

The Role of the Ventral Brainstem in Vestibulosympathetic and Vestibulorespiratory Reflexes

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I. Introduction

Vestibular receptors located in the inner ear detect linear and angular acceleration imposed on the head and thus provide signals to the central nervous system that indicate head position and the direction and velocity of head movements (1,2). By combining vestibular inputs with signals from neck receptors, the central nervous system can distinguish head and whole-body movements and thus produce appropriate compensatory reflexes (3,4); examples are vestibuloocular reflexes that assist in maintaining fixed gaze during head rotations and vestibulospinal reflexes that compensate for postural perturbations (1,2).

Disturbances in homeostasis can also occur during movement and changes in posture, and there is evidence to suggest that the vestibular system participates in correcting these perturbations. For example, vestibulosympathetic reflexes are presumably involved in compensating for orthostatic hypotension (5). Vestibular effects on respiratory outflow have also been reported (6-9). Vestibular actions on expiratory and inspiratory motoneurons could assist in adjustments of breathing during changes in posture that place adverse mechanical restraints on respiratory muscles. In addition, such vestibulorespiratory

reflexes could aid in cardiac return during movements that might lead to orthostatic hypotension (10,11) or be involved in the production of movements and the maintenance of postures that require the participation of respiratory muscles (12-14).

This review will first describe evidence indicating that the vestibular system influences both sympathetic and respiratory outflow; it will then discuss the role of the ventral brainstem in producing these effects.

II. Vestibular Influences on Sympathetic and Respiratory Outflow

Electrical stimulation of the vestibular nerve in the cat produces evoked potentials bilaterally in the phrenic, abdominal, intercostal, and sympathetic nerves (5,9). Examples are shown in Figure 1. Such potentials can typically be elicited by short stimulus trains (two or three shocks) at intensities less than half those that produce current spread to the closest nontarget nerve, the facial nerve. Vestibulorespiratory responses in the phrenic nerve usually have a latency of 7 to 11 ms; the latency for the lumbar abdominal nerves is 10 to 18 ms. Furthermore, the amplitudes of the responses are a sizable fraction of the amplitude of ongoing spontaneous respiratory discharges (9). Thus, the vestibular system appears to make a significant contribution to the excitability of respiratory motoneurons via a fairly direct neural pathway. In contrast, vestibulovagal reflexes recorded from the splanchnic nerve have much longer

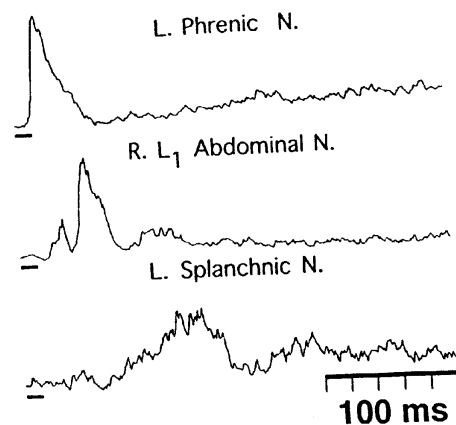


Figure 1 Evoked potentials recorded simultaneously from the left phrenic, right abdominal, and left splanchnic nerves following stimulation of the left vestibular nerve in a decerebrate and paralyzed cat. Fifty-four sweeps were averaged. The potentials were elicited by a train of five shocks (3 ms intershock interval; time of delivery indicated by bar). The amplification is the same for each waveform.

latencies, typically of the order of 60 to 100 ms (5,9). This is not to say, however, that the central processing time for vestibulovagal interactions is long, since the recording site was several centimeters distal to the spinal cord in these studies and the conduction time along the unmyelinated and finely myelinated fibers of the splanchnic nerve is lengthy (15).

Effects on respiratory and sympathetic outflow produced by stimulation of the vestibular nerve can be abolished by making electrolytic or chemical lesions affecting the medial vestibular nucleus or the adjacent parts of the inferior nucleus (9,16-18). Thus, it is clear that the autonomic activity produced by vestibular nerve stimulation is the result of activation of vestibular afferents and is *not* due to stimulus spread to nontarget nerves. Furthermore, it is likely that the medial and/or inferior vestibular nuclei are involved in producing the responses.

Evidence that the vestibular system influences sympathetic outflow also comes from studies employing natural stimulation of the vestibular system. Caloric stimulation of the labyrinth in rabbits has been reported to elicit effects on blood pressure that are abolished by lesions placed in the vestibular nuclei (16,17). Furthermore, Doba and Reis (19) showed that transection of the vestibular nerves in paralyzed, chloralose-anesthetized cats significantly impairs compensation for orthostatic hypotension produced by 30° or 60° head-up tilt, suggesting that the vestibular system is involved in correcting posturally related changes in blood pressure. This finding is supported by our recent experiments in decerebrate cats. We delivered sinusoidal head rotations in multiple vertical planes (in animals with upper cervical dorsal root rhizotomies to remove inputs from neck receptors) and found that maximal sympathetic outflow recorded from the splanchnic nerve was elicited by nose-up pitch, the direction most likely to produce orthostatic hypotension (20). Since the position of the trunk was not altered, it seems unlikely that the sympathetic reflexes elicited by head rotation were due to nonvestibular inputs from such sources as baroreceptors or abdominal receptors.

III. The Role of the Ventral Brainstem in Producing Vestibulo-Sympathetic Reflexes

Many of the bulbospinal neurons in the cat relaying descending signals to sympathetic preganglionic neurons are found in two regions located partly or entirely in the ventral brainstem: the subretrofacial rostral ventrolateral medulla (21-23) and the caudal medullary raphe nuclei (24-26). Over two-thirds of the neurons in these areas with slowly conducting (≤ 10 m/s for the subretrofacial rostral ventrolateral medulla and ≤ 5 m/s for the raphe nuclei) projections to the thoracic spinal cord responded to electrical stimulation of the vestibular nerve (27,28). The responses of one such neuron are illustrated in the left panel of Figure 2; Figure 3A shows the location in the ventral brainstem of slowly

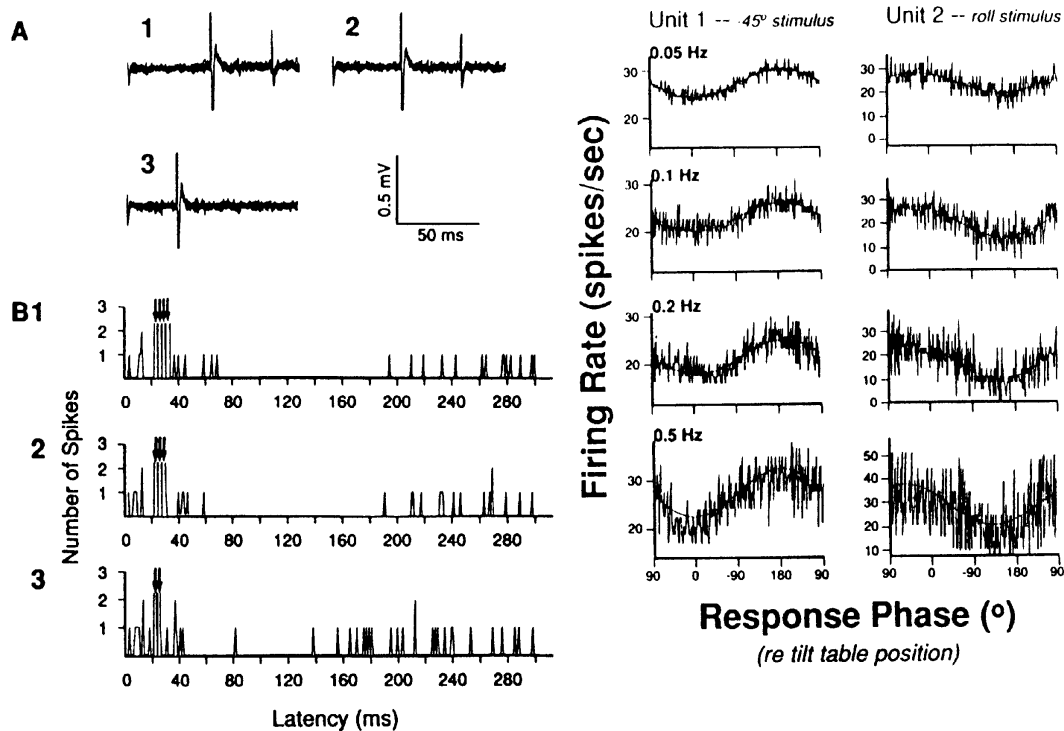


Figure 2 Left panel: Responses of a subretrofacial neuron with a slowly conducting projection to the thoracic spinal cord to stimulation of the vestibular nerve. (A) Collision test showing antidromic driving from the upper thoracic spinal cord. The conduction velocity was 3 m/s. (B) Poststimulus time histograms showing the responses of the same cell to stimulation of the vestibular nerve. Arrows denote the times when stimuli were delivered, and responses to stimulus trains of three different lengths are shown. (From Ref. 27.) Right panel: Histograms showing responses of raphe neurons to sinusoidal natural vestibular stimulation in vertical planes. The traces are averages of binned spike data indicating the response to several frequencies of tilt; the stimulus frequency tested is indicated above each pair of waveforms. Superimposed on each trace is the sinusoid fit to the fundamental of the response. Unit 1 is a raphespinal neuron that responded best to tilt near the plane of the left anterior and right posterior semicircular canals (-45° in our orientation scheme). The stimulus amplitude was 7.5° in all runs. The response phase remained near table position at all frequencies and response gain only increased by a factor of 1.6 as frequency was increased from 0.05 to 0.5 Hz. This behavior is similar to that of otolith afferents. Unit 2 was not tested for a projection to the spinal cord. Like unit 1, it had response phases near table position and almost equivalent response gains across stimulus frequencies and was also classified as mainly having otolith input. (From Ref. 30.)

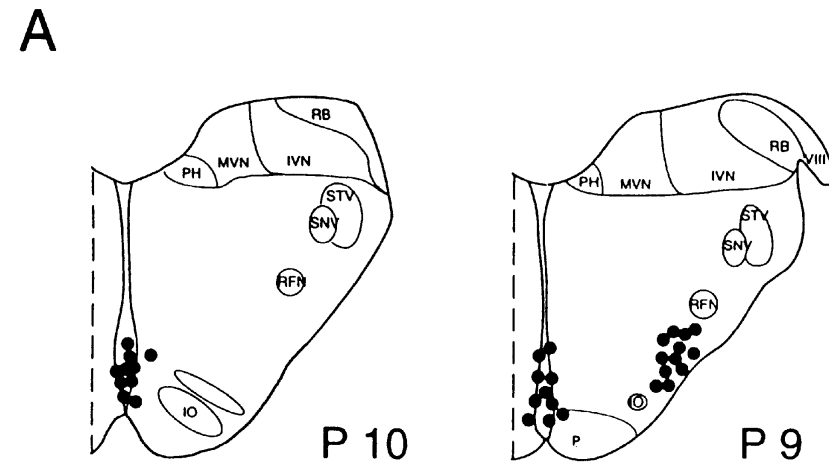


Figure 3 (A) Locations of cells with slowly conducting projections to the thoracic spinal cord that responded to stimulation of the vestibular nerve. The numbers below each drawing indicate the distance (in millimeters) posterior to stereotaxic zero. The laterally located subretrofacial neurons had conduction velocities ≤ 10 m/s, whereas the medially placed raphe neurons had conduction velocities between 1 and 4 m/s. (Data from Refs. 27 and 28.) (B) Neurons that responded to natural vestibular stimulation. The medially located raphe neurons had projections to the spinal cord, whereas the laterally located subretrofacial neurons were not tested for a spinal projection. Filled circles indicate cells that were classified as receiving inputs predominantly from otolith receptors, and open circles denote cells with other inputs (from semicircular canals or convergent inputs from otoliths and canals). (Data from Refs. 29 and 30.) *Abbreviations*: EC, external cuneate nucleus; IO, inferior olivary nucleus; IVN, inferior vestibular nucleus; LVN, lateral vestibular nucleus; MVN, medial vestibular nucleus; P, pyramid; PH, prepositus hypoglossi; RB, restiform body; RFN, retrofacial nucleus; SA, stria acustica; SNV, spinal trigeminal (Vth) nucleus; STV, spinal trigeminal tract; VII, facial nucleus, VIII, vestibulocochlear nerve.

conducting bulbospinal neurons with vestibular input. Most slowly conducting subretrofacial-spinal neurons (21) and many slowly conducting raphespinal neurons (25,26) make direct connections with sympathetic preganglionic neurons; the presence of vestibular inputs to a large fraction of these cells suggests that the vestibular system is likely to have an important role in cardiovascular control.

Subretrofacial and raphespinal neurons were also tested for responses to natural vestibular stimulation. Sinusoidal rotations in multiple vertical planes as well as horizontal rotations were employed in these experiments; the animals used were baroreceptor-denervated, vagotomized, and had a cervical spinal

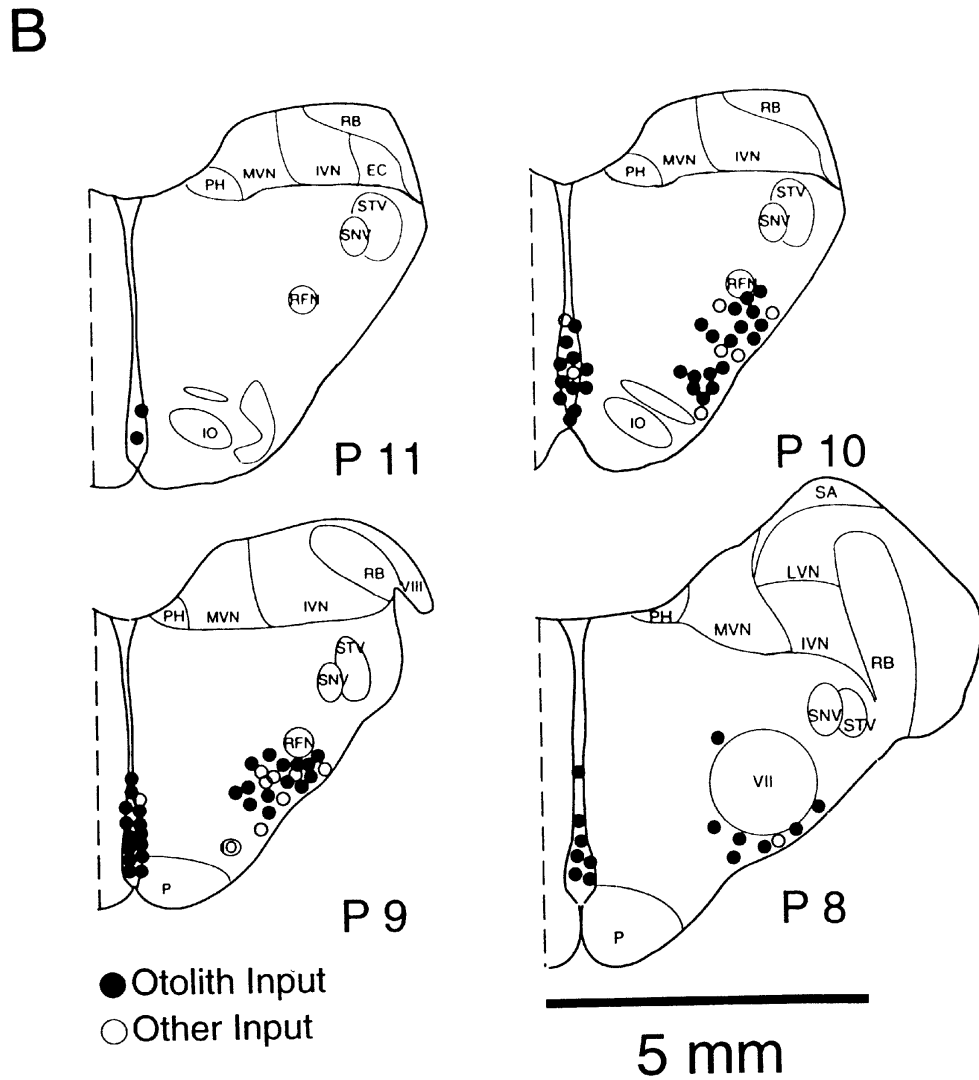


Figure 3 Continued

transection to prevent inputs from tilt-sensitive receptors outside of the labyrinth from influencing the studied neurons. The responses of two raphe neurons to natural vestibular stimulation are shown in the right panel of Figure 2; Figure 3B shows the locations in the ventral brainstem of subretrofacial and raphe neurons that responded to natural vestibular stimulation. The majority of neurons had response properties typical of cells with inputs from otolith organs; only a few had responses similar to those of afferents from semicircular canals (29,30). Figure 4A indicates the types of vertical vestibular in-

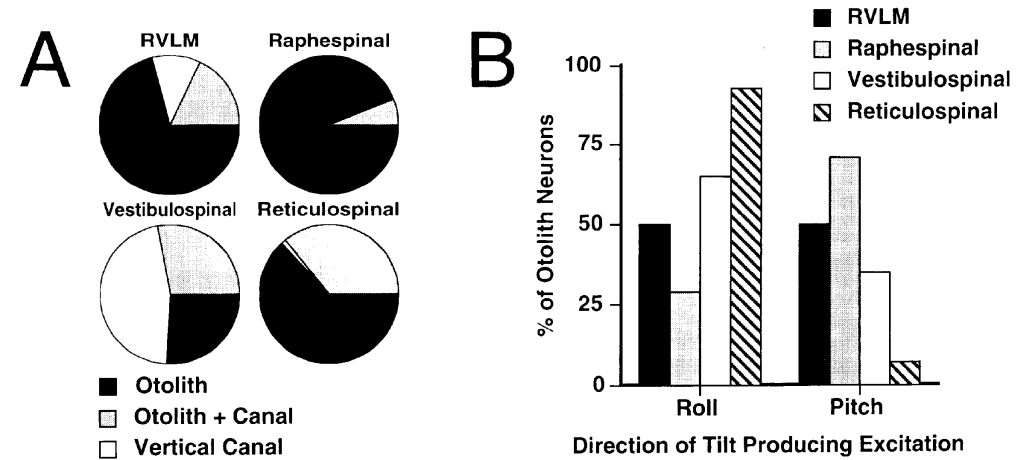


Figure 4 (A) Comparison of the predominant type of vertical vestibular inputs to neurons in the subretrofacial rostral ventrolateral medulla (RVLM), raphespinal neurons, vestibulospinal neurons, and medial pontomedullary reticulospinal neurons. (RVLM data are from Ref. 29, raphespinal data are from Ref. 30, reticulospinal data are from Ref. 31, and vestibulospinal responses represent the pooled data of Refs. 32 and 33.) (B) Direction of tilt producing maximal excitation of subretrofacial RVLM, raphespinal, medial reticulospinal, and vestibulospinal neurons that were classified as receiving predominant otolith input. *Pitch* refers to nose-up or nose-down rotations, whereas *roll* indicates ear-down tilt.

puts (i.e., from the anterior and posterior semicircular canals and otolith organs) to raphespinal neurons and cells in the subretrofacial rostral ventrolateral medulla and, for comparison, shows the types of inputs to vestibulospinal and medial reticulospinal neurons which presumably are involved in motor control. The otolith-dominant pattern of vertical vestibular inputs to the cardio-regulatory areas is shared by medial reticulospinal neurons (31); in contrast, many vestibulospinal neurons receive inputs from the vertical semicircular canals (32,33). Furthermore, raphe and subretrofacial neurons (29, 30) differ from both vestibulospinal and reticulospinal neurons (34-36) in lacking a significant input from the horizontal semicircular canals. The otolith-dominant pattern of vestibular inputs to cardio-regulatory areas in the ventral brainstem is in keeping with the idea that these regions are involved in compensation for posturally related changes in blood pressure. Because otolith receptors respond to linear accelerations, including gravity, they signal static head position; in contrast, semicircular canals are primarily activated during angular head movements and provide information concerning the direction and velocity of those movements (1,2). Compensation for postural changes requires specific information about static body position, and it would be expected that such compensation calls for selective inputs from otolith organs.

Figure 4B shows the direction of vertical tilt producing maximal excitation of subretrofacial, raphespinal, vestibulospinal, and medial reticulospinal neurons classified as receiving predominant otolith input. A large fraction of neurons in cardiorespiratory areas in the ventral brainstem responded best to nose-up or nose-down tilt (pitch). In contrast, vestibulospinal and reticulospinal neurons that mainly had otolith inputs responded best to roll (ear-down tilt). The preference of raphespinal and subretrofacial neurons for activation by pitch rotations is consistent with their presumed involvement in compensation for orthostatic hypotension, which can result from nose-up body rotations in the cat (which has a long longitudinal axis).

The differences in the responses of subretrofacial and raphespinal neurons from those of both reticulospinal and vestibulospinal neurons to natural vestibular stimulation suggest that cardiorespiratory areas in the ventral brainstem receive their vestibular input through specific circuits and not through diffuse connections with the vestibular nuclei or reticular formation. This is further evidence to suggest that vestibular inputs to subretrofacial and raphe neurons serve a particular physiological function and are *not* present just to modulate the background firing rate of cells in the ventral brainstem.

IV. The Roles of the Ventral Brainstem in Producing Vestibulorespiratory Reflexes

The neural underpinnings of vestibular influences on respiration have not been as well investigated as the circuitry producing vestibul sympathetic reflexes. However, recently we tested neurons with inspiratory-related activity that were located in the ventrolateral reticular formation near nucleus ambiguus for their responses to electrical stimulation of the vestibular nerve. The locations of the neurons studied are shown in Figure 5. Only 2/21 cells responded at all to stimulation of the vestibular nerve on either side, and the effects on these two neurons were extremely weak, despite the fact that large evoked potentials were simultaneously recorded from the phrenic nerve. Thus, the ventral respiratory group does not appear to play a substantial role in transmitting vestibular signals to inspiratory motoneurons. Furthermore, the dorsal respiratory group (located in the ventrolateral part of nucleus solitarius) also fails to receive appreciable vestibular input (37), suggesting that neurons besides those in the two major groups that are involved in imparting rhythmic activity on inspiratory motoneurons (the dorsal and ventral respiratory groups) must be responsible for vestibular effects on phrenic outflow. Although the descending pathways responsible for vestibulorespiratory reflexes are yet to be determined, they may include vestibulospinal and/or reticulospinal tracts, which are known to make synapses on motoneurons in the vicinity of respiratory motoneurons (1,2).

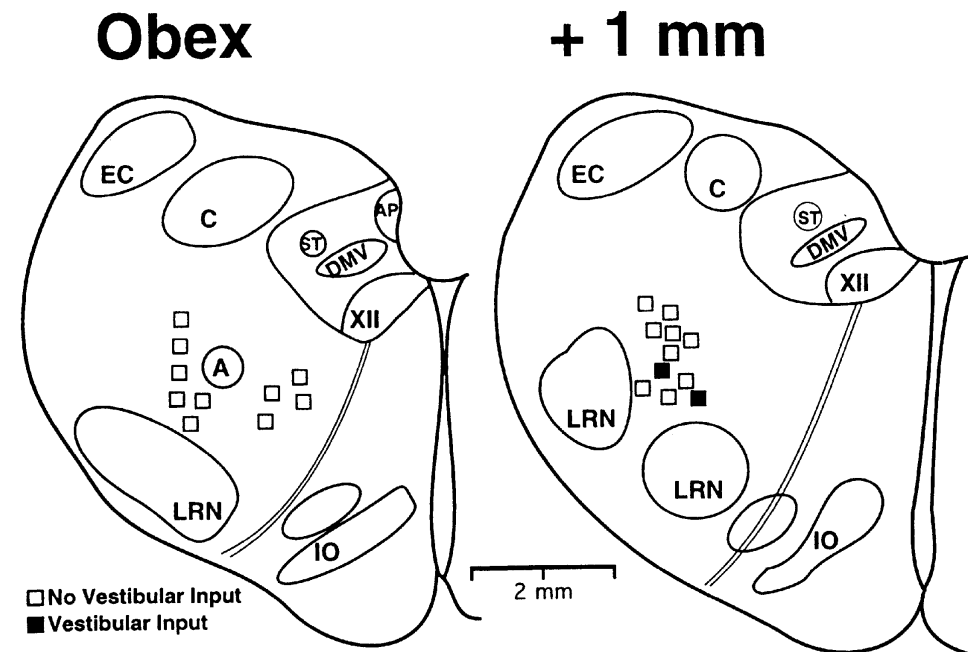


Figure 5 Locations of neurons with inspiratory-related activity that were tested for responses to vestibular nerve stimulation. *Abbreviations:* A, nucleus ambiguus; AP, area postrema; C, cuneate nucleus; DMV, dorsal motor nucleus of the vagus; EC, external cuneate nucleus; IO, inferior olivary nucleus; LRN, lateral reticular nucleus; ST, solitary tract; XII, hypoglossal nucleus.

V. Summary

The vestibular system influences both sympathetic and respiratory outflow. Two regions of the cat ventral brainstem, the subretrofacial rostral ventrolateral medulla and ventral portions of the caudal medullary raphe nuclei, appear to be involved with relaying vestibular signals to sympathetic preganglionic neurons. These areas receive their vestibular inputs mainly from otolith organs, and many ventral brainstem neurons respond better to nose-up or nose-down rotations (pitch) than to ear-down rotations (roll). Thus, the responses of neurons in ventral medullary cardiorespiratory areas to natural vestibular stimulation are consistent with their participation in compensation for orthostatic hypotension. In contrast, ventrally located neurons near nucleus ambiguus with inspiratory-related activity receive little vestibular input, suggesting that other neuronal groups must be involved in generating vestibulorespiratory reflexes.

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The Role of the Caudal Ventrolateral Medulla in the Reflex Bronchodilation Arising from the Hindlimb

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I. Introduction

The caudal ventrolateral medulla plays an important role in mediating the reflex airway dilation evoked by stimulation of thin fiber afferents arising from the hindlimb of anesthetized dogs. This paper focuses on the central neural pathways and neurotransmitters that are likely to play a role in causing the reflex airway dilation arising from the hindlimb. Particular attention has been paid to two areas. The first is the dorsal horn, the site of the first synapse in the reflex arc causing these autonomic effects. The second is the caudal ventrolateral medulla, whose participation in this reflex arc also appears to be vital.

II. Reflex Responses Arising from Hindlimb Afferents

Static contraction of the hindlimb muscles of anesthetized cats and dogs has been shown to increase arterial pressure, heart rate, and ventilation (1-3). In cats, McCloskey and Mitchell (2) demonstrated that these contraction-induced cardiovascular and ventilatory responses resulted from the activation of group

III and IV muscle afferents but not from the activation of group I and II afferents. Similarly, Tibes (4) found that the cardiovascular and ventilatory responses to dynamic contraction of the hindlimb muscles of anesthetized dogs were due to stimulation of group III and IV afferents.

Contraction of the hindlimb muscles in both anesthetized cats and dogs has also been shown to dilate the airways. Longhurst (5) demonstrated that static contraction of the hindlimb muscles of anesthetized cats reflexly relaxes tracheal smooth muscle. Similarly, Kaufman and Rybicki (6) showed that both static and repetitive twitch contractions of the gracilis muscles of anesthetized dogs reflexly decreased tracheal smooth muscle tone. Kaufman et al. (7) extended their previous findings to include total lung resistance as a functional index of airway caliber by demonstrating that both types of contractions also reflexly decreased total lung resistance. The reflex decreases in total lung resistance evoked by contraction were unaffected by propranolol but were blocked by atropine, suggesting that the contraction induced airway dilation resulted from the withdrawal of a tonic cholinergic outflow to airway smooth muscle.

The afferent arm of this reflex arc is believed to comprise group III and IV muscle afferents but not group I and II afferents. Stimulation of group I and II afferents with succinylcholine has been shown to have no effect on tracheal smooth muscle tone, whereas stimulation of group III and IV afferents with capsaicin has been shown to reflexly decrease tracheal smooth muscle tone (8,9). Rybicki and Kaufman (10) demonstrated that electrical stimulation of the gracilis muscle afferents at current intensities that activate group I and II afferents elicited no change in total lung resistance, whereas stimulation of these muscle afferents at current intensities that activate group III and IV afferents decreased total lung resistance. This reflex decrease in total lung resistance evoked by electrical stimulation of muscle afferents was due to a withdrawal of tonic cholinergic input to airway smooth muscle.

III. The Dorsal Horn

Thin fiber hindlimb muscle afferents enter the spinal cord via the dorsal roots and synapse in the superficial laminae of the dorsal horn (11,12). The neurotransmitters released onto second-order dorsal horn neurons from contraction-activated group III and IV hindlimb afferents is unknown; however, potential candidates include glutamate and substance P.

Primary afferents synapsing in the dorsal horn are known to contain glutamate (13,14), and neurons in the superficial laminae of the dorsal horn have been shown to be depolarized by glutamate (15). The role of glutamate in the spinal transmission of the cardiovascular and ventilatory responses evoked by stimulation of hindlimb afferents, however, has received little attention. Recently, Hill et al. (16) demonstrated that the reflex cardiovascular responses to static contraction of the hindlimb of chloralose-anesthetized cats were unaf-

ected by intrathecal injection of *N* methyl-D-aspartate (NMDA) receptor antagonists but were attenuated by intrathecal injection of a non-NMDA receptor antagonist.

Considerable evidence has been accumulated suggesting that substance P plays a role in the spinal transmission of the cardiovascular and ventilatory responses evoked by stimulation of hindlimb afferents. In anesthetized cats, both electrical stimulation of group III and IV afferents in the sciatic nerve and static contraction of hindlimb muscles have been shown to evoke release of substance P in the dorsal horn (17-19). Further, intrathecal injections of either a peptide antagonist to substance P (20,21) or an antibody to this peptide (22) attenuated the reflex pressor and ventilatory responses to static contraction of the hindlimb muscles in anesthetized cats. Similarly, microinjection of a substance P antagonist into the gray matter of the spinal cord has also been shown to attenuate the reflex pressor response to static contraction (23).

Hill et al. (16) have extended their findings regarding the role of substance P and glutamate receptors in the spinal transmission of the reflex cardiovascular responses to static contraction of the hindlimb muscles in anesthetized cats. These investigators reported that the reflex pressor response to static contraction was nearly abolished by intrathecal injection of a substance P antagonist and a non-NMDA receptor antagonist. They speculated that substance P and glutamate are the major neurotransmitters in the first synapse of the exercise pressor reflex arc. The role of the dorsal horn in the reflex arc causing airway dilation when hindlimb afferents are stimulated remains to be determined.

IV. The Ventrolateral Medulla

The role played by neurons in the ventrolateral medulla in the regulation of cardiovascular function has been examined extensively. For example, both electrical and chemical stimulation of the caudal ventrolateral medulla (CVLM) have been shown to evoke depressor responses in anesthetized animals (24,25), although, in a few instances, chemical stimulation of this area has been shown to evoke a pressor response (26,27).

The ventrolateral medulla has also been shown to play a role in the control of airway tone. For example, Haxhiu et al. (28) demonstrated that topical application of nicotine to the intermediate area in cats evoked tracheal constriction, whereas application of GABA evoked tracheal dilation. Haselton et al. (29) showed that microinjection of DL-homocysteic acid into the lateral aspect of the rostral ventrolateral medulla increased total lung resistance and tracheal smooth muscle tension in chloralose-anesthetized dogs. In addition, the increase in total lung resistance evoked by DL-homocysteic acid was not affected by β -adrenergic blockade but was abolished by muscarinic blockade.

The cells of origin of the vagal efferent fibers whose activation causes constriction of airway smooth muscle appear to be located in the nucleus ambiguus.

In cats, McAllen and Spyer (30) demonstrated that cells in the nucleus ambiguus could be antidromically invaded from the pulmonary branches of the vagus nerves. Additionally, cell body labeling in the nucleus ambiguus following application of horseradish peroxidase to the cut central ends of the pulmonary branches of the vagus has been reported in cats (31) and dogs (32,33). Similarly, Wallach et al. (34) found cell body labeling in this nucleus when horseradish peroxidase was applied to the cut central end of the superior laryngeal nerve in the dog.

The role of the CVLM in the control of airway tone has also been examined. Connelly et al. (35) demonstrated that both electrical and chemical stimulation of the CVLM significantly decreased total lung resistance (TLR) in chloralose-anesthetized dogs. The bronchodilation evoked by stimulation of the CVLM was unaffected by β -adrenergic blockade but was abolished by muscarinic blockade. These investigators reported that the CVLM contains a pool of neurons whose excitation evokes a bronchodilation by withdrawing cholinergic input to the airways.

V. Hindlimb Afferents and the Ventrolateral Medulla

Electrophysiological evidence links input from hindlimb afferents with the ventrolateral medulla. Iwamoto and Kaufman (36) demonstrated that activation of group III and IV afferents by intra-arterial injection of capsaicin and by static muscular contraction of the triceps surae muscles in cats stimulated cells in the CVLM. Similarly, Bauer et al. (37) showed that cells in the ventrolateral medulla responded to muscular contraction of the hindlimb of anesthetized cats and that many of these cells projected to the intermediolateral cell column. Moreover, these cells exhibited a discharge related to either sympathetic nerve activity or the cardiac cycle. These cells described by Bauer et al. (37) appeared to be located more rostrally than those described by Iwamoto and Kaufman (36) and may play a role in the control of sympathetic outflow.

Although the activity of neurons in the ventrolateral medulla increased in response to activation of hindlimb afferents, it is not clear from the above studies whether these neurons play a role in mediating the reflex-induced cardiovascular and ventilatory responses evoked by stimulation of hindlimb afferents. There is, however, evidence suggesting that the CVLM plays an important role in the mediation of reflex-induced cardiovascular and ventilatory responses arising from the hindlimb. For example, electrolytic destruction of the CVLM has been shown to attenuate the reflex pressor responses to either electrical stimulation of afferent fibers in the sciatic nerve (38) or to static muscular contraction (39). Stornetta et al. (40), however, suggested that the attenuation of these reflex pressor responses resulted from destruction of fibers of passage in the CVLM.

An intact CVLM appears to be necessary for the full expression of the bronchodilation evoked reflexly from hindlimb afferents. In anesthetized cats, topical application of lidocaine to the ventrolateral medullary surface has been shown to abolish the reflex tracheal dilation evoked by electrical stimulation of the sciatic nerve (41). Similarly, chemical inactivation of cell bodies in the CVLM has been shown to attenuate the reflex decrease in TLR evoked by stimulation of hindlimb afferents. Using chloralose-anesthetized dogs, Padrid et al. (42) demonstrated that microinjection of either ibotenic acid or cobalt chloride into the CVLM attenuated the bronchodilation evoked by static muscular contraction and by electrical stimulation of C fibers in the sciatic nerve. Ibotenic acid destroyed cell bodies but not axons (43), whereas cobalt chloride prevented neurotransmitter release by blocking presynaptic calcium channels (44). These findings suggested that cells in the CVLM served as interneurons in the reflex arc that dilates the airways in response to afferent stimulation arising from the hindlimb.

Although the findings of Padrid et al. (42) implicated cell bodies located in the CVLM in the reflex control of airway caliber, the pharmacological mechanism by which hindlimb afferents evoked these reflex bronchomotor effects was not investigated. We therefore examined the role played by excitatory amino acids in the CVLM in the reflex airway dilation arising from the hindlimb of anesthetized dogs. In preliminary experiments, using the broad-spectrum glutamate antagonist kynurenic acid, we found that blockade of glutamatergic receptors located in the CVLM reversibly attenuated the reflex bronchodilation evoked by electrical stimulation of C fibers in the sciatic nerve (45). We also found that these injections of kynurenic acid potentiated the pressor response to sciatic nerve stimulation. From this work, however, it is not clear whether NMDA or non-NMDA receptors were involved. To distinguish which of the two excitatory amino acid receptor subtypes in the CVLM were responsible for evoking this reflex airway dilation, we examined the role played by NMDA and non-NMDA receptors in the CVLM in the reflex airway dilation arising from the hindlimb.

Decreases in TLR were evoked by electrical stimulation of the sciatic nerve at current intensities shown to recruit C fibers and by static contraction of the gastrocnemius muscles of both hindlimbs in chloralose-anesthetized dogs. Once we established control levels for the reflex airway dilation evoked by these maneuvers, we bilaterally microinjected 50 nL of a NMDA or non-NMDA receptor antagonist into the CVLM. We then repeated stimulation at 15- to 30-min intervals for 2 h and recorded the bronchomotor and cardiovascular effects. We used (\pm)-3-(2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP; 25 mM) and (\pm)-2-Amino-5-phosphono-valeric acid (AP5; 50 mM) to block NMDA receptors and 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX; 39 μ M) to block non-NMDA receptors (46).

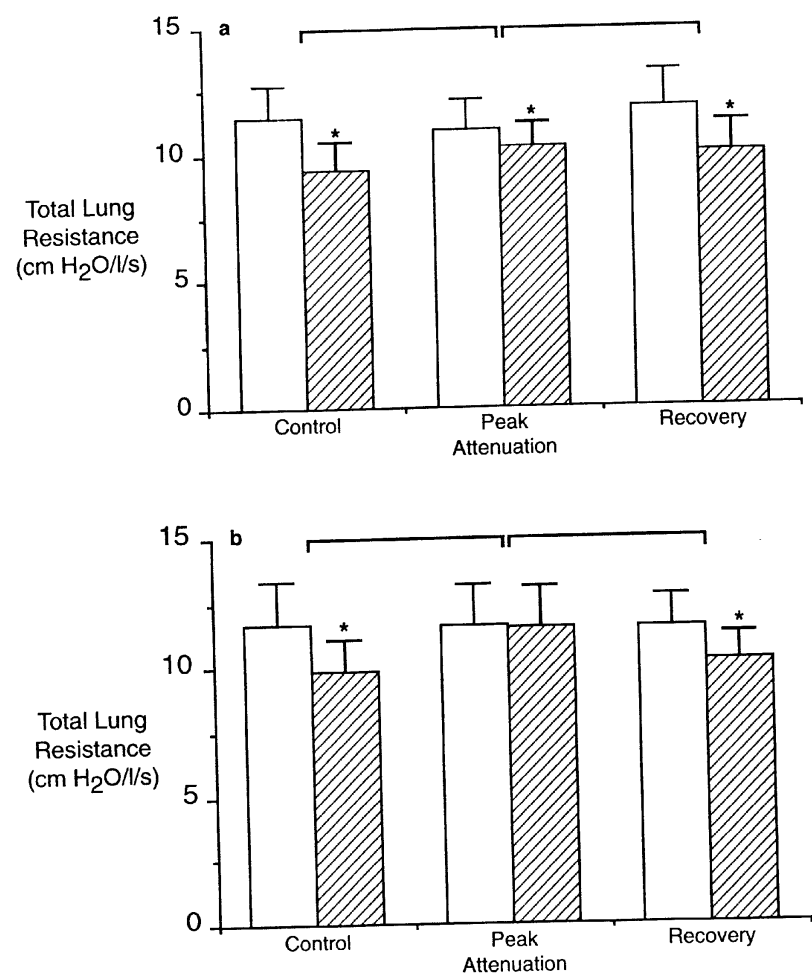


Figure 1 Effects of bilateral microinjection into the CVLM of either CPP (25 mM; 50 nL) or AP5 (50 mM; 50 nL) on the reflex airway dilation evoked by (a) electrical stimulation of the sciatic nerve ($n = 9$) and by (b) static contraction of both gastrocnemius muscles ($n = 5$). Blockade of NMDA receptors in the CVLM reversibly attenuated the reflex decrease in total lung resistance evoked by both maneuvers. Data are reported as means \pm SE. Asterisks represent statistically significant differences ($P < 0.05$) between baseline (open bar) and its corresponding stimulation effect (hatched bar). Horizontal brackets connect control, peak attenuation, and recovery values that are significantly different ($P < 0.05$) from each other.

We found that bilateral microinjection into the CVLM of either CPP (25 mM; 50 nL) or AP5 (50 mM; 50 nL) reversibly attenuated the decrease in total lung resistance that was evoked by electrical stimulation of C fibers in the sciatic nerve (Fig. 1a). These injections also augmented the pressor response to stimulation. Similarly, bilateral microinjection of AP5 (50 mM; 50 nL) into the

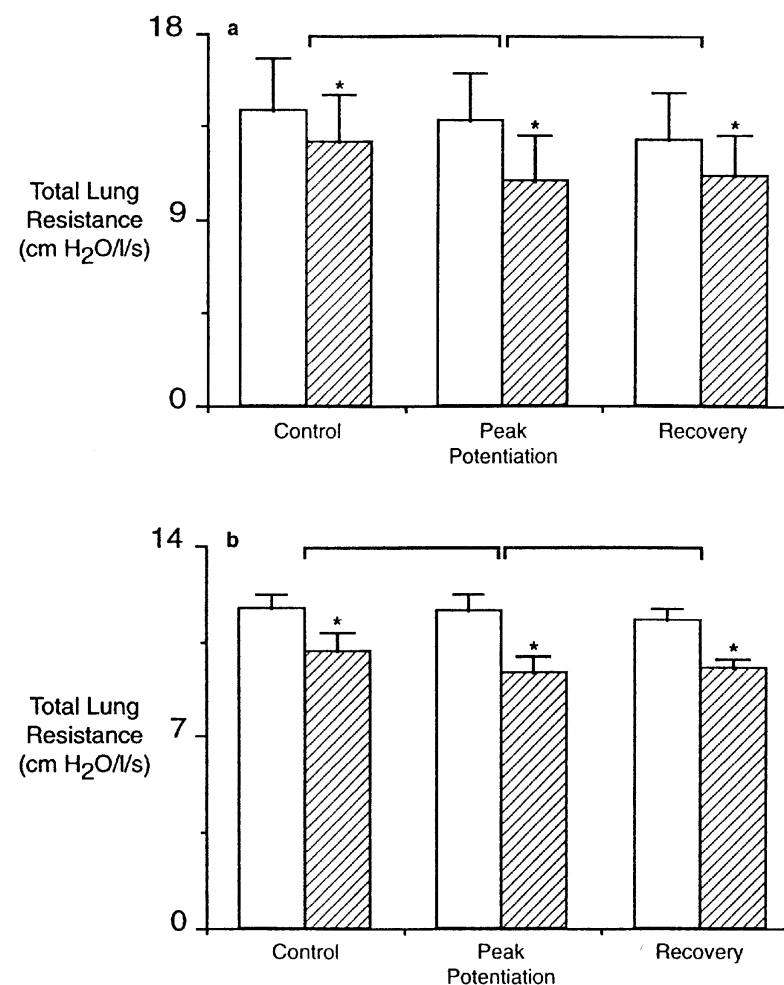


Figure 2 Effects of bilateral microinjection into the CVLM of CNQX (39 μ M, 50 nL) on the reflex airway dilation evoked by (a) electrical stimulation of the sciatic nerve ($n = 6$) and by (b) static contraction of both gastrocnemius muscles ($n = 5$). Blockade of non-NMDA receptors in the CVLM reversibly augmented the reflex decrease in total lung resistance evoked by both maneuvers. Data are reported as means \pm SE. Asterisks represent statistically significant differences ($P < 0.05$) between baseline (open bar) and its corresponding stimulation effect (hatched bar). Horizontal brackets connect control, peak potentiation, and recovery values that are significantly different ($P < 0.05$) from each other.

CVLM reversibly attenuated or abolished the decrease in total lung resistance elicited by static contraction of both gastrocnemius muscles (Fig. 1b). In contrast, bilateral microinjection into the CVLM of CNQX (39 μ M; 50 nL) reversibly augmented the reflex decrease in total lung resistance that was evoked by either sciatic nerve stimulation (Fig. 2a) or by static muscular contraction (Fig. 2b).

Bilateral microinjections of xanthurenic acid (100 mM; 50 nL) into the CVLM had no effect on the decrease in total lung resistance that was evoked by sciatic nerve stimulation, and bilateral microinjection of either AP5 or CNQX into the dorsal medulla did not simulate the effects reported above. These findings suggest that NMDA receptors, but not non-NMDA receptors, located in the CVLM play the predominant role in mediating the reflex-induced bronchodilation arising from the hindlimb.

VI. Summary

Sensory input arising from hindlimb afferents has been shown to have marked reflex effects on the cardiovascular and respiratory systems. Both static contraction and electrical stimulation of hindlimb afferents can evoke increases in blood pressure, heart rate, and ventilation, as well as dilate the airways. The afferents responsible for inducing these reflex cardiovascular and respiratory effects appear to be group III and IV afferents. The CVLM has been shown to participate in the reflex airway dilation evoked by stimulation of these thin fiber afferents arising from the hindlimb. The pharmacological mechanism in the CVLM by which hindlimb afferents evoke this reflex airway dilation may in part be due to stimulation of excitatory amino acid receptors, specifically NMDA receptors.

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