects were likely due, at least in part, to block of the Hering-Breuer reflex because vagal lung inflation afferents terminate in the ventrolateral NTS (27, 29, 58). The DRG contains several types of neurons, including pump cells and late-inspiratory neurons, that are thought to be involved in mediating the Hering-Breuer reflex (20, 22). However, interruption of vagal lung inflation afferent input to DRG neurons cannot fully explain the pro-
The increase in central respiratory rate that occurred in response to chemical inactivation of the LTF offers new information on the control of the respiratory rhythm. Future studies should be designed to determine the site and respiratory neuronal types acted on by LTF neurons to maintain respiratory rate and depth within the physiological range. In addition, the results of the current study in combination with recent studies (45, 46) have shown that the LTF is involved in mediating changes in SND produced by activation of baroreceptor, chemoreceptor, and vagal lung inflation afferents. A common denominator in these studies is that these afferent fibers terminate in the NTS. This leads to the novel hypothesis that, at least in the cat, the LTF is an obligatory link in all reflex pathways affecting SND and arterial pressure in which the primary afferent terminates in the intermediate or caudal portion of the NTS.

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