48

A “Pneumotaxic Center” in the Ventrolateral Pons of Rats

THOMAS E. DICK and SHARON K. COLES

School of Medicine
Case Western Reserve University
Cleveland, Ohio

JOZEF S. JODKOWSKI

Institute of Physiological Sciences
Medical Academy of Warsaw
Warsaw, Poland

The “pneumotaxic center” in the pons plays a critical role in determining respiratory phase duration in a number of species. Lumsden reported in 1923 that apneustic breathing, a breathing pattern with prolonged inspiratory phase, follows transection separating the rostral pons from the medullary respiratory central pattern generator in rabbit, cat, dog, and monkey, especially in vagotomized animals (1,2). Even in humans, apneustic breathing has been associated with pontine lesions (3). However, there is a paucity of data regarding its presence in rats. Indeed, a report has questioned the existence of a pneumotaxic center in rats (4).

In other species, the rostral dorsolateral pons has been identified as the pneumotaxic center. Electrophysiological studies have characterized the medial parabrachial and Kölliker-Fuse nuclei as the specific neuronal substrates for the pneumotaxic center in human (3), cat (5–16), opossum (17), guinea pig (18), and rabbit (19,20). The physiological criteria for characterizing an area as a pneumotaxic center were derived from data obtained in lesion, stimulation, and recording studies and are listed in Table 1. For example, bilateral lesions in the dorsolateral pons led to apneustic breathing in vagotomized animals, i.e., similar to Lumsden’s transection experiments (5–10,19). Small unilateral

Table 1 Criteria for a "Pneumotaxic Center"

Lesion:
1. Bilateral: Apneustic breathing in vagotomized anesthetized animal
2. Unilateral: Instability in breathing pattern

Stimulation: Response dependent on pattern of stimulation
1. Single electrical pulses
   A. Phrenic nerve: short-latency inhibition or excitation
   B. Hypoglossal nerve: short-latency excitation
   C. Phase switching especially inspiration to expiration
2. Trains of electric pulses or injection of excitatory chemical
   A. Change in amplitude in phrenic nerve activity
   B. Change in breathing frequency

Recording: Various patterns of respiratory-modulated activity
1. Respiratory-modulated phasic activity
2. Phase-spanning activity
   A. Peak activity near phase transition
   B. Activity starts in one phase and ends in another
3. Respiratory-modulated tonic activity
   A. Peak activity occurs during a phase and not associated with transition
   B. No quiescent period

lesions destabilize respiration, as indicated by increased variability in the respiratory pattern and by increased time necessary for the respiratory cycle to return to its normal cycle length following a perturbation (11). Depending on the stimulus site, single pulses can elicit short-latency inhibition or excitation in phrenic nerve activity and can cause phase switching (12,13). Similarly, trains of stimulus pulses or microinjection of excitatory amino acids can increase or decrease breathing frequency and phrenic nerve activity (14,17). Recordings of neuronal activity have shown various patterns of respiratory-modulated activities, including phase-spanning activity and respiratory-modulated tonic activity (15,16,18).

Results from preliminary (21,22) as well as recent (23) studies have identified an area in the dorsolateral pons in rats that meets some of these criteria. In particular, microinjections of excitatory amino acids in the dorsolateral pons result in both increases and decreases in phrenic nerve activity (21,22). Potent effects were cessation of phrenic nerve activity with DL-homocysteic acid in the Kölliker-Fuse nucleus (22). In addition, bilateral lesions in the dorsolateral pons lead to apneustic breathing (23). Therefore, we hypothesized that rats are similar to other species and contain a pontine area that fulfills the criteria of a pneumotaxic center. Having identified the criteria in Table 1, we addressed our hypothesis using electrophysiological techniques, specifically (a) short-latency inhibition of phrenic nerve activity following single-pulse stimulation, (b) apneustic breathing with bilateral lesions, (c) changes in breathing frequency and/or phrenic nerve amplitude with stimulus trains and/or chemical injections, and (d) recording of respiratory modulated activity.

We used adult male rats ($n = 20$, Sprague-Dawley, 310 to 440 g). Animals were anesthetized with Equithesin (0.3 mL/100 g, IP) which is a mixture of chloral hydrate and pentobarbital (5.55 g and 1.215 g respectively per 125 mL double-distilled water). In addition to the anesthetic agent, atropine sulfate (0.5 mg/100 g, S.C.) and pancuronium bromide (0.1 mg/100 g/h, IV) were administered to reduce secretions and to paralyze the animals, respectively. Body temperature was maintained at 38°C with a heating pad. The left femoral artery and vein were cannulated to measure blood pressure and to administer drugs, respectively. A tracheostomy was performed and animals were ventilated. Animals were placed in a stereotaxic frame so that the dorsal surface of the parietal plates was horizontal. The right phrenic nerve was dissected, transected, and desheathed from a dorsal approach and the nerve was placed on a bipolar recording electrode. Phrenic nerve activity was amplified and filtered 0.1 to 3 KHz (Grass P511) and was sent to a moving averager (CWE, Inc.) where it was rectified and integrated (Paynter Filter, time constant 50 ms). The raw phrenic neural activity and the output from the moving averager were recorded on paper (Astromed DASH 8) and tape (Hewlett-Packard). In all but one animal, the vagi were transected and in three of these animals the right vagus was placed on a stimulating electrode. In the animal without vagal transection, the vagi were cut after the electrolytic lesions were produced.

Experimental protocol involved initially identifying the region of interest by determining a site from which single stimulus pulses evoked short-latency inhibition of phrenic nerve activity. Initially, tungsten electrodes (0.005 in, AM Systems) were placed stereotaxically at 9.05 to 9.25 mm caudal to bregma and 2.5 to 2.6 mm lateral to the midline. Electrode depth was referenced to the dorsal cerebral/cerebellar surface. Testing for short-latency inhibition of phrenic nerve activity began at a depth of 4500 μm and consisted of stimulating the tissue with trains of stimuli (10 Hz at 50 μA). If a short-latency inhibition was recorded at 50 μA, we then decreased the current to determine the lowest stimulus current necessary to evoke a response. We tested the tissue systematically by positioning the electrode progressively deeper in increments of 250 μm initially and 100 μm when the threshold stimulus went below 50 μA. If an electrode track was made without evoking an effect or without decreasing the threshold stimulus below 50 μA, then the electrode was repositioned.

Once an effective site was located, one of various protocols was followed. In the first protocol ($n = 13$), we lesioned the identified site bilaterally either electrolytically ($n = 8$, two of which received only unilateral lesions; the last two animals in Table 2) or chemically ($n = 5$). Electrical lesions were DC pulses, 2 to 10 mA, and 5-s pulse duration. For chemical lesions, we replaced the tungsten microelectrode with a glass micropipette containing either 1 m*M* ibotenic acid ($n = 3$) or 10 m*M* muscimol ($n = 2$) in a 2% fast green buffered physio-

Table 2 Changes in Variables of Breathing Pattern Before and After Pontine Lesion in Anesthetized, Vagotomized Rats[a]

Ti (s)		Te (s)		Ttot (s)		Resp rate (bpm)	
Before	After	Before	After	Before	After	Before	After
0.23	0.37	0.90	0.47	1.13	0.84	52.9	71.4
0.01	0.02	0.04	0.02	0.05	0.03		
0.31	3.25	0.87	1.15	1.17	4.40	51.1	13.6
0.01	0.14	0.01	0.05	0.02	0.19		
0.22	1.99	1.16	3.77	1.38	5.76	43.5	10.4
0.00	0.42	0.05	0.22	0.05	0.34		
0.36	1.33	0.69	0.92	1.05	2.25	57.0	26.6
0.00	0.08	0.02	0.03	0.02	0.11		
0.23	1.41	0.97	2.91	1.20	4.32	50.0	13.9
0.01	0.25	0.01	0.38	0.02	0.63		
0.44	0.49	1.05	0.70	1.49	1.19	40.4	57.0
0.00	0.01	0.07	0.02	0.07	0.02		
0.26	0.53	1.17	1.05	1.43	1.59	42.1	37.8
0.00	0.02	0.04	0.01	0.04	0.03		

[a]Values: mean and standard deviation of three consecutive breaths.

logical saline solution. Chemical lesions were produced by pressure injection at the stereotaxic coordinates identified by microstimulation. The amount injected was measured directly by observing the displacement of the meniscus marking its displacement, using a reticle. Phrenic nerve activity was recorded for at least 30 min following the last lesion.

In the second protocol ($n = 6$), the tungsten microelectrode was replaced by a glass micropipette containing either 10 m*M* D-glutamate ($n = 2$) or 10 m*M* L-glutamate ($n = 4$). Both solutions were made with 2% fast green in buffered saline. The D-glutamate injections were 200 nL, which ranged from 2 to 10 times the amount of L-glutamate injections (20 to 100 nL) and served as controls.

Recently, we initiated a third protocol ($n = 1$) in which neural activity in the ventrolateral pons was recorded. Activity was recorded by a glass micropipette filled with 4 m*M* NaCl, tip size less than 2 μm and an impedance between 10 and 25 MΩ. After recording single-unit activity, the glass micropipette was replaced with a tungsten microelectrode ($Z = 5$ MΩ). A lesion was made at the site where respiratory-modulated noise was recorded via the tungsten microelectrode.

At the end of the experiment, the brainstem was removed and placed in 10% formalin and stored for at least 48 h. At least 24 h before sectioning, the tissue was blocked and placed in a 40% sucrose solution. Frozen sections (50 μm thick) were cut in the frontal plane. The sections were examined microscopically and the extent of the lesions was mapped.

We analyzed the data by measuring the duration of inspiratory phase (Ti) and the duration of the respiratory cycle (Ttot) using phrenic nerve activity as the indicator of the respiratory cycle: Ti was measured from the onset of phrenic nerve activity to its offset and Ttot was measured from the onset of one cycle to the onset of the next cycle. Measurements were made for three consecutive cycles and post-lesion measurements were made after the breathing pattern stabilized. The Ti/Ttot ratio was calculated for each animal before and after lesioning. Statistical difference was tested using the paired Student's *t*-test and was accepted if the probability for the null hypothesis was less than 0.01.

In all animals, we located a site in the ventrolateral pons between 7500 and 8500 μm below the dorsal cerebral surface from which single stimulus pulses evoked a short-latency inhibition of phrenic nerve activity (Fig. 1). The latency was measured from the onset of the stimulus pulse to the decrease of phrenic nerve activity and was less than 5 ms.

Bilateral electrolytic lesions at the sites identified by stimulation altered respiratory pattern profoundly (Fig. 2, Table 2). Duration of inspiration (Ti) increased between 1.2- and 10-fold (Table 2). In 5 animals, the duration of expiration (Te) increased also, but to a lesser extent than Ti (Table 2). In 2 animals, Te was decreased (Table 2). As a net result, the ratio of Ti to the total duration of the respiratory cycle (Ttot), an index of apneustic breathing, increased significantly in all ($n = 7$, including the two with unilateral lesions) animals (Table 2). Even after unilateral lesions, Ti was prolonged (Fig. 2B, and the last two animals in Table 2).

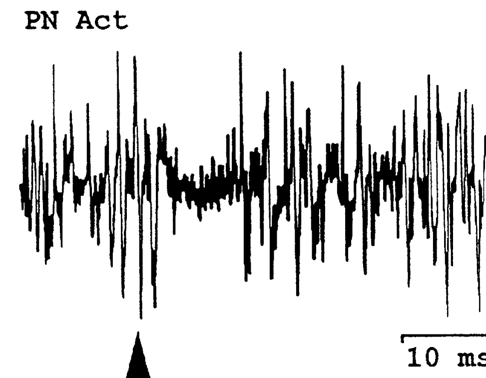

Figure 1 Short-latency inhibition of phrenic nerve activity by pontine stimulation. Latency was measured from the onset of the stimulus pulse and was 3.4 ms in this case. Stimulus pulses were delivered 8500 μm below the dorsal cerebral surface. Arrowhead = onset of the stimulus pulse (10 μA, 1 ms); PN Act = phrenic nerve activity; ms = milliseconds.

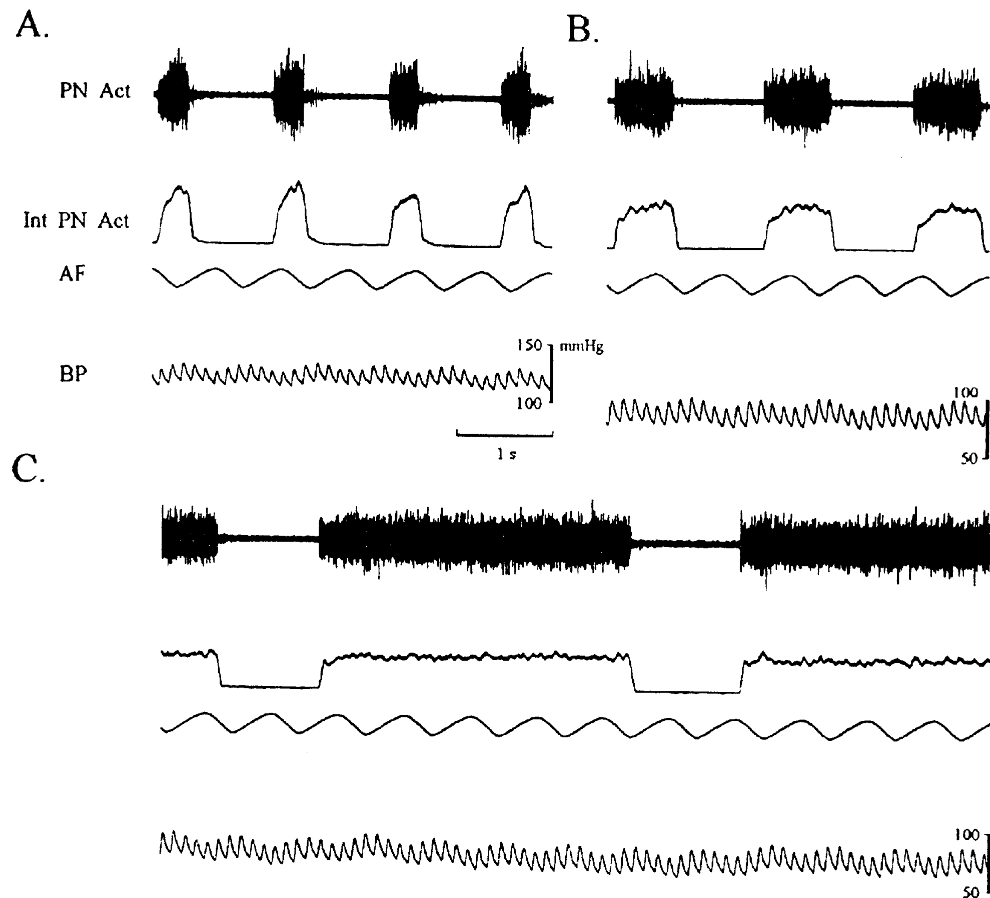

Figure 2 Effect of electrolytic lesion (10 mA, 5 s) in the ventrolateral pons of an anesthetized vagotomized rat. (A) Pons intact. (B) Stable breathing pattern after unilateral lesion. (C) Stable breathing pattern after contralateral lesion. Breathing frequency slowed due to the prolongation of inspiration (i.e., apneustic breathing). Arterial blood pressure decreased from 120 to 75 mm Hg approximately 10 s after the first lesion and remained relatively stable after the second lesion with the exception of the marked respiratory modulation. Abbreviations: PN Act = phrenic nerve activity; Int. PN Act = integrated phrenic nerve activity; AF = airflow; BP = blood pressure.

The effects of the lesions on the breathing pattern were dependent on bilateral vagal transection. When large bilateral lesions were formed in a vagally intact animal ($n = 1$), Ti was prolonged slightly, but apneusis developed more completely after vagal transection. Further, in both animals tested, continuous stimulation (10 Hz) of the proximal end of a transected vagus nerve terminated Ti and restored partially the normal breathing pattern.

Histological examination revealed that electrical lesion sites were in the ventrolateral quadrant of the pons (dotted area, Fig. 3). Lesions were lateral

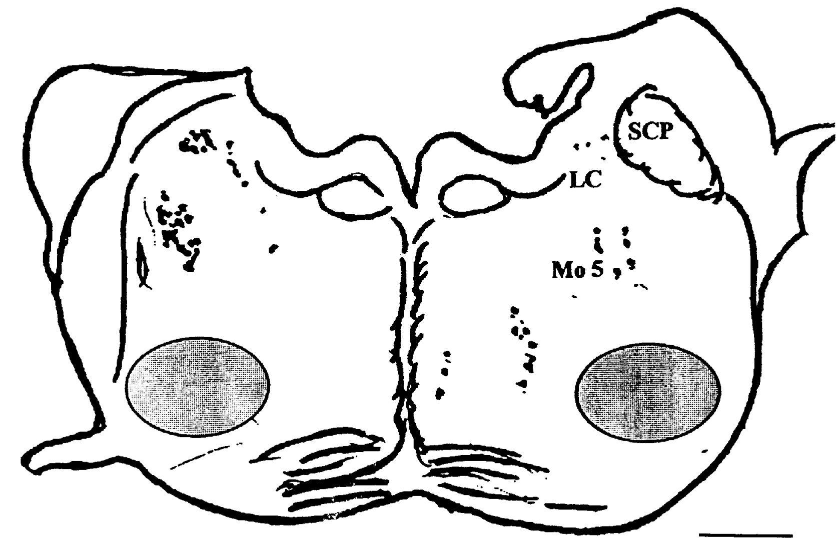

Figure 3 Drawing of coronal section of the pons. The cross-hatched areas represent damaged areas from all of the animals. On the left side, the electrode track was visible above the lesion. The distance from bregma is an approximate measure (9.05 mm caudal) and the scale bar is 11 mm. Abbreviations: LC = locus ceruleus; Mo 5 = motor nucleus of the trigeminal nerve; SCP = superior cerebellar peduncle.

to the nucleus of the trapezoid body and dorsolateral to superior olivary nucleus (the superior olivary nucleus was included in the large lesions). At its rostral end, the lesions were ventrolateral to the motor nucleus of the fifth cranial nerve (CN-V). At its caudal end, the lesion was medial to exit of the CN-VII fibers and included the A5 area. Even though the rostrocaudal extent of a single lesion was not greater than 0.5 mm, the rostrocaudal extent of all the lesions ranged from -8.7 to -10.0 mm relative to bregma.

Electrolytic stimulation and lesioning are notorious for activating both cell bodies and fibers of passage. This region of the brainstem (i.e., ventrolateral pons) contains both fibers of passage from the dorsolateral pons, the area classically defined as the pneumotaxic center, as well as cells of the A5 cell group (24,25). In addition, even in cat, axonal projections from the commissural subnuclei of the solitary tract have been shown to terminate in the ventrolateral pons (26). Therefore, we pursued these initial studies by lesioning and stimulating this area chemically as well as recording cellular activity there.

Chemical lesions consisted of both ibotenic acid (1 m*M*) and muscimol (10 m*M*) bilateral microinjections. Ibotenic acid is an excitatory amino acid agonist that causes depolarization blockade. Muscimol is a long-acting gamma-aminobutyric acid (GABA) agonist that inhibits neuronal activity directly. Both compounds act preferentially on cell bodies, block neural activity, and effectively lesion the site. Therefore, after identifying a site in the ventrolateral pons

from which a short-latency inhibition could be elicited, the tungsten electrode was replaced by a glass micropipette containing either ibotenic acid or muscimol.

Ibotenic acid injections were made bilaterally near the ventrolateral pontine surface (ranging from 7800 to 8200 μm below the dorsal cerebral surface) (Fig. 4). The response to the injections was time-dependent. Initially, the amplitude of phrenic nerve activity increased, and both Ti and Te prolonged (Fig. 4B). Subsequently, phrenic nerve amplitude decreased and Ti prolonged further (Fig. 4C). Blood pressure did not change consistently. However, blood pressure increased transiently during the injection of ibotenic acid.

Muscimol injections were made near the ventral surface of the brainstem (7800 and 8000 μm below the surface). The chemical injection had both immediate (an apnea) and latent (progressive development of apneusis) effects on breathing pattern but no effect on blood pressure until the end of the experiment, at which time blood pressure decreased.

The response to excitatory amino acid injections was also time-dependent but on a shorter time scale. The immediate response to injections of L-glutamate was a termination of phrenic nerve activity, an "off switch." Subsequently,

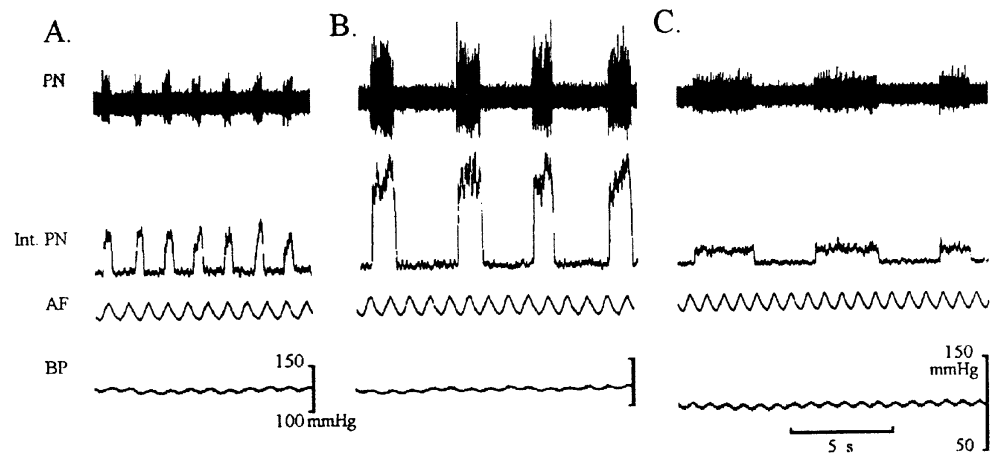

Figure 4 Effect of ibotenic acid (1 m*M*) injections in the ventrolateral pons in an anesthetized, vagotomized rat. (A) Respiration and blood pressure in vagotomized intact rat. (B) Approximately 5 min following bilateral injections of ibotenic acid (1 m*M*). The injections were approximately 15 min apart and were not of equal size (50 nL on one side and 20 nL on the contralateral side). The increase in the amplitude of phrenic nerve activity occurred after unilateral injection, but the change in timing e.g., the increase in Ti and Te, did not occur until after the contralateral injection. (C) Apneustic breathing pattern approximately 90 min after last injection. Mean arterial blood pressure is approximately 100 mm Hg. Abbreviations: PN Act = phrenic nerve activity; Int. PN = integrated phrenic nerve activity; AF = airflow; BP = blood pressure.

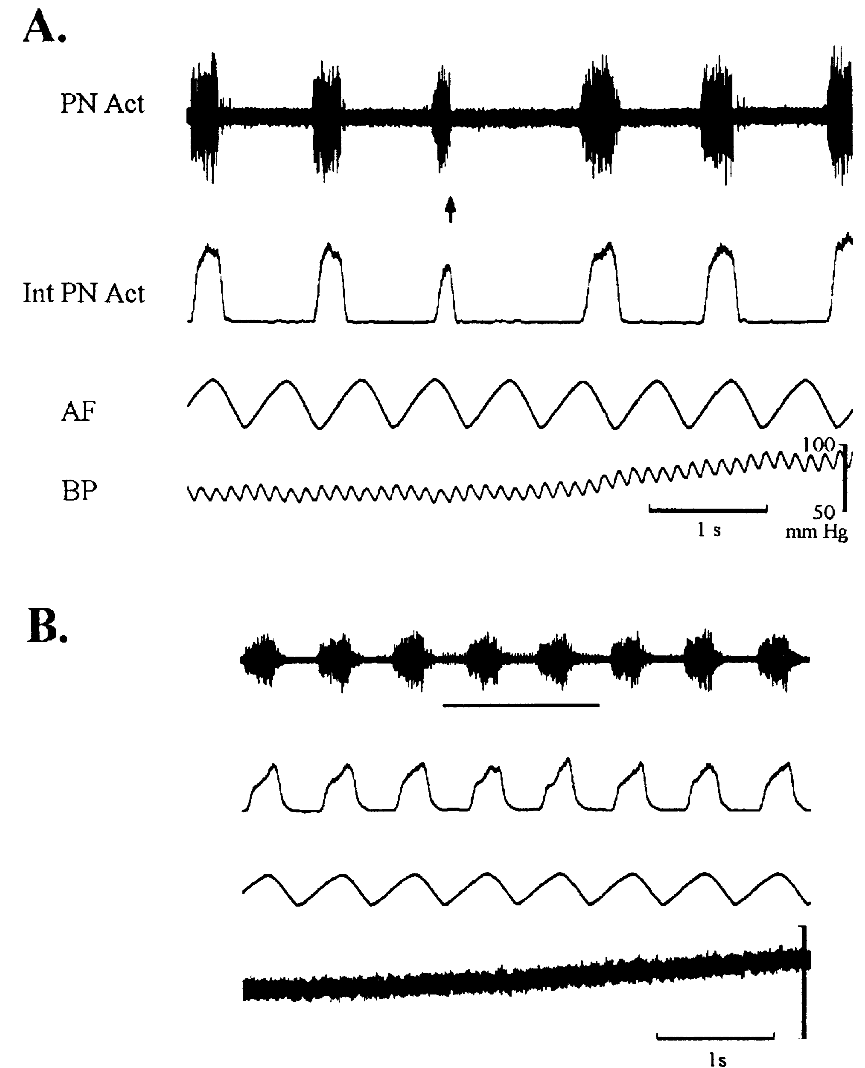

Figure 5 Effect of unilateral excitatory amino acid injection into the ventrolateral pons in rat. (A) L-glutamate (10 m*M*, 100 nL) was injected in the ventrolateral pons as a single bolus injection during inspiration. Phrenic nerve activity ceased immediately and subsequently was slightly prolonged. Te was prolonged in the shortened breath but not in the subsequent cycles. (B) D-glutamate (10 m*M*, 300 nL) was injected as a series of numerous injections (marked by bar). No change in the respiratory pattern was apparent, only a subsequent rise in blood pressure. Time (1s) and blood pressure (50 to 100 mm Hg) bars are the same in panels A and B. Abbreviations: PN Act = phrenic nerve activity; Int. PN Act = integrated phrenic nerve activity; AF = airflow; BP = blood pressure.

Ti increased transiently (Fig. 5A). Blood pressure increased as well during this subsequent increase in Ti (Fig. 5A). The respiratory response was stereospecific, indicating a dependence on an interaction between L-glutamate and its receptors. Injections of D-glutamate did not elicit a phase switching or a prolongation of Ti (Fig. 5B).

Extracellular recordings of neurons in the ventrolateral pons revealed various patterns of activity (Fig. 6). These cells were recorded with glass micropipettes filled with 4 *M* NaCl and the amplifier was AC-coupled. Unfortunately these recordings cannot distinguish between fibers of passage and cell bodies. Nevertheless, they verify a presence of highly modulated respiratory activity in the ventrolateral pons. This is in counterdistinction to respiratory-modulated sympathetic activity that has been recorded recently in the ventrolateral pons in the A5 region (23).

In conclusion, in the anesthetized, vagotomized adult rat, bilateral lesions localized specifically to ventrolateral pons resulted in apneustic breathing, i.e., a pattern in which principally Ti is lengthened. This breathing pattern was dependent on bilateral vagal transection and was partially reversed by vagal stimulation. Thus, our study confirms that a pneumotaxic center, as defined by Lumsden's classic experiments (1,2), exists in rats. Further, we believe that activity from ventrolateral pontine structures interacts with vagal input to determine respiratory phase duration in mammals.

Our physiological findings agree with observations made in a variety of species including rodents (1–3,5–10,12–23) and fulfill the criteria for a pneumotaxic center (Table 1). However, these data disagree with those of Monteau et al. (4), who reported no changes in breathing pattern following pontine transection in anaesthetized, vagotomized rats. They concluded that in adult rat pontine influences upon respiration, if any, were negligible (4). In contrast, we report apneustic breathing in rats following pontine lesions. These data contradict those of Monteau et al. (4) and support those of Wang et al. (23). We conclude that pontine influences on breathing in rats are similar to those in other mammalian species. We speculate that the failure of Monteau et al. (4) to observe changes in the breathing pattern following pontomedullary transection may be related to either incomplete transections or a strain difference among the rodent species. The transections illustrated in their report appear to be incomplete, leaving neural tissue intact in the ventral pons, which may include pathways connecting pontine and medullary respiratory networks (see Fig. 2 in Ref. 4; lines indicating transections do not go to the bottom of tissue section). In addition, a difference was reported recently between Wistar and Sprague-Dawley strains of rats regarding the breathing pattern changes following MK-801 (27). In Sprague-Dawley rats, like other animal species, apneusis followed MK-801 injections; whereas in Wistar rats, apneusis was not evident (27). We and Wang et al. (23) used Sprague-Dawley rats; Monteau et al. (4) did not identify strain.

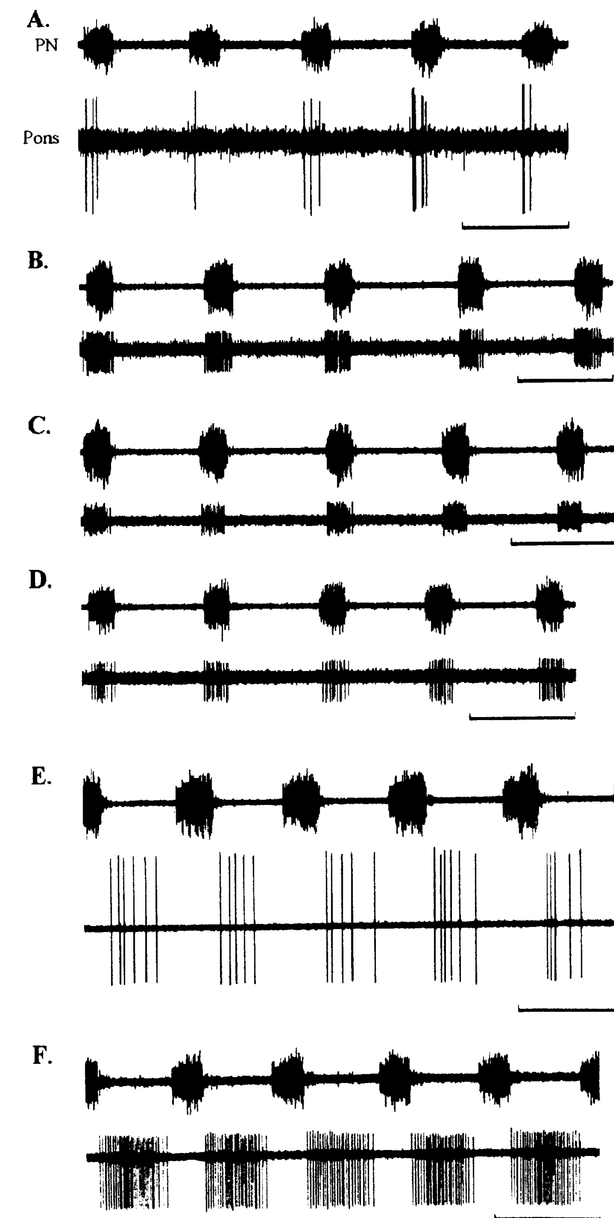

Figure 6 Extracellular recordings of highly modulated respiratory activity in the ventrolateral pons of bilaterally vagotomized rats. Tracings are paired with raw phrenic nerve activity (PN) above the pontine activity (Pons). (A) Early-inspiratory or inspiratory-decrementing activity recorded at 7400 μm below the dorsal cerebral surface. (B, C, and D) Inspiratory activities recorded 7700, 7740, and 8700 μm deep. (E) Post-inspiratory or expiratory-decrementing activity recorded 7200 μm deep. (F) Expiratory activity, 7200 μm deep. Time bar equals 1 s.

The unusual aspect of our finding is that the lesion was located in the ventrolateral pons. The lesion site was identified in vivo by determining the lowest stimulus that evoked short-latency inhibition of phrenic nerve activity. The neural elements activated by electrical stimulus pulses depend on numerous variables and fibers rather than cells that may have been preferentially activated. However, both our chemical lesioning and stimulating experiments support the hypothesis that cells in this region can modify the breathing pattern.

Electrical stimulation does provide a discrete onset time for latency measurements. The onset latency of the response recorded in the phrenic nerve activity was less than 5 ms. This short-latency response indicates a paucisynaptic if not a direct pontospinal pathway. These data are also similar to those of the cat, in which the response of phrenic nerve activity was as short (4 to 7 ms), indicating a pontospinal projection (12,13). In a recent study in which A5 neurons were antidromically activated by thoracic spinal cord stimulation, the mean response latency was greater than 25 ms (24). This discrepancy indicates multiple populations of projecting neurons from and through this area.

As indicated above, it is generally accepted that the neuronal substrate for Lumsden's pneumotaxic center is located in dorsolateral pons, specifically the medial parabrachial and Kölliker-Fuse nuclei (1–3,5–22). Indeed, previous reports indicate that this area is important in rodents as well (21–23). Only a few reports indicate that other structures in the lateral and ventrolateral pons may play any role in control of respiration, and most of these studies were performed in neonatal and fetal preparations (28–30). In an early study in the adult cat, electrolytic lesions in the ventrolateral pons in at least one cat resulted in apneustic breathing also (Fig. 8 in Ref. 9). Further, a recent study, also in the adult cat (26), indicated that second-order respiratory afferents projected to and terminated in the ventrolateral pons in addition to the dorsolateral pons. We speculated that the electrolytic lesions in the ventrolateral pons destroyed tracts between dorsolateral pontine nuclei and medullary respiratory groups and neurons in the A5 area. A potential role for neurons in the A5 group in adult rats is supported by our data from microinjections of muscimol and excitatory amino acid agonists near the ventral pontine surface.

This is the first study to report strong respiratory-modulated phasic activity in the ventrolateral quadrant in rats. These are initial recordings, and it remains unclear if they were obtained from fibers or cell bodies. Weak respiratory-modulated tonic activity has been recorded in the A5 cell group (24). The relationship between these cell populations is unclear. There were similarities and differences in both animal protocols. For instance, both utilized vagotomized, adult Sprague-Dawley rats but differed in anesthetic agents. We used Equithesin, a mixture of pentobarbital and chloral hydrate, and they induced anesthesia with halothane and maintained it with urethane. However, the cellular nature of their recordings was verified by evoking an excitatory response to locally injected L-glutamate.

Even in these initial recordings from one animal, it is apparent that different types of respiratory-modulated activity exist in the ventrolateral pons. We recorded not only from inspiratory and expiratory cells but also from early inspiratory and postinspiratory neurons. These latter two groups have been identified as representing the "kernel" of the medullary respiratory pattern generator (31). In addition, whether fibers or cells, the recorded activity could provide the neural substrate for the modulation reported by Guyenet et al. (24).

In summary, this study confirms that, in at least Sprague-Dawley rats, a pneumotaxic center exists. Further, our working hypothesis is that activity in the ventrolateral pons modulates the breathing pattern. Finally, the results in this paper and those previously published (9,26) raise the possibility that the pontine respiratory network may include structures in the ventrolateral pons even in other species.

Acknowledgments

We thank Mr. Philip Martinak for assistance in data analysis and histological processing of the tissue and Ms. Cheryl Diane Giliam-Walker for secretarial assistance in preparation of the manuscript. We acknowledge gratefully the support of the National Heart, Lung and Blood Institute, HL-42400 to TED and of the American Heart Association to SKC.

References

1. Lumsden T. Observations on the respiratory centres in the cat. J Physiol (Lond) 1923; 57:153–160.
2. Lumsden T. Observations on the respiratory centres. J Physiol (Lond) 1923; 57: 354–367.
3. Plum F, Posner JB. Diagnosis of Stupor and Coma, 2nd ed. Philadelphia: Davis, 1972.
4. Monteau R, Errchidi S, Gauthier P, et al. Pneumotaxic centre and apneustic breathing: Interspecies differences between rat and cat. Neurosci Lett 1989; 99:311–316.
5. Berger AJ, Herbert DA, Mitchell RA. Properties of apneusis produced by reversible cold block of the rostral pons. Respir Physiol 1978; 33:323–337.
6. Caille D, Vibert J-F, Hugelin A. Apneusis and apnea after parabrachial of Kölliker-Fuse N lesion: Influence of wakefulness. Respir Physiol 1981; 45:79–95.
7. von Euler C, Marttila I, Remmers JE, Trippenbach T. Effects of lesioning the parabrachial nucleus on the mechanisms for central and reflex termination of inspiration in the cat. Acta Physiol Scand 1976; 96:324–337.
8. Feldman JL, Gautier H. The interaction of pulmonary afferents and pneumotaxic center in control of respiratory pattern in cats. J Neurophysiol 1976; 39:31–44.
9. Ngai SH, Wang SC. Organization of central respiratory mechanisms in the brain stem of the cat: Localization by stimulation and destruction. Am J Physiol 1957; 190:343–349.

10. St. John WM. Characterization of the tidal volume regulating functions of the pneumotaxic center. Respir Physiol 1973; 18:64–79.
11. Oku Y, Dick TE. Phase resetting of the respiratory cycle before and after unilateral pontine lesion in cat. J Appl Physiol 1992; 72:721–730.
12. Cohen MI. Switching of the respiratory phases and evoked phrenic responses produced by rostral pontine electrical stimulation. J Physiol (Lond) 1971; 217:133-158.
13. Dick TE, Haxhiu MA, Cherniack NS. Salivary secretion elicited by activation of the parabrachial nuclei in the cat. J Auton Nerv Syst 1992; 39:19–28.
14. Denavit-Saubié M, Riche D, Champagnat J, Velutti JC. Functional and morphological consequences of kainic acid microinjections into a pontine respiratory area of the cat. Neuroscience 1981; 5:1609–1620.
15. Bianchi AL, St. John WM. Medullary axonal projections of respiratory neurons of pontile pneumotaxic center. Respir Physiol 1982; 48:357–373.
16. Cohen MI, Wang SC. Respiratory neuronal activity in pons of cats. J Neurophysiol 1959; 22:33–50.
17. Farber JP. Effects on breathing of rostral pons glutamate injection during opossum development. J Appl Physiol 1990; 69:189–195.
18. Glogowska M, Gromysz H. Respiratory activity of the pons and its influence on breathing in the guinea pig. Acta Neurobiol Exp 1988; 48:125–135.
19. Gromysz H. The effects of pontine or bulbar transection on the respiratory pattern in the rabbit. Acta Neurobiol Exp 1984; 44:239–247.
20. Hukuhara T Jr, Goto K, Takano K, Hattanmaru Y. Localization and functional organization of bulbar and pontine respiratory neurons in the rabbit. Adv Physiol Sci 1981; 10:579–586.
21. Fournier M, Feldman JL. Microinjections of excitatory amino acids into the region of the Kölliker-Fuse nucleus alter phrenic motoneuronal activity in the rat (abstr). Soc Neurosci 1988; 14:183.
22. Holmes HR, Herbert H, Saper CB. Parabrachial subnuclei in respiratory control (abstr). Soc Neurosci 1987; 13:1586.
23. Wang W, Fung M-L, St. John WM. Pontile regulation of ventilatory activity in the adult rat. J Appl Physiol 1993; 74:2801–2811.
24. Guyenet PG, Koshiya N, Huangfu D, et al. Central respiratory control of A5 and A6 pontine noradrenergic neurons. Am J Physiol 1993; 264:R1035–R1044.
25. Herbert H, Moga MM, Saper CB. Connections of the parabrachial nucleus with the nucleus of the solitary tract and medullary reticular formation in the rat. J Comp Neurol 1990; 293:540–580.
26. Otake K, Ezure K, Lipski J, Wong She RB. Projections from the commissural subnucleus of the nucleus of the solitary tract: An anterograde tracing study in the cat. J Comp Neurol 1992; 324:365–378.
27. Connelly CA, Otto-Smith MR, Feldman JL. Blockade of NMDA receptor-channels by MK-801 alters breathing in adult rats. Brain Res 1992; 596:99–110.
28. Di-Pasquale E, Morin D, Monteau R, Hilaire G. Serotonergic modulation of the respiratory rhythm generator at birth: An in vitro study in the rat. Neurosci Lett 1992; 143:91–95.
29. Hilaire G, Monteau R, Errchidi S. Possible modulation of the medullary respiratory rhythm generator by the noradrenergic A5 area: An in vitro study in the newborn rat. Brain Res 1989; 485:325–332.

30. Johnston BM, Gluckman PD. Lateral pontine lesions affect central chemosensitivity in unanesthetized fetal lambs. J Appl Physiol 1989; 67:1113–1118.
31. Smith JC, Ellenberger H, Ballanyi K, et al. Pre-Bötzinger complex: A brainstem region that may generate respiratory rhythm in mammals. Science 1991; 254:726–729.