

# The Ventral Medullary Network and Airway Control

**MUSA A. HAXHIU and  
NEIL S. CHERNIACK**

School of Medicine  
Case Western Reserve University  
Cleveland, Ohio

**ARTHUR D. LOEWY**

Washington University School of Medicine  
St. Louis, Missouri

## I. Introduction

An adequate gas exchange in the lungs and sufficient supply of O<sub>2</sub> to tissues depends on the patency of the airways, as they serve as a conduit for the tidal movement of gases entering and leaving the lung.

The airways, extending from nares to alveoli, have a complex geometry. Their size and resistance to airflow may be influenced by numerous factors, including changes in neural drive to the upper airway dilating muscles, airway vasculature, and airway smooth muscle.

The chemosensitive elements located in the vicinity of the ventral medullary surface play a critical role in the regulation of breathing and blood pressure (1–7). Neurons localized in this chemosensitive region are also involved in the control of airway size and in coordination of airway, ventilatory, and cardiovascular defense responses (8–13). This article summarizes the findings of our studies on the importance of the structures near the ventrolateral medullary surface in the regulation of airway geometry and discusses the neuroanatomical and functional findings of neuronal circuitry involved in the control of parasympathetic outflow to the airways.

## II. The Role of Ventrolateral Medulla in Regulation of Nasal Patency

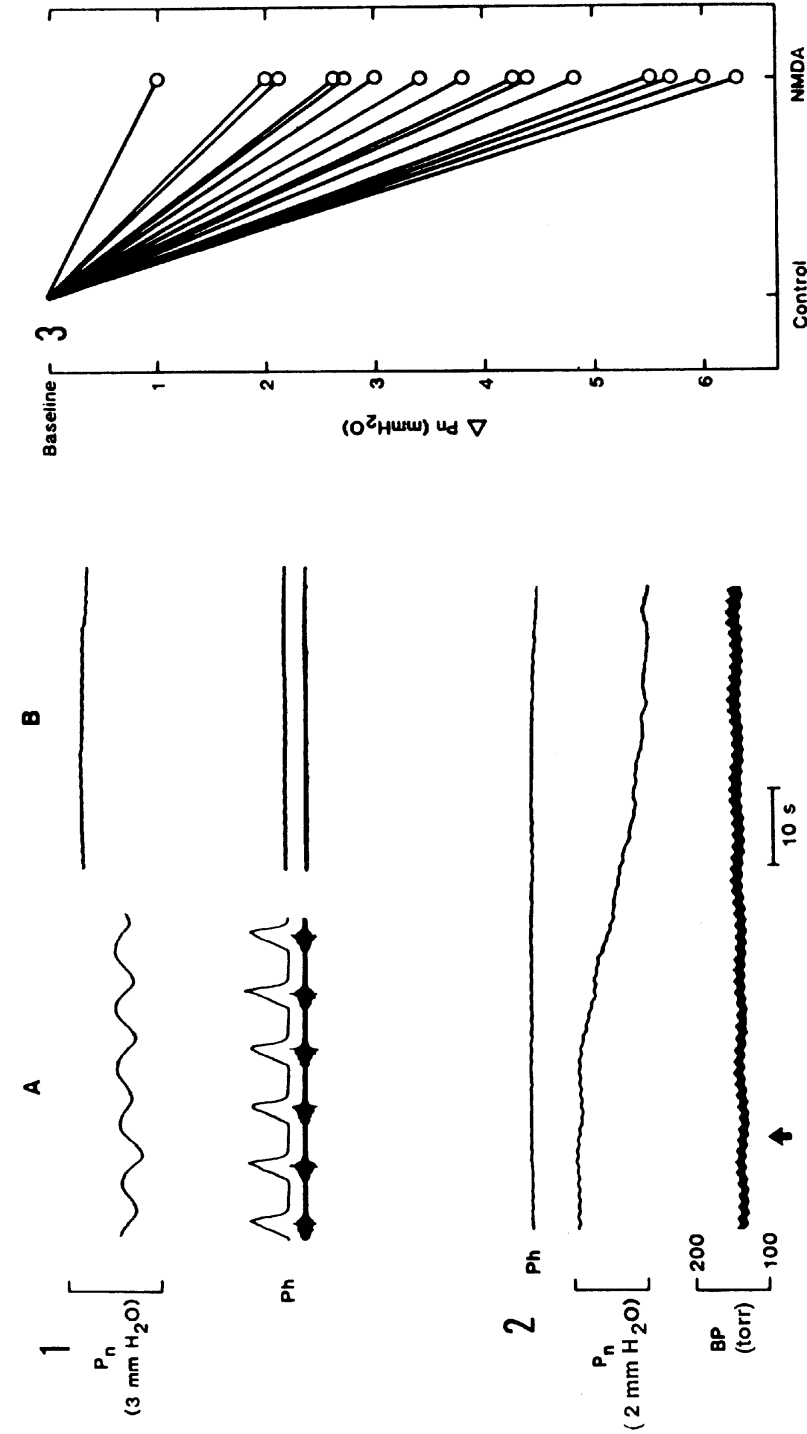
The volume of blood in the mucosal vascular bed largely determines nasal caliber. This blood volume fluctuates synchronously with breathing and is predominantly regulated by the sympathetic nervous system (14–16). The nasal vasculature also has nerve supplies, cholinergic and noncholinergic; but the significance of these innervations in the regulation of nasal resistance is not clear (17,18).

In anesthetized cats, we examined whether structures located near the surface of ventrolateral medulla are involved in the control of nasal patency and in coordination of changes in nasal caliber with changes in respiratory activity. The influences of skeletal muscle activation on the size of nasal passages were eliminated by paralyzing the animals. Changes in the pressure across the nose at a constant flow were used to indicate modulations in nasal caliber (for details, see Refs. 19 and 20).

In the first series of seven experiments in cats, we examined the importance of the ventrolateral medullary surface in the response of nasal resistance to elevated  $\text{CO}_2$ . When the animal was ventilated with 100%  $\text{O}_2$  at end-tidal  $\text{PCO}_2$ , close to the level found during spontaneous breathing (end-tidal  $\text{PCO}_2 = 35$  to 40 torr), phasic oscillations in nasal resistance could be observed. Hyperventilation with 100%  $\text{O}_2$  to apnea increased nasal resistance and abolished respiratory-related changes in nasal pressure (Fig. 1, panel 1). Ventilating the lungs with 6%  $\text{CO}_2$  and 94%  $\text{O}_2$  resulted in the reappearance of phrenic nerve activity associated with a concomitant fall in nasal pressure. The nasal pressure, on average, decreased by 31% (range, 20% to 61%). Prior administration of lidocaine to the intermediate area of the ventral surface of medulla blocked the  $\text{CO}_2$ -induced nasal pressure changes, indicating that the nasal vasculature response to hypercapnia is mediated through the ventrolateral medullary structures (19).

In the second series of 15 experiments, we assessed the importance of the ventrolateral medulla in the regulation of nasal patency by topical application of *N*-methyl-D-aspartic acid (NMDA), a synthetic excitatory amino acid that acts on soma-dendritic membranes (20). In all animals studied, application of NMDA to the intermediate area of the ventral surface, while the cat was ventilated with 100%  $\text{O}_2$  at rate sufficient to produce neural apnea, induced the appearance of phrenic nerve activity and a decrease in nasal pressure. Lower concentrations of NMDA often caused a decrease in nasal pressure without affecting phrenic nerve activity (Fig. 1, panel 2). Individual data for the effects of NMDA applied to intermediate area of the ventrolateral medullary surface (VMS) are presented in Figure 1, panel 3.

The response to NMDA could be diminished or abolished by the application to the ventral medullary surface of the NMDA antagonist 2-amino-5-



phosphonovalerate (2-APV) or the local anesthetic lidocaine. Carotid sinus denervation and posthypothalamic decerebration did not alter the nasal and phrenic nerve responses to NMDA; however, cervical sympathetic denervation decreased but did not abolish these responses, both in intact and in bilaterally adrenalectomized animals. These data suggest that pathways other than the sympathetic participate in the changes in nasal resistance induced by activation of neurons located within the intermediate area.

In a third series of seven experiments, we examined the role of cholinergic agents in the regulation of nasal patency by topical application of nicotine on the intermediate area of the ventral medullary surface. In six of these animals, studies were performed before and after application of lidocaine to the field. Application of nicotine resulted in a 62% fall in nasal resistance (range = 42% to 59%). After lidocaine administration, the same dose of nicotine caused only a 4% decrease (range 0% to 10%) in nasal pressure. However, application of hexamethonium increased nasal pressure by 42% (19). The relevance of these findings will be discussed in relation to changes induced by interventions to the VMS on the other segments of the airways.

### III. A Role for the Ventral Surface of the Medulla (VMS) in Regulation of Pharyngeal Patency

In the obstructive sleep apnea syndrome, the pharyngeal passage seems to be the predominant site of airway closure (21-24). During inspiration, this compliant structure is subjected to negative intra-airway pressure. Alterations in its caliber may result from differences in the activity of upper airway dilating and chest wall pumping muscles.

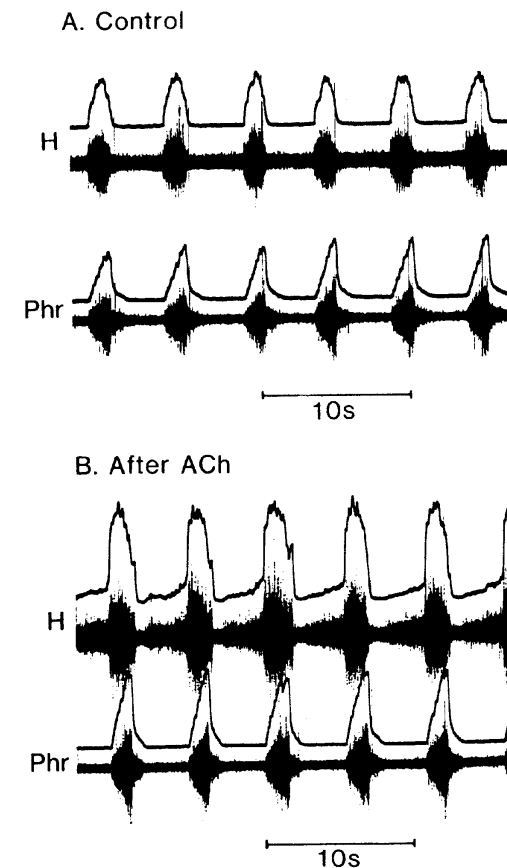
Motoneurons of the hypoglossal nerve that innervate the genioglossus muscle exhibit phasic discharge coincident with breathing (25,26). While the phrenic nerve activity of anesthetized or awake unsedated cats typically rises steadily during inspiration and then declines abruptly, the activity pattern of the hypoglossal nerve is characterized by a rapid rise, peaking in early inspiration, followed by a plateau or downward slope during the remainder of inspiration.

The early peaking pattern of hypoglossal nerve activity, which closely resembles the pattern of inspiratory airflow, may serve to dilate or stiffen the upper airways before they are subjected to the greatest transmural forces produced by inspiratory contraction of the diaphragm. Changes in this pattern or any other change that produces an imbalance in the activities of upper airway dilating and chest wall pumping muscles may induce the occurrence of obstructive apneas during sleep.

The pattern of electrical activity of the hypoglossal nerve or the genioglossus muscle that it innervates is shaped by vagally mediated inhibition, which preferentially depresses the activity of hypoglossal motoneurons in midinspiration. Thus, after vagotomy or when lung inflation is prevented, the pattern of

hypoglossal motoneuron discharge changes and reaches maximum near the end of inspiration. In addition to pulmonary stretch receptors, other afferent inputs (i.e., from the central chemosensory system) may also modulate within-breath discharge patterns of upper airway dilating muscles (like the genioglossus) and of cranial nerves (like the hypoglossus).

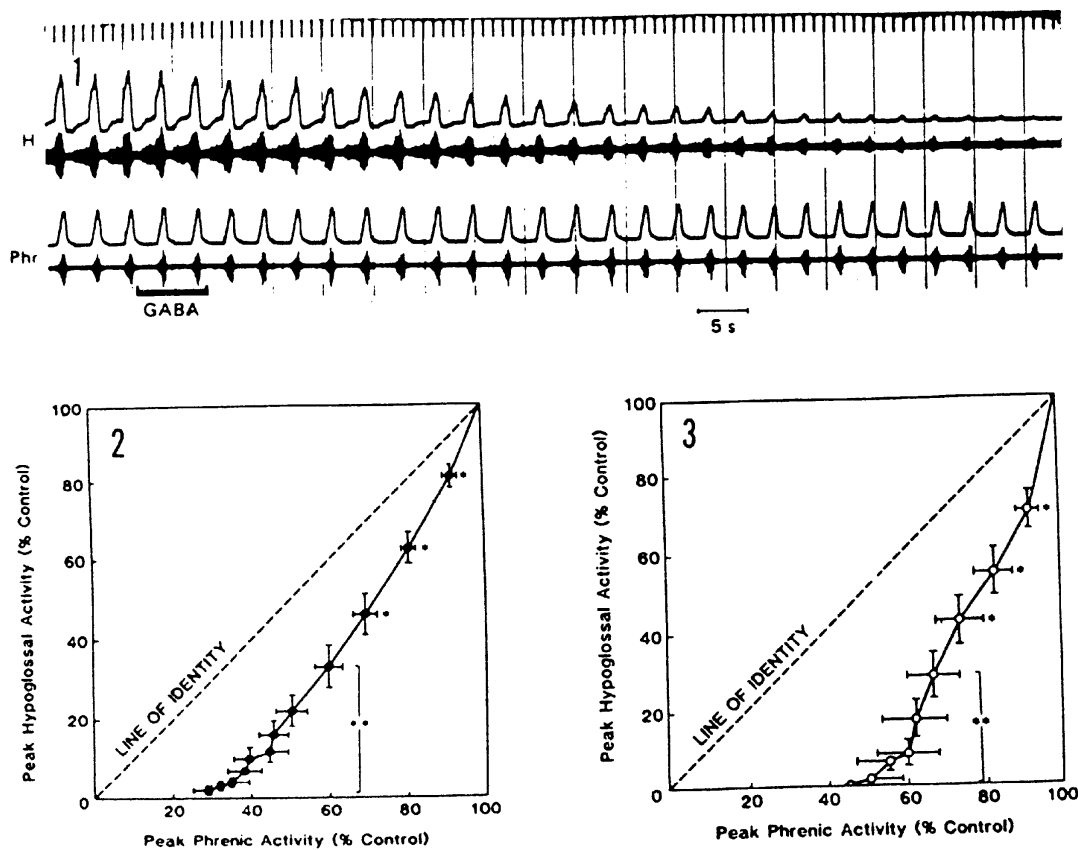
In cats, we investigated the effects of changes in central respiratory drive produced by activation of central cholinergic and GABAergic neurons located in the vicinity of the ventrolateral medulla (26-29) on the phasic pattern and the activity of the hypoglossal and phrenic nerves after vagotomy, eliminating lung mechanoreceptor influences. Experiments were performed in anesthetized, paralyzed, vagotomized, and artificially ventilated animals. The effects of cholinergic agents (acetylcholine, carbachol, methacholine, physostigmine) were



**Figure 2** The effect of acetylcholine (ACh) applied to intermediate area of the VMS on hypoglossal (H) and phrenic nerve (Phr) activity in an anesthetized, paralyzed, and vagotomized cat artificially ventilated with 7% CO<sub>2</sub> in O<sub>2</sub>. (A) Control period; (B) after application of ACh. (From Ref. 28.)

examined in 19 animals. All the cholinergic agents applied to the intermediate area increased tonic and phasic hypoglossal activity significantly. Atropine applied topically to the same medullary areas blocked the respiratory effects of locally administered acetylcholine. In addition, after the administration of cholinergic agents, hypoglossal nerve discharge began earlier and peaked sooner than did phrenic nerve activity. The maximal amplitude of hypoglossal activity was attained while phrenic nerve discharge was still augmenting, shown in Figure 2. The effects of the drugs were relatively greater on hypoglossal nerve activity than on phrenic discharge. Similar effects were obtained by administration of nicotine.

On the other hand, cooling of the intermediate areas of the VMS and topical application of GABA<sub>A</sub> receptor agonists (GABA, muscimol) or benzodiaze-



**Figure 3** Panel 1: An example of the effect of GABA on Phr and H activities. Panels 2 and 3: relationships between mean changes of phrenic and hypoglossal nerve activities induced by local cooling (20°C) (panel 2) and by topical application of GABA<sub>A</sub> receptor agonists (GABA and muscimol) on intermediate area of VMS (panel 3). Data are expressed as percent of control ( $\bar{X} \pm \text{SEM}$ ; \*;  $P < 0.05$ ; \*\*;  $P < 0.001$ ). (Modified from Ref. 29.)

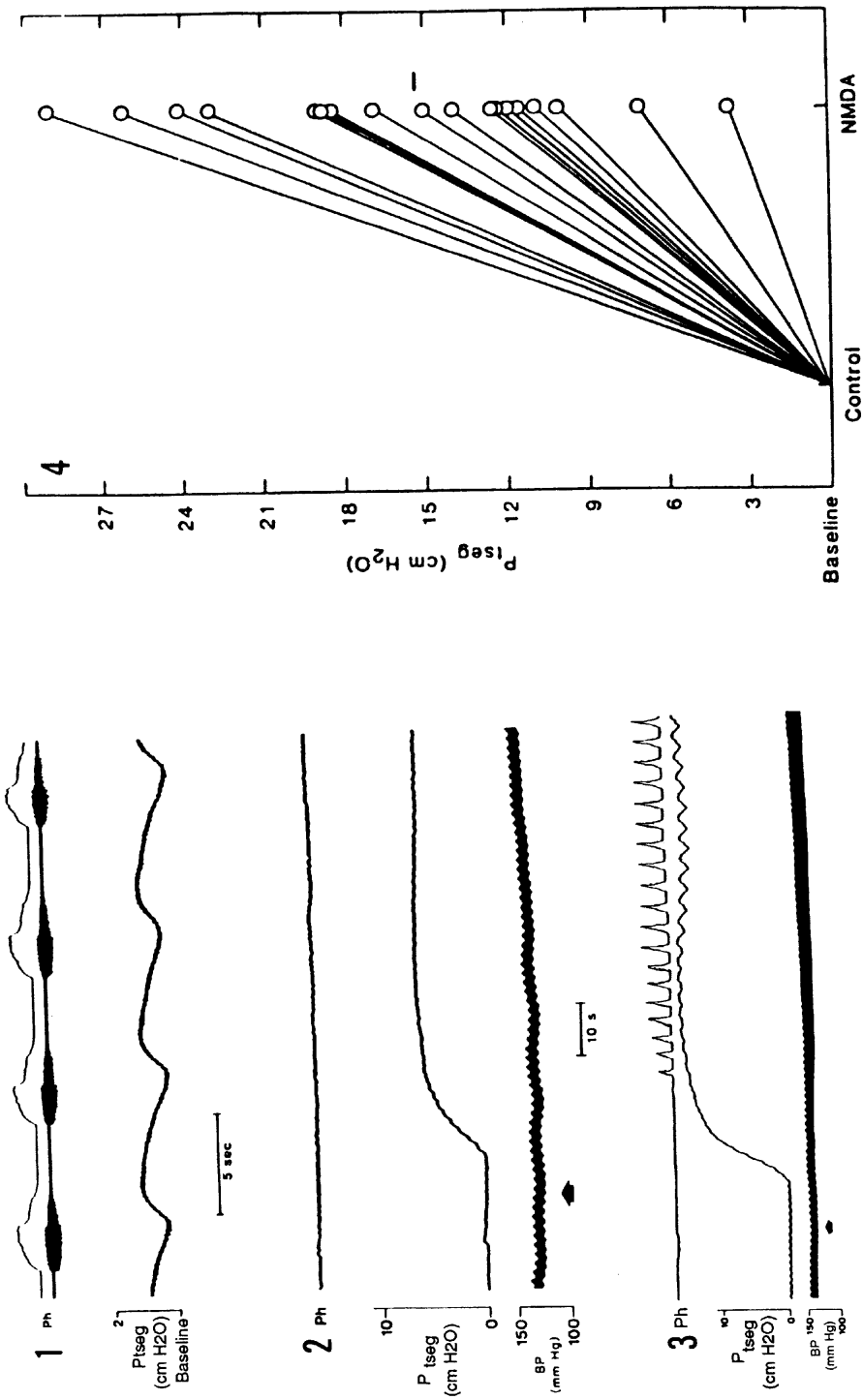
lines depressed respiratory activity. The depressive effects were more pronounced on hypoglossal than on phrenic nerve discharge (Fig. 3). Furthermore, the reduction in hypoglossal nerve activity was associated with a change in the pattern of hypoglossal nerve discharge, which often had its onset later than that of the phrenic nerve and ended near the end of phrenic inspiratory activity.

In addition, the effect of neurons near the ventral medullary surface on respiratory reflex responses to pulmonary C-fiber stimulation was studied. In eight experiments, activation of ventral medullary neurons close to the surface and just lateral to the pyramidal tract by topical application of NMDA significantly increased phrenic activity; NMDA application decreased the duration of apnea induced by administration of phenyldiguinaide or capsaicin into the right atrium ( $14.6 \pm 2.65$ ;  $4.1 \pm 1.75$ ;  $P < 0.05$ ). Likewise, cooling the intermediate area to 20°C markedly prolonged the capsaicin- or phenyldiguinaide-induced hypoglossal nerve apnea compared to the phrenic nerve activity. Cooling the caudal areas had less effect, while cooling the rostral area had the least effect. Cooling of the intermediate area to 15°C had no effect on the increase in laryngeal constrictor activity of recurrent laryngeal nerve produced by capsaicin, which occurred during phrenic apnea (30).

#### IV. The Ventral Medullary Network and Control of Bronchial Caliber

Airway smooth muscle tone can be modulated centrally by the parasympathetic outflow generated within the medulla oblongata. In part these changes in airway caliber seem to be related to changes in respiratory activity (31). Since structures near the VMS can produce profound effects on respiration, it seems reasonable to believe that they also might be capable of modifying airway smooth muscle tone. In the first series of experiments, we examined the effects of agents applied to VMS on tracheal tone: NMDA and nicotine, which stimulate respiratory activity, and gamma aminobutyric acid (GABA), which depresses respiratory activity. In chloralose-anesthetized, paralyzed, artificially ventilated cats, airway smooth muscle tone was assessed by measuring pressure changes in a rostral bypassed segment of the trachea, while phrenic nerve activity was monitored simultaneously (10).

Phasic oscillations in tracheal segment pressure were recorded at an end-tidal CO<sub>2</sub> pressure close to the level found during spontaneous breathing (PET<sub>CO2</sub> = 35 to 40 torr). Usually tracheal pressure began to increase near the end of inspiration, reached a peak in early expiration, and started to decrease when postinspiratory activity of the phrenic nerve disappeared (Fig. 4, panel 1). The magnitude of the oscillations varied from cat to cat and was inversely related to phrenic nerve frequency. Hypercapnia increased peak phrenic activity and mean tracheal pressure. Hypocapnia induced by hyperventilation of the cat with O<sub>2</sub> decreased phrenic nerve activity and tracheal tone and abolished the



**Figure 4** Panel 1: Tracheal pressure (P<sub>tseg</sub>) and phrenic nerve activity (Phr) in paralyzed, artificially ventilated cat at end-tidal CO<sub>2</sub> above apneic point. Panels 2 and 3: Responses to local application of lower (panel 2) and higher (panel 3) concentration of NMDA in a cat ventilated with 100% O<sub>2</sub> to neural apnea. Panel 4: individual data of all studied cats. 1: NMDA application; BP: arterial blood pressure. (Modified from Ref. 33.)

phasic oscillations in the pressure of the tracheal segment; cooling the VMS caused similar changes. Vagotomy reduced both mean tracheal tone and abolished the oscillations in tracheal pressure.

Application of pledgets containing NMDA produced tracheal constriction without the appearance of phasic phrenic nerve activity (Fig. 4, panel 2) (33). Higher concentrations raised tracheal tone and reactivated phrenic nerve activity (Fig. 4, panel 3). Application to the entire VMS either 2-APV, (10<sup>-6</sup> mol) or the local anesthetic lidocaine (2%) 60 s before the application of pledgets containing NMDA prevented the increase in tracheal tone and the elevation of phrenic activity. Intravenous administration of a cholinergic antagonist, atropine methyl nitrate (0.5 mg/kg), blocked tracheal responses to NMDA but did not affect the increase in phasic phrenic nerve activity. These findings suggest that stimulation of neurons near the surface of the VMS within the intermediate area increases the parasympathetic outflow to the airways.

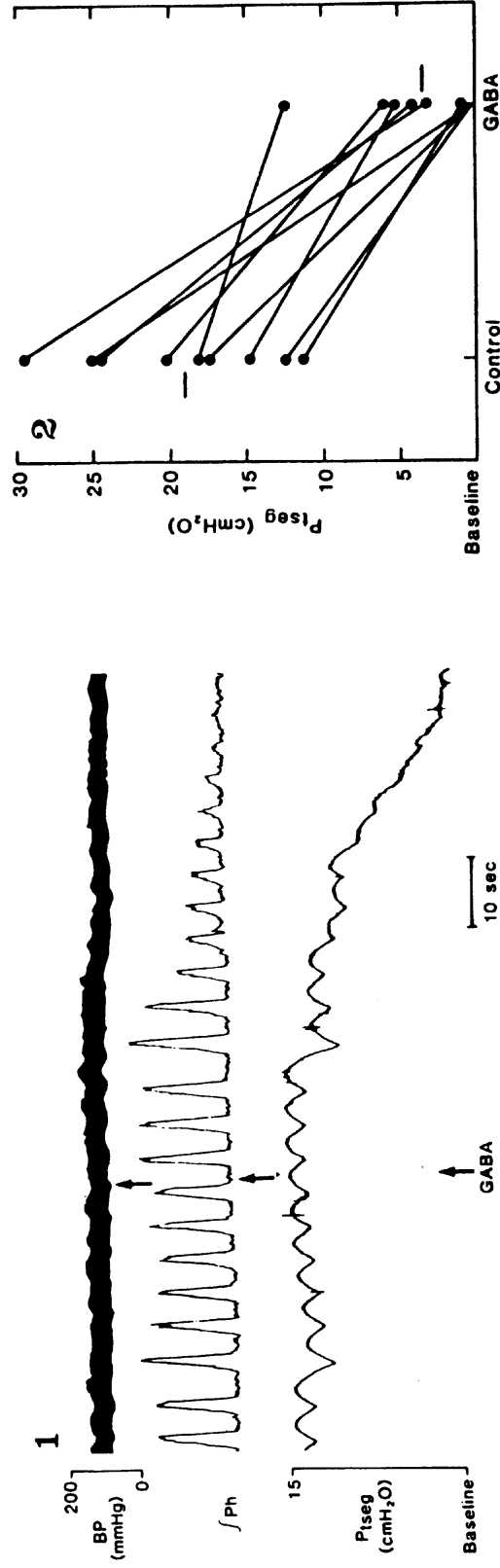
The responses to nicotine applied topically on the intermediate area in doses of 2 × 10<sup>-8</sup> to 2 × 10<sup>-7</sup> mol were determined in 11 artificially ventilated cats. Nicotine, like NMDA, produced an increase in the tracheal segment pressure and a reappearance of phrenic nerve activity (Fig. 5). It should be noted that the increase in tracheal smooth muscle tone seemed to precede the increase in phrenic nerve activity.

The tracheal constrictor response to medullary application of nicotine was abolished by local administration of 2% lidocaine to the VMS even when the carotid sinus nerve was intact. Furthermore, in one cat, focal cooling of the intermediate area to 20°C completely abolished the effect of nicotine applied to the caudal area, while rewarming to 37°C restored the increased tracheal tone. In addition, the prior application to the VMS of the nicotinic antagonist hexamethonium bromide, performed in two cats, eliminated the tracheal response to topically administered nicotine.

The effect of GABA on tracheal pressure was also studied in nine animals. When GABA was applied on the VMS of anesthetized cats ventilated with 7% CO<sub>2</sub> in O<sub>2</sub>, tracheal segment pressure fell (Fig. 5, panel 1) so that, on the average, it was only 4 ± 1 cm H<sub>2</sub>O greater than its baseline level during O<sub>2</sub> ventilation (Fig. 5, panel 2). Phrenic nerve activity decreased at the same time. The decrease in tracheal pressure and phrenic nerve activity were both statistically significant (*P* < 0.01). These effects of GABA on tracheal pressure were blocked by the prior application of bicuculline methiodide in four cats studied. Similarly, administration of benzodiazepines and cooling of the intermediate area of the VMS diminished the response to elevated CO<sub>2</sub> (8,32).

## V. Influences of the VMS on Airway Responses to Stimulation of Peripheral Chemoreceptors

The intermediate area of the VMS may integrate central chemoreceptor information with that from peripheral chemoreceptors (34).



**Figure 5** An example (panel 1) and single data (panel 2) of the effects of GABA topically applied to the intermediate area of the VMS on tracheal pressure (Ptseg), phrenic nerve activity (Phr), and arterial blood pressure (BP) in paralyzed and artificially ventilated cats with 7% CO<sub>2</sub> in O<sub>2</sub> (panel 2). 1: GABA application. (Modified from Ref. 10.)

In anesthetized animals, carotid stimulation results in airway constriction which is abolished by vagotomy (35,36). In series of experiments, we determined the influence of the intermediate area of the VMS on respiratory and airway responses to carotid body stimulation by hypoxia (11). Anesthetized, paralyzed, and artificially ventilated cats were examined to minimize the reflex effects of changes in pulmonary mechanoreceptor activity and of PCO<sub>2</sub> on tracheal tone. Respiratory activity was assessed from the moving averaged phrenic electroneurogram, and tracheal constriction (as measured with a saline-filled balloon placed in the rostral cervical trachea) was used as an index of airway responses.

Anesthetized, paralyzed cats were hyperventilated with 100% oxygen to produce phrenic neural apnea. Switching the inspired gas from 100% O<sub>2</sub> to 12% O<sub>2</sub> increased tracheal pressure in 11 of 12 cats but caused phrenic activity to reappear in only 6 of the animals. Ventilation with 6% O<sub>2</sub> increased tracheal tone significantly prior to the appearance of phrenic activity. Neither the tracheal pressure nor the phasic phrenic electroneurogram responded to hypoxia after the carotid sinus nerves were cut. When the intermediate area of the VMS was cooled to 20°C prior to ventilation with the hypoxic gases, both tracheal and phrenic responses were significantly diminished. Both returned to the same levels after rewarming. Cooling of the intermediate area also significantly diminished the increase in tracheal pressure and phrenic nerve activity caused by 6% O<sub>2</sub>. Cooling of the intermediate area blunted tracheal and phrenic responses to carotid body stimulation by sodium cyanide (NaCN).

These results support the idea that the intermediate area modulates the parasympathetic reflex responses of the trachea (8,9). The pathways through which the intermediate area modulates the respiratory response to carotid body stimulation is not entirely clear. Schläpke et al. (1979) found that coagulation of the intermediate area reduced the ventilatory response to hypoxia by 50% in spontaneously breathing animals (5). Millhorn et al. (1982) also studied the effect of carotid sinus nerve (CSN) stimulation at various intermediate area temperatures. When baseline phrenic activity was held constant by varying the CO<sub>2</sub> at different intermediate area temperatures, the effect of CSN stimulation on minute phrenic activity was reduced by 50% when the intermediate area was cooled to 20°C. They concluded that the intermediate area is part of a common pathway from both central and peripheral chemoreceptors and that it is involved in the integration of both inputs (34).

From the results of these studies, we cannot state conclusively whether cooling of the intermediate area removed an excitatory effect of the central chemoreceptors on phrenic nerve discharge even when the CO<sub>2</sub> level was below the apneic threshold. There is a possibility that tonically active neurons in the intermediate area project to both the respiratory controller and to the airway-related preganglionic vagal neurons and, in turn, that respiratory related neurons modulate parasympathetic outflow to the airways.

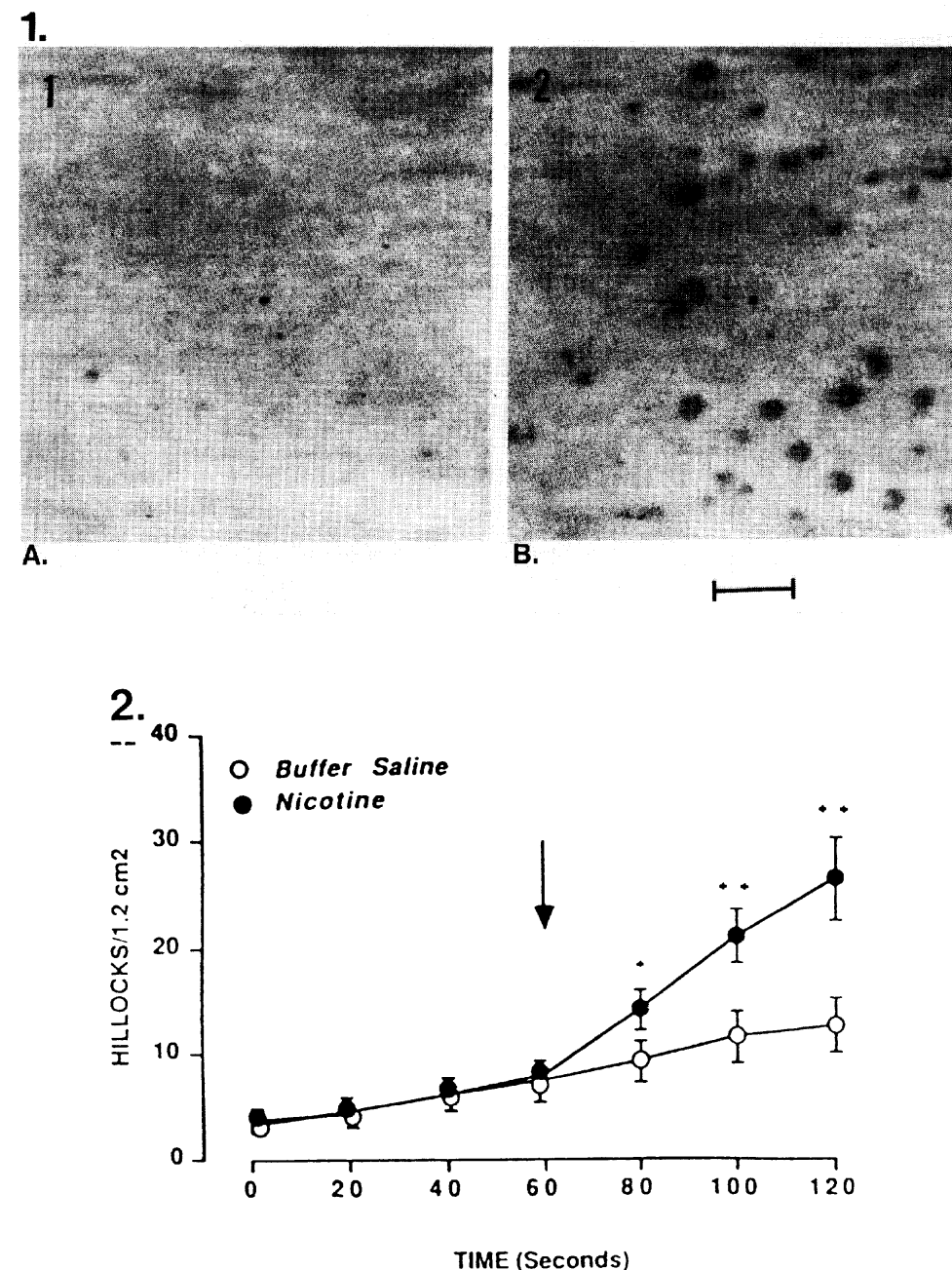
## VI. Influence of the VMS in Tracheal Gland Secretion

Many stimuli that affect smooth muscle tone also influence airway submucosal gland function. For example, changes in airway tone induced by hypoxia or reflexly initiated by stimulation of sensory nerve endings are associated with an increase in fluid secretion from tracheal submucosal glands (37-40). Because structures near the VMS can produce profound effects on cholinergic outflow to the airway smooth muscle, it seems reasonable that they also might be capable of modifying tracheal secretions. Therefore, in another study, we evaluated the possibility that structures near the VMS may influence secretion of fluid from tracheal submucosal glands (41,42).

We examined the changes in the number of hillocks of secretion appearing from submucosal glands in an exposed field of tracheal epithelium (1.2 cm<sup>2</sup>) coated with tantalum dust before and after interventions on the VMS. Experiments were performed in  $\alpha$ -chloralose-anesthetized dogs paralyzed and ventilated with 40% O<sub>2</sub>. Stimulation of nicotinic receptors by application of a pledget containing nicotine in 11 dogs caused a significant elevation in tracheal gland secretion in the subsequent 60 s, compared with a control period in which buffered saline was applied (Fig. 6). Prior application of lidocaine or hexamethonium bromide to the VMS blocked the effect of topically applied nicotine. The central effects of nicotine were diminished by atropine methyl-nitrate given intravenously. In addition, lidocaine application to the VMS or focal cooling of intermediate areas to between 20 and 15°C significantly decreased the rate of secretion reflexly produced by capsaicin-induced stimulation of pulmonary C-fiber receptors or by mechanical stimulation of the carina and larynx. These findings suggest that the ventral medulla contains cells near its surface that influence tracheal fluid secretion and modulate reflex responses of airway submucosal glands, probably by altering the level of general excitation within central respiratory integrating circuits.

## VII. Transneuronal Labeling Studies of Ventral Medullary Circuitry Involved in Regulation of Parasympathetic Outflow to the Airways

The pathways connecting ventral medullary neurons with the airway-related vagal preganglionic cell bodies have not been described. This is because most contemporary neuroanatomical methods, due to their inherent properties, cannot provide information regarding the location or chemical nature of the central neurons that innervate specific functional classes of autonomic preganglionic neurons. Recently, we have circumvented this problem by using the viral transneuronal labeling method that utilizes a weakened strain of pseudorabies virus (PRV), a neurotropic herpes virus, to produce controlled infections that



**Figure 6** Panel 1: Video images of the effects of topical application of nicotine to intermediate area of VMS on tracheal submucosal gland secretion. (A) tracheal epithelium at the end of 60 s control period; (B) tracheal epithelium 60 s after topical application of nicotine. Horizontal bar, 1 mm. Panel 2: Average results ( $\bar{X} \pm \text{SEM}$ ) of the effects of nicotine on submucosal gland secretion. (Modified from Ref. 42.)

spread in a hierarchical manner within functionally related sets of neurons (43). Since this virus travels preferentially in a retrograde direction (i.e., from axon terminal to the cell body), it is possible to use it as a cell body marker of hierarchical chains of central nervous system (CNS) neurons that regulate autonomic motor systems. The utility of this method rests on experimental evidence indicating that the Bartha strain of PRV has the properties of a specific retrograde transneuronal marker (44–47).

The viral transneuronal labeling method works in the following manner. An injection of PRV (or other types of herpesvirus) is made in a particular end organ; then it is taken up and transported in a retrograde fashion to the cell bodies of the first-order autonomic preganglionic neurons that innervate this tissue. An active infection occurs within these neurons. Next, the infection spreads to the second-order neurons that synapse on these infected cells. In principle, if enough time is allowed for this infectious process to develop more fully, it is possible to label (i.e., infect) third- (or even higher) level neurons that form a specific CNS motor network. However, in practice, only a few reports have verified that this technique can be used to label neurons beyond those of the second-order level, because as the infection proceeds, there is a high probability that a nonspecific central viremia will develop. The infected neurons are easily visualized with standard immunohistochemical techniques, and this technique has been used to localize the CNS cell groups that innervate a variety of different autonomic and somatic motor outflows.

Since most functional investigations of the airway conducting system have used the trachea as a model system, we decided to study the CNS innervation of this organ. Furthermore, the rationale for selecting this particular airway segment stems from the fact that all the organs that form the tracheobronchial tree originate from a common embryonic structure, the respiratory diverticulum, and thus are likely to retain certain basic similar patterns in both their peripheral and central innervation.

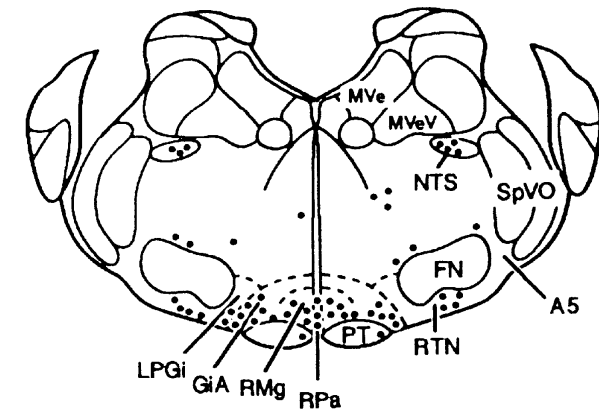
Experiments were performed in C<sub>8</sub>-level spinal rats in order to eliminate the possibility that PRV injected into the tracheal wall entered the CNS via the sympathetic nervous system. The results were compared with the findings of experiments in which cholera toxin  $\beta$ -subunit (CTb) was used as the retrograde marker to determine the location of the parasympathetic preganglionic neurons that innervate the trachea (for details, see Ref. 43).

The results showed that PRV-labeled first-order parasympathetic preganglionic neurons were observed in the same medullary sites as found in the CTb experiments – namely, in a compact part of the nucleus ambiguus, the area ventral to it, and the rostralmost part of medial nucleus tractus solitarius. In the ventral medulla, an extensive rostral caudal network of PRV-labeled neurons extended from the pontomedullary border to the decussation of the pyramidal tract. Within this network, five superficial individual cytoarchitectonic groupings were recognized: the lateral medullary surface, the lateral parapyramidal nucleus, the parapyramidal nucleus, the raphe pallidus, and the retro-

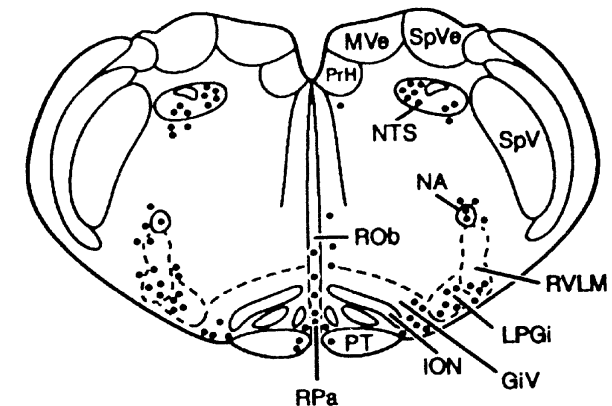
trapezoid nucleus. In addition, two deeper-lying ventral medullary nuclei were labeled as well: the gigantocellular and lateral paragigantocellular reticular nuclei.

Many of these neurons were located literally on or next to the VMS (Fig. 7). This network of PRV-labeled neurons were continuous with a more medial

Bregma -10.80mm



Bregma -11.80mm



**Figure 7** Distribution of cell body labeled in the rat brain following PRV injections into the tracheal wall. A5, A5 catecholamine cell group; DMV, dorsal motor nucleus of vagus F; FN, facial nucleus; GiA, gigantocellular reticular nucleus, alpha part; GiV, gigantocellular reticular nucleus, ventral part; ION, inferior olivary nucleus; LPG, lateral paragigantocellular reticular nucleus; Mdd, medullary reticular nucleus; MVe, medial vestibular nucleus; MVeV, medial vestibular nucleus, ventral part; NA, nucleus ambiguus; NTS, nucleus tractus solitarius; PT, pyramidal tract; ROb, raphe obscurus nucleus; RPa, raphe pallidus nucleus; RTN, retrotrapezoid nucleus; RVLm, rostro-ventrolateral reticular nucleus; SpV, spinal trigeminal nucleus; SpVO, spinal trigeminal nucleus, oral part; XII, hypoglossal nucleus. (Modified from Ref. 44.)

