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Role of the Rostral Ventrolateral Medulla (RVLM) in the Patterning of Vestibular System Influences on Sympathetic Nervous System Outflow to the Upper and Lower Body

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Abstract

Research on animal models as well as human subjects has demonstrated that the vestibular system contributes to regulating the distribution of blood in the body through effects on the sympathetic nervous system. Elimination of vestibular inputs results in increased blood flow to the hindlimbs during vestibular stimulation, because it attenuates the increase in vascular resistance that ordinarily occurs in the lower body during head-up tilts. Additionally, the changes in vascular resistance produced by vestibular stimulation differ between body regions. Electrical stimulation of vestibular afferents produces an inhibition of most hindlimb vasoconstrictor fibers and a decrease in hindlimb vascular resistance, but an initial excitation of most upper body vasoconstrictor fibers accompanied by an increase in upper body vascular resistance. The present study tested the hypothesis that neurons in the principal vasomotor region of the brainstem, the rostral ventrolateral medulla (RVLM), whose projections extended past the T10 segment, to spinal levels containing sympathetic preganglionic neurons regulating lower body blood flow, respond differently to electrical stimulation of the vestibular nerve than RVLM neurons whose axons terminate rostral to T10. Contrary to our hypothesis, the majority of RVLM neurons were excited by vestibular stimulation, despite their level of projection in the spinal cord. These findings indicate that the RVLM is not solely responsible for establishing the patterning of vestibular-sympathetic responses. This patterning apparently requires the integration by spinal circuitry of labyrinthine signals transmitted from the brainstem, likely from regions in addition to the RVLM.

Keywords

Cardiovascular system; Vasoconstriction; Blood pressure; Blood flow; Orthostatic hypotension

Introduction

Over the past decades, research on animal models as well as human subjects has demonstrated that the vestibular system contributes to regulating the activity of the sympathetic nervous system (SNS), as reviewed in (Yates 1992; Yates and Kerman 1998; Yates and Miller 1998; Ray and Monahan 2002; Ray and Carter 2003; Carter and Ray 2008). Sympathetic preganglionic neurons located in the thoracic and upper lumbar spinal cord provide inputs to sympathetic postganglionic neurons, which are situated in paravertebral sympathetic ganglia near the spinal cord or prevertebral sympathetic ganglia that are positioned peripherally near the viscera. In turn, postganglionic sympathetic neurons

provide inputs to glands and smooth muscle (Harati 1993; Sved and Ruggiero 1996). The functions of the SNS are diverse, and include affecting pupillary diameter, heart rate and contractility, arterial resistance, gastrointestinal motility, bladder activity, sexual function, sweating, and piloerection (Harati 1993; Sved and Ruggiero 1996). Amongst the most important roles of the SNS is the regulation of blood pressure.

Blood pressure is dependent on cardiac output and the resistance to blood flow provided by peripheral vasculature, which is referred to as “total peripheral resistance” (TPR) (Rushmer 1976; Hall 2011). A variety of factors influence cardiac output, including heart rate, the amount of blood returning to the heart from the periphery (preload), and the pressure that must be developed in the chambers of the heart to overcome the peripheral “load” that the heart must pump against (afterload) (Rushmer 1976; Hall 2011). TPR is mainly altered by constriction of smooth muscles in arterioles. The constriction of arteriole smooth muscle is regulated by a variety of mechanisms, including the SNS, local paracrine factors such as products of skeletal muscle metabolism, and hormones like angiotensin 2 (Rushmer 1976; Hall 2011). In addition to raising central blood pressure, constriction of smooth muscle in arterioles profoundly diminishes blood flow to downstream capillaries. Thus, raising blood pressure by increasing TPR comes at the cost of depriving oxygen and nutrients to the tissues perfused by constricted arterioles (Rushmer 1976; Hall 2011). Few arterioles are innervated by the parasympathetic nervous system; as such, the SNS plays the predominant role in mediating nervous system influences on blood distribution in the body (Harati 1993; Sved and Ruggiero 1996).

Anatomical studies conducted in rats showed that sympathetic preganglionic neurons have a general topographic organization in the spinal cord; preganglionic neurons in the upper thoracic cord regulate sympathetic outflow to the upper body, whereas those in the lower thoracic and lumbar spinal cord regulate sympathetic outflow to the lower body (Strack et al. 1988; Llewellyn-Smith 2009). Strack et al. (Strack et al. 1988) first demonstrated this topographic organization by injecting fluorescent tracers into different prevertebral ganglia. The presence of a topographical organization of sympathetic preganglionic neurons was confirmed in subsequent transneuronal tracing studies involving the injection of neurotropic viruses into particular targets. For example, injection of retrogradely-transported viruses into the hindlimb (Ugolini 1992; Lee et al. 2007) or tail (Smith et al. 1998) infected sympathetic preganglionic neurons confined to the lower thoracic and upper lumbar segments.

Patterning of sympathetic outflow elicited by vestibular stimulation

There is no apparent physiological rationale for the vestibular system to provide influences on many targets of the SNS, with the exception of blood vessels, whose resistance to blood flow is an important determinant of blood pressure. Some changes of posture, such as standing in humans and head-up rotations of quadrupeds, can result in the pooling of blood in the periphery, which causes a decrease in return of blood to the heart and thus hypotension (Wieling and von Lieshout 1993; Mano 2001). The SNS must produce a rapid net increase in peripheral vascular resistance during such movements, in order to maintain stable blood pressure (Rushmer 1976; Wieling and von Lieshout 1993; Mano 2001). It makes sense for the vestibular system to work in a feed-forward fashion and trigger changes in vascular resistance at the onset of postural alterations that could result in peripheral blood pooling, so that perturbations of blood pressure never occur (Jian et al. 1999; Yavorcik et al. 2009).

Studies in animals have borne out that the vestibular system has the strongest influences on the sympathetic efferents that regulate blood flow. When vestibular afferents are stimulated electrically, changes in activity can be recorded across a variety of sympathetic nerves. The magnitude of the responses is particularly large for the renal nerve, which is comprised

almost entirely of vasoconstrictor efferents, and relatively small for the hypogastric nerve, which is made up largely of non-vasoconstrictor efferents (Kerman and Yates 1998). Furthermore, vestibulo-sympathetic responses (VSR) are strongly attenuated or abolished by increases in blood pressure (Kerman and Yates 1998), which selectively diminish the excitability of sympathetic efferents that innervate vascular smooth muscle (Bahr et al. 1981; Bahr et al. 1986; Janig and McLachlan 1992). These observations support the notion that vestibular system influences on the SNS are patterned according to the innervation targets of sympathetic efferents.

Vasoconstriction does not need to be equivalent in every vascular bed during postural alterations in order to maintain stable blood pressure. Blood pooling is likely to occur in the lower extremities during head-up rotations, so it is necessary for the SNS to induce powerful vasoconstriction in the lower body during such movements to limit this pooling (Rushmer 1976; Wieling and von Lieshout 1993; Mano 2001). The drop in arterial perfusion of the hindlimb is matched to the decrease in venous return that occurs due to gravitational effects on blood in the lower body (Yavorcik et al. 2009). Since blood pooling is unlikely in the upper body during head-up postural alterations, vasoconstriction does not need to be as powerful in body regions above the heart as in regions below it. We recently demonstrated that a bilateral vestibular neurectomy attenuates the increase in vascular resistance that ordinarily occurs in the hindlimbs of conscious cats during head-up rotations (Wilson et al. 2006; Yavorcik et al. 2009)(see Fig. 1). The deficits in adjusting hindlimb blood flow were pronounced during the first week after removal of labyrinthine inputs, but gradually dissipated (Wilson et al. 2006). In contrast, forelimb vascular resistance was similar during head-up movements before and after the removal of labyrinthine inputs, indicating that the vestibular system has distinct influences on lower and upper body vasculature (Wilson et al. 2006).

Studies employing electrical stimulation of vestibular afferents in cats have also demonstrated that VSR are patterned in accordance with the anatomical location of the blood vessels that are innervated. Delivery of a train of current pulses to the VIIIth cranial nerve elicited a decrease in hindlimb vascular resistance but an increase in forelimb vascular resistance (Kerman et al. 2000a), as illustrated in Fig. 2A. Similar response patterning was also demonstrated through experiments where VSR were recorded from sympathetic efferent fibers innervating blood vessels in skeletal muscles (muscle vasoconstrictor fibers, MVC) of the face, forelimb, and hindlimb (Kerman et al. 2000b). Simultaneous recordings were performed from MVC in the lower body (coursing in the peroneal nerve of the hindlimb) and the upper body (coursing in either the radial nerve of the forelimb or the facial nerve). Most hindlimb muscle MVC were inhibited by electrical stimulation of vestibular afferents, whereas most forelimb and facial MVC exhibited a response consisting of early, powerful excitation followed by weak prolonged inhibition, as shown in Fig. 2B (Kerman et al. 2000b). Such a response pattern of sympathetic efferents would evoke the increase in hindlimb blood flow (as vascular smooth muscle dilates) and decrease in forelimb blood flow (as vascular smooth muscle constricts) that is observed when current pulses are applied to the VIIIth nerve (Kerman et al. 2000a).

Despite the practicality of the SNS producing regionally-specific changes in blood flow, the findings (Kerman et al. 2000a; Kerman et al. 2000b; Wilson et al. 2006; Yavorcik et al. 2009) suggesting that this patterning exists have been controversial. A general dogma, based upon studies in human subjects, is that patterning of blood flow mediated by the SNS is in accordance with tissue type, but not the location of the tissue within the body. Mild unloading of cardiopulmonary afferents in humans resulted in an increase in the discharges of MVC in both the arms and legs (Rea and Wallin 1989), but no change in the activity of cutaneous vasoconstrictor fibers (Vissing et al. 1994). Mental stress produced parallel

increases in arm and leg MVC activity (Carter et al. 2005), but variable changes in cutaneous blood flow depending on the ambient temperature (Wallin 1990). The variety of inputs evoked by head-down neck flexion in human subjects produced parallel changes in arm and leg MVC activity (Monahan and Ray 2002). Although such studies show that some stimuli evoke similar changes in MVC activity in several body regions, the experiments do not establish that the nervous system lacks the capacity to elicit anatomically-patterned alterations in blood flow; they just show that certain inputs fail to elicit the patterning. For example, a recent study in women showed that forelimb and hindlimb MVC responses to head-down flexion can diverge in certain phases of the menstrual cycle (Lawrence et al. 2010); hormonal changes have not shown to affect patterning of responses to other stimuli in humans. As such, vestibular signals may be atypical in their capacity to selectively alter sympathetic outflow to blood vessels in particular body regions.

Regulation of sympathetic outflow by the rostral ventrolateral medulla (RVLM)

A large body of physiological evidence from a variety of species suggests that the RVLM, a dense longitudinal column of cells located near the ventral surface of the medulla, ventral to the retrofacial nucleus, plays the predominant role in controlling blood pressure and the distribution of blood in the body (Dampney et al. 1987a; Dampney 1990; Dampney 1994; Bourassa et al. 2009). Stimulation of the RVLM produces large increases in blood pressure and blood flow to a variety of vascular beds (Dampney et al. 1982; Dampney et al. 1985; McAllen 1986a; McAllen 1986b; Dampney et al. 1987a; Dampney and McAllen 1988; McAllen and Dampney 1989; McAllen and Dampney 1990; McAllen et al. 1994), but in the cat does not affect dilation of the pupils, piloerection, or sudomotor responses, and does not elicit appreciable changes in gastrointestinal motility (McAllen 1986a). The firing of RVLM neurons is powerfully inhibited by stimulation of baroreceptors (Barman and Gebber 1985; McAllen 1986b; Dembowski and McAllen 1990; McAllen et al. 1994; McAllen et al. 1997; McAllen et al. 2001), and bilateral destruction or inhibition of this region produces a profound drop in blood pressure similar to that observed after transecting the cervical spinal cord (Feldberg and Guertzenstein 1976; Dean and Coote 1986; Stein et al. 1989). Furthermore, bilateral inhibition or destruction of the RVLM causes a loss of baroreceptor reflexes (Dampney 1981; Reis et al. 1984; Granata et al. 1985). Axons that are labeled by injection of anterograde tracers into the RVLM of cats terminate mainly in the intermediolateral cell column and intermediate zone (central autonomic region) of the thoracic and upper lumbar spinal gray matter, and are sparse in the dorsal and ventral horns (Dampney et al. 1987b). This evidence suggests that the RVLM mainly provides inputs to sympathetic preganglionic neurons and nearby interneurons, and not the spinal neurons most directly involved with sensory or motor functions (Dampney et al. 1987b). In addition, retrograde tracing studies have shown that most, if not all, of the neurons in the feline RVLM project to the spinal cord (Miura et al. 1983; Dampney et al. 1987a; Dampney et al. 1987b; Polson et al. 1992). The axons of RVLM neurons course bilaterally in the dorsolateral and lateral funiculi of the spinal white matter of cats (Barman and Gebber 1985; Dampney et al. 1987b). The principal influences of descending projections from the RVLM to the spinal cord are excitatory (Pilowsky and Goodchild 2002; Stornetta 2009).

In addition to mediating baroreceptor reflexes, existing data suggest that the RVLM provides the major pathway through which vestibular signals are conveyed to sympathetic preganglionic neurons. Previous studies showed that a majority of RVLM neurons whose axons could be antidromically activated from the white matter of the upper thoracic spinal cord responded to electrical stimulation of the vestibular nerve (Yates et al. 1991). Virtually all RVLM neurons with activity modulated by vestibular nerve stimulation also received inputs from baroreceptors (Yates et al. 1991). Furthermore, bilateral chemical lesions of the

RVLM abolished changes in SNS activity elicited by stimulation of vestibular afferents (Yates et al. 1995b).

The prior evidence demonstrating that vestibular stimulation elicits regionally-specific changes in blood flow suggest that RVLM neurons should have the appropriate connectivity with spinal sympathetic preganglionic neurons to elicit anatomically-patterned responses. The RVLM is a highly heterogeneous region in terms of inputs and neuroanatomical phenotypes of neurons; this heterogeneity raises the possibility that the RVLM can differentially regulate sympathetic outflow to different body regions (Bourassa et al. 2009; Goodchild and Moon 2009). Several studies in cats revealed that individual RVLM neurons provide inputs to select spinal cord segments, and thus have the capacity to modify sympathetic outflow to particular body regions. For example, Barman and Gebber (Barman and Gebber 1985) demonstrated that a subset of RVLM neurons whose axons could be antidromically activated from the T3 spinal gray matter lacked a descending branch coursing in the white matter to more caudal segments. Polson et al. (Polson et al. 1992) showed that large injections of HRP in the vicinity of sympathetic preganglionic neurons in one spinal segment labeled only a fraction of RVLM neurons. However, another study in felines suggested that RVLM neurons are organized topographically with regards to the tissue that they influence, but not the anatomical location of the tissue in the body (Dampney and McAllen 1988). When microinjection of sodium glutamate was used to activate small populations of RVLM neurons, no differences could be found between sites that caused vasoconstriction in forelimb and hindlimb muscles (McAllen and Dampney 1990), despite the fact that injection sites that specifically altered blood flow to particular tissues (e.g., viscera vs. muscle or skin) were readily identifiable (Dampney and McAllen 1988; McAllen and Dampney 1990; Dean et al. 1992). This work is equivocal, however, since it did not exclude the possibility that neurons that regulate forelimb and hindlimb blood flow could be comingled in the RVLM. In light of previous data regarding the patterning of VSR, it seems warranted to challenge the notion that the RVLM lacks the capacity to coordinate regionally-specific changes in blood flow.

Goals of the present study

This study tested the hypothesis that the responses of RVLM neurons to vestibular stimulation differ depending on whether the cell projects to rostral spinal cord segments providing SNS influences on the upper body, or more caudally to spinal segments containing lower body sympathetic preganglionic neurons. For this purpose, recordings were made from RVLM neurons during antidromic stimulation of the lateral and dorsolateral spinal white matter in the T1 and T10 spinal segments. After a spinally-projecting neuron was isolated, we determined its responses to electrical stimulation of the labyrinth. We employed current pulses to activate vestibular afferents (as opposed to rotations of the body axis) because the stimulus produces stereotyped changes in SNS activity that remain consistent over a multi-hour recording session (Yates et al. 1993; Kerman et al. 2000a; Kerman et al. 2000b). Furthermore, there was no concern that electrical vestibular stimulation activated afferents outside of the labyrinth, as could have occurred if natural vestibular stimulation was employed (Yates and Miller 1994).

Methods

All of the procedures used in this study conformed to the *Guide for the Care and Use of Laboratory Animals*, and were approved by the University of Pittsburgh's Institutional Animal Care and Use Committee.

General surgical procedures

Experiments were performed on 11 purpose-bred adult cats of either sex weighing 3.2 — 5.8 kg obtained from Liberty Research (Waverly, NY, USA). Each animal was initially anesthetized with isoflurane (5% for induction, 1.5–2.5% for maintenance; Baxter Healthcare, Deerfield, IL, USA) vaporized in oxygen. The level of anesthesia was titrated such that blood pressure, which was monitored using a transducer (Millar Instruments, Houston, TX, USA) inserted through a femoral artery into the abdominal aorta, remained < 100 mm Hg. Anesthesia was adequate to prevent both spontaneous and reflexive movements. The trachea was intubated, and cannulae were placed in both femoral veins for drug administration. Dexamethasone (1 mg/kg, i.v.) and atropine (0.1 mg/kg, i.m.) were administered to minimize brain edema and to reduce secretions in the airways, respectively. Rectal temperature was maintained at 37–38°C using a DC-powered heating lamp and pad.

Each animal was decerebrated at the midcollicular level after bilateral ligation of the common carotid arteries. The vestibular nerves on each side were prepared for bipolar electrical stimulation, as described in a previous manuscript (Yates et al. 1993). The tympanic bulla was exposed on both sides using a ventrolateral approach and opened to expose the promontory of the middle ear. The promontory was opened, and a Ag-AgCl ball electrode insulated except at the tip was inserted in the direction of the vestibule. A second electrode was placed 1–2 mm away, in the vicinity of the oval window. The electrodes were covered with warm paraffin wax and fixed in place with dental cement. The effectiveness of electrodes in activating vestibular afferents was confirmed by noting that stimulation (using a 50-shock train of 0.2 msec pulses each separated by 3 msec) evoked eye movements and neck muscle contractions. We also determined the stimulus intensity that elicited facial movements, which were the consequence of current spread to the facial nerve. During the recording session, the electrode on each side that generated the highest threshold facial movements served as the cathode.

The animals were placed in a stereotaxic frame and supported using hip pins and a clamp placed on the dorsal process of an upper thoracic vertebra. A craniotomy was performed and the caudalmost aspect of the cerebellum was aspirated to expose the dorsal surface of the medulla. Subsequently, two small laminectomies were performed to expose the T1 and T10 spinal segments. After opening the dura, two monopolar stainless steel floating electrodes insulated except for ~0.5 mm at the tip were placed bilaterally in both T1 and T10. The electrodes were inserted through the dorsal root entry zone to a depth of ~2 mm, so the tips would lie near the intermediolateral cell column; Fig. 3A illustrates the electrode placements in one animal. Four electrodes were placed in each segment (two on each side) to minimize the possibility that we failed to activate any descending projections of RVLM neurons. Since the exposed electrode surface was large (0.5 mm), this placement should have effectively activated fibers in the dorsolateral and lateral funiculi (where the axons of RVLM neurons are known to course (Barman and Gebber 1985; Dampney et al. 1987b)), particularly those entering the intermediolateral cell column.

Anesthesia was removed after all surgical procedures were completed. The animal was then paralyzed using gallamine triethiodide (Sigma, St. Louis, MO, USA; initial injection of 10 mg/kg i.v., maintained by hourly injections of 5 mg/kg) and artificially ventilated with room air (20–25 cycles/min). End-tidal CO₂ was maintained at 3–5%. If hypotension occurred, mean blood pressure was increased to above 90 mm Hg using an intravenous infusion of phenylephrine in saline at the rate of 0.005 to 0.01 mg/kg/min.

At the end of the experiment, animals were killed by the intravenous injection of 120 mg/kg sodium pentobarbital. The brainstem and the T1 and T10 spinal cord segments were removed and fixed in 10% formaldehyde solution.

Electrophysiological procedures

At the beginning of each recording session, we determined the minimal stimulus intensity delivered to the inner ear on each side that evoked field potentials recordable from the medial longitudinal fasciculus in the caudal medulla. This intensity was deemed to be the threshold (T) for activating vestibular afferents.

Subsequently, recordings from RVLM neurons were initiated. For this purpose, penetrations were conducted from 3.5–6.5 mm rostral to the obex and 3.5–5.5 mm lateral to the midline using a 5 M Ω tungsten microelectrode (Frederick Haer Company, Bowdoin, ME, USA). Landmarks on the exposed surface of the fourth ventricle were used to guide electrode placement. Only spontaneously active neurons were targeted for study. Furthermore, we did not characterize responses for neurons with respiratory-related activity, which likely participated in regulating breathing and not blood pressure, since the ventral respiratory group is positioned just dorsal to the RVLM (Lois et al. 2009). During recordings, monopolar current pulses of 0.5 msec width and ~0.5 mA intensity were delivered to the electrodes positioned in T1 to provide antidromic stimulation; the anode was attached to muscle adjacent to the stimulated spinal cord. The presence of a spinal projection was confirmed using collision, as illustrated in Fig. 3B. Subsequently, the current intensity required to antidromically activate the neuron from each electrode positioned in T1 was determined. We next attempted to antidromically activate the neuron from each of the electrodes positioned in T10. Stimulus intensities of over 1.5–2 mA were delivered before concluding that a neuron lacked a projection to T10. After establishing whether a neuron projected as far caudally as T10, we determined its responses to stimulation of the vestibular nerve on each side. We initially delivered a train of 5 shocks (each of 0.2 msec duration, separated by 3 msec) at 5T intensity to each inner ear; the stimulus trains were repeated at 1 Hz. Subsequently, we determined the minimal number of pulses required to elicit a response, as well as the lowest effective stimulus intensity when using a 5-shock train. A lesion was placed at 1–2 recording sites in each animal by delivering a 0.1 mA continuous current through the microelectrode for 30 sec.

Although RVLM neurons receive baroreceptor inputs (Barman and Gebber 1985; McAllen 1986b; Dembowsky and McAllen 1990; McAllen et al. 1994; McAllen et al. 1997; McAllen et al. 2001), we did not attempt to determine whether the activity of units sampled was altered by baroreceptor stimulation. It was necessary to ligate the carotid arteries in order to perform a decerebration, which prevented the carotid sinus baroreceptors from being activated by changes in blood pressure. Moreover, we noted that the process of opening the tympanic bulla for placing stimulating electrodes in the labyrinth often resulted in denervation of the adjacent carotid sinus or damage to the glossopharyngeal nerve, which carries baroreceptor afferents to the brainstem (Yates et al. 1991). As such, methods such as stretch of the carotid artery were ineffective in causing a change in blood pressure.

Activity recorded by the microelectrode was amplified by a factor of 10,000 and filtered with a bandpass of 300–10,000 Hz. The output of the amplifier was sampled at 10,000 Hz using a Micro1401 mk 2 data collection system and Spike-2 version 6 software (Cambridge Electronic Design, Cambridge, UK) running on an Intel Macintosh computer with the Microsoft Windows XP operating system and “Boot Camp” program installed. When determining the responses of a neuron to stimulation of the vestibular nerves, the output of the amplifier was also fed into a window discriminator for the delineation of spikes from single units. The discriminator output was sampled at 10,000 Hz as described above.

Histological procedures and statistical analyses

After tissue fixation, the brainstem was cut transversely at 100 μm and the T1 and T10 spinal cord were cut transversely at 40 μm using a freezing microtome. Tissue sections were stained using thionine. Spinal cord sections were examined microscopically to confirm that the tips of stimulating electrodes were positioned in the vicinity of the lateral funiculus. Photographs of brainstem sections were captured using a digital stereomicroscope and Motic[®] (Xiamen, China) Images Advanced Software; Adobe Illustrator software (Adobe systems, San Jose, CA) was used to generate drawings of the sections. Recording sites were reconstructed on these drawings with reference to the locations of electrolytic lesions, the relative positions of electrode tracks, and microelectrode depths.

Statistical analyses were performed using Prism 5 software (GraphPad Software, San Diego, CA, USA). Pooled data are presented as means $\pm$ one standard error.

Results

Ninety-eight spontaneously active RVLM neurons that projected to the spinal cord were identified. Eighty (82%) of these cells responded to stimulation of the vestibular nerve on at least one side; 45 of the 80 neurons could be antidromically activated from T10, whereas 35 were activated from T1 but not T10, as indicated in Table 1. The median current intensity required to antidromically activate a neuron from T1 was 500 μA . The median antidromic threshold from T10 was lower: 250 μA . Since we used stimulus intensities 6–8 times higher than this median value before concluding that a neuron lacked a projection to T10, we are confident that we did not misidentify whether a neuron had a projection to the lower thoracic cord. Furthermore, the vast majority of neurons could be activated antidromically from multiple electrodes at a particular level, including those on the contralateral side, indicating that current reached the entirety of the spinal white matter. Sixty-two (78%) of the spinally-projecting RVLM neurons with labyrinthine inputs could be activated at lower threshold from the ipsilateral side, whereas 15 (19%) appeared to have a projection on the contralateral side. The laterality of the projection for the other three units could not be established, as the antidromic thresholds for the left and right sides were similar.

The minimal current intensity delivered to the inner ear that activated vestibular afferents, as reflected in the threshold for eliciting field potentials recordable from the medial longitudinal fasciculus, was 53 ± 5 μA (median of 50 μA). In contrast, the minimal current intensity that elicited facial movements (when a 50-shock stock train was delivered prior to paralysis) was 480 ± 67 μA (median of 400 μA). On average, MLF field potentials could be elicited using stimulus intensities that were $15 \pm 2\%$ of those that resulted in stimulus spread to the facial nerve. Every unit whose firing rate was affected by inner ear stimulation had response thresholds that were lower than the current intensity needed to evoke facial movements. The mean minimal current intensity delivered to the vestibular nerve that elicited a change in activity of RVLM neurons was 163 ± 8.7 μA .

Responses to stimulation of vestibular afferents

Four response types were distinguished amongst the RVLM neurons that received labyrinthine inputs: excitation with a sharp onset latency and early peak (*E-short*), excitation that built slowly after the stimulus and had a long duration (*E-diffuse*), excitation combined with inhibition (*EI*), and inhibition (*I*). Examples of these response patterns are provided in Fig. 4. Table 1 indicates the number of neurons with each response type that projected to T1 (but not T10) and to T10. Responses to vestibular stimulation were similar across RVLM neurons, despite the level of the spinal cord their axon reached. For the 35 neurons whose axons were only activated from T1, the following types of responses to vestibular

stimulation were observed: E-short (12 units, 34%), E-diffuse (7 units, 20%), E-I (14 units, 40%), I (2 units, 6%). Amongst the 45 neurons projecting to T10, the following response profiles were noted: E-short (14 units, 31%), E-diffuse (8 units, 18%), E-I (17 units, 38%), I (6 units, 13%). A χ^2 test verified that the distribution of response types was not significantly different for neurons whose axons terminated rostral to T10, and those that reached T10 ($p=0.73$). Invariably, when a unit responded to stimulation of both the left and right labyrinth, the response characteristics from both sides were similar. We determined the latency and duration of responses for the side that elicited the most robust changes in firing rate.

Table 1 indicates the mean spontaneous firing rates and the latency and duration of responses to vestibular stimulation for RVLM neurons. The distribution of response latencies for the neurons is indicated in Fig. 5. Spontaneous firing rates were calculated from data collected upon first encountering a cell, as spontaneous activity could increase or decrease markedly over time as vestibular stimulation was repeated. Such variations in spontaneous activity are evident in Fig. 4 (during the period prior to stimuli), when more than one run is illustrated for a particular cell. The spontaneous firing rates that we noted in decerebrate animals were much higher than previously reported in barbiturate-anesthetized animals (~3 spikes/sec [Barman and Gebber 1985]), which minimized the risk that vestibular-elicited inhibition would not be detected (since baseline activity was high, decreases in activity due to inhibition should have been apparent). The mean latencies of responses to vestibular stimulation were comparable across unit types, with the exception that changes in activity occurred later for E-diffuse neurons than for others. Only one cell had a response latency measured from the effective shock < 6 msec. The response latencies for most neurons were 6–18 msec after the last shock of the shortest train that elicited a change in firing rate.

The locations of the RVLM neurons whose activity was analyzed are shown in Fig. 6. Neurons with different response types were intermingled in the RVLM, as were neurons with projections of different lengths. For example, neurons whose axons extended to T10 were located on average at 4.5 ± 0.1 mm lateral to the midline and 5.2 ± 0.1 mm rostral to the obex, whereas those with terminations rostral to T10 were located on average at 4.5 ± 0.1 mm lateral to the midline and 5.1 ± 0.1 mm rostral to the obex. As such, there was no evidence that neurons regulating sympathetic outflow to a particular body region were clustered in a particular subarea of the RVLM.

Discussion

Previous studies showed that vestibular stimulation elicits distinct changes in sympathetic outflow to the upper and lower body (Kerman et al. 2000a; Kerman et al. 2000b; Wilson et al. 2006; Yavorcik et al. 2009). Electrical stimulation of vestibular afferents produces an inhibition of most hindlimb MVC and a decrease in hindlimb vascular resistance, but an excitation of most upper body MVC accompanied by an increase in upper body vascular resistance (Kerman et al. 2000a; Kerman et al. 2000b). We thus hypothesized that RVLM neurons whose projections extended past the T10 segment, to spinal levels containing sympathetic preganglionic neurons regulating lower body blood flow (Ugolini 1992; Lee et al. 2007), would respond differently to electrical stimulation of the vestibular nerve than RVLM neurons whose axons terminated rostral to T10. In particular, since a majority of RVLM neurons have excitatory connections in the spinal cord (Pilowsky and Goodchild 2002; Stornetta 2009), we predicted that most RVLM units whose axons could be antidromically activated from T10 would be inhibited by vestibular stimulation (without preceding excitation), whereas RVLM cells whose axons were activated from T1 but not T10 would be excited by delivery of current to the labyrinth. The present data failed to

support our hypothesis and predictions: there was no indication that the responses of RVLM neurons to electrical stimulation of the vestibular nerve differed based on the length of their projection in the spinal white matter. Strikingly, the responses to vestibular stimulation of a large majority of RVLM neurons whose axons reached at least T10 included prominent excitatory components, whereas the activity of hindlimb MVC and hindlimb vascular resistance decreased when electrical stimuli were applied to the labyrinth (Kerman et al. 2000a; Kerman et al. 2000b). These findings suggest that a disparity exists between the effects of vestibular stimulation on sympathetic outflow to the lower body and the activity of RVLM neurons that seemingly control that outflow.

An important caveat is that we did not demonstrate that the RVLM neurons studied made direct connections with sympathetic preganglionic neurons. Despite the fact that RVLM neurons in the cat are known to provide extensive inputs to sympathetic preganglionic neurons (Dampney et al 1987a), some of the cells included in the sample could have terminated on interneurons in the thoracic spinal cord that were not directly involved with controlling autonomic function. However, at the very least we expected that a high proportion of RVLM neurons with projections past T10 would be inhibited by vestibular stimulation. The strong preponderance of excitatory responses observed suggests that inhibitory VSR in the hindlimb are mediated at least in part by inputs from regions in addition to the RVLM to sympathetic preganglionic neurons.

The view that the RVLM plays an overarching role in regulating vascular resistance developed from observations that lesions of the RVLM silence the activity of sympathetic efferents that innervate vascular smooth muscle, and abolish neural reflexes that affect blood flow (Feldberg and Guertzenstein 1976; Dampney 1981; Reis et al. 1984; Granata et al. 1985; Dean and Coote 1986; Stein et al. 1989), including VSR (Yates et al. 1995b). However, this could simply be due to the fact that without tonic excitatory drive from the RVLM, SNS excitability becomes too low for inputs from other regions to modulate the firing of sympathetic nerves. Thus, while prior results demonstrate that the RVLM is critically important in maintaining baseline SNS activity, they do not establish that the area is solely responsible for regulating sympathetic outflow to blood vessels. Patterning of blood flow to different body regions could be the result of integration by sympathetic preganglionic neurons of inputs from several regions of the nervous system.

A variety of studies have established that spinal interneurons participate in regulating SNS activity (Barman and Gebber 1984; Tang et al. 2003; Tang et al. 2004; Schramm 2006). The transformation of descending signals from the RVLM by spinal interneurons is one potential mechanism for the patterning of VSR, despite the fact that RVLM neurons with projections to the upper and lower thoracic spinal cord had similar responses to vestibular stimulation. However, this explanation requires that existence of a sophisticated spinal pattern generator; there is no current evidence to suggest that such a spinal pattern generator plays an appreciable role in regulating SNS activity.

Another possibility is that brainstem areas in addition to the RVLM are involved in shaping the properties of VSR. Studies in rodents involving the retrograde transneuronal transport of neurotropic viruses by sympathetic efferent fibers that innervate blood vessels in the hindlimb (Lee et al. 2007) or kidney (Schramm et al. 1993; Cano et al. 2004) have shown that a number of brainstem areas in addition to the RVLM provide inputs to sympathetic preganglionic neurons: the medullary raphe nuclei, rostral ventromedial medulla, A5 adrenergic cell group region, locus coeruleus, nucleus subcoeruleus, and the paraventricular nucleus of the hypothalamus. Several of these regions receive vestibular inputs (for review, see (Balaban and Yates 2004)), and thus could mediate labyrinthine influences on SNS activity. Further studies will be required to determine the role of brain areas other than the

RVLM in the patterning of blood flow to different body regions during vestibular stimulation.

The present data also provide insights into the neural circuitry that produces VSR. Antidromic responses of feline vestibular nucleus neurons to stimulation within the ipsilateral or contralateral medial reticular formation have a latency of 0.6–3.0 msec (Peterson and Abzug 1975). The latencies of the responses of vestibular nucleus neurons to vestibular nerve stimulation using methodology similar to that employed in this study range from 0.8–3.1 msec, with response latencies < 1.5 msec considered to be monosynaptic (Wilson et al. 1968). Thus, the vast majority of RVLM neurons, which had response latencies > 6 msec, likely received labyrinthine inputs through a polysynaptic circuit. Neurons in the lateral medullary reticular formation (Yates et al. 1995a) and caudal ventrolateral medulla (Steinbacher and Yates 1996) project to the RVLM and respond to vestibular nerve stimulation at shorter latencies than RVLM cells. As such, both regions potentially mediate VSR. Other areas may also participate in relaying labyrinthine signals to the RVLM, including the parabrachial nucleus (Balaban 2004). In particular, convergence of labyrinthine signals relayed through multiple regions to single RVLM neurons could explain the activity of E-diffuse cells, whose activity increased slowly after vestibular nerve stimulation.

In summary, we started this study with the hypothesis that the patterning of sympathetic outflow to different body regions elicited by the vestibular system is mediated by a relatively simple circuitry, and is reflected in distinct responses to vestibular stimulation of RVLM neurons projecting to different levels of the thoracic spinal cord. Instead, we discovered that despite the predominant function of the RVLM in regulating baseline SNS activity, this region is apparently not solely responsible for establishing the patterning of vestibular-sympathetic responses. Other areas of the central nervous system apparently participate in eliciting the patterning of blood flow to different regions of the body, including the patterning produced by labyrinthine stimulation.

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Figure 1.

The effects of removal of vestibular inputs on femoral artery blood flow and vascular resistance during 60° head-up tilts. The tilt was initiated at 0 sec, and reached maximal amplitude ~2 sec later. The plots reflect averaged data from seven animals. When vestibular inputs were present (*blue lines*), femoral blood flow rapidly decreased during head-up rotations, as femoral vascular resistance increased. In the first week following the removal of vestibular inputs (*W1, red lines*), the tilt-related reduction in blood flow and the increase in vascular resistance were attenuated. During the subsequent three weeks of data collection (*W2–4, green lines*), the deficits in adjusting hindlimb blood flow during head-up rotations remained present, but were less prominent. Symbols indicate post-lesion changes in blood flow and vascular resistance during tilt that were significantly different from those recorded when vestibular inputs were present. For points where no symbols are present, no significant differences were found. Error bars indicate one S.E.M. Data from (Wilson et al. 2006).

Figure 2.

A: Average change in resistance of the femoral (*blue line*) and brachial (*red line*) arteries elicited by stimulation of vestibular afferents using a train of 50 shocks (*indicated by a bar*). Resistance was calculated as blood pressure divided by the blood flow velocity signal, which was simultaneously measured from both arteries. Values are expressed as a percentage of baseline vascular resistance, which was calculated over the 20-sec period preceding each stimulus train. Data from (Kerman et al. 2000a). **B:** Averaged vestibular-elicited responses recorded from muscle vasoconstrictor efferents (MVC); these responses consisted of inhibition (*blue line*) or strong excitation followed by weaker inhibition (*red line*). Inhibitory responses predominated for hindlimb MVC, whereas excitatory responses predominated for upper body MVC. Responses were elicited by a train of five shocks delivered to the vestibular nerve (onset indicated by an arrow), and were pooled by averaging individual poststimulus histograms (50 msec bin width). Prior to averaging, counts within each bin were normalized with respect to the mean activity level of the unit during the pre-stimulus period. Horizontal dotted lines indicate baseline activity levels, while solid horizontal lines correspond to zero activity levels. Data from (Kerman et al. 2000b).

Figure 3.

A: Locations of the tips of stimulating electrodes in the spinal cord of one animal. Two electrodes, spaced 4–5 mm apart along the rostral-caudal axis, were inserted on each side of the T1 and T10 spinal cord. In these reconstructions, the locations of electrode tips in a particular spinal segment are denoted on the same section by red circles. **B:** Collision test verifying that the responses of an RVLM neuron to stimulation of the T1 white matter were antidromic. In this example, a 200- μ A stimulus was delivered to the ipsilateral white matter. Three consecutive trials are indicated as sweeps of different colors; arrows designate action potentials. When the interval between a spontaneously occurring action potential and the stimulus was < 4 msec, collision block was observed.

Figure 4.

Examples of responses to stimulation of the vestibular nerve.

A, B: responses of a neuron that was excited at short latency by vestibular stimulation (*E-short unit type*). In **A**, a train of 3 shocks at 5T (250 μ A) intensity was applied to the ipsilateral labyrinth; in **B**, a single shock at the same intensity was used. **C, D:** responses of a neuron that exhibited long and diffuse excitation following vestibular stimulation (*E-diffuse unit type*). 5-shock (**C**) and 2-shock (**D**) trains were delivered to the contralateral labyrinth at 5T (350 μ A) intensity. **E, F:** responses of a neuron consisting of excitation followed by inhibition (*E-I unit type*). The responses were elicited by a train of 3 shocks (**E**) and a single shock (**F**) at 5T (200 μ A) intensity applied to the ipsilateral labyrinth. **G, H:** responses of a neuron that was inhibited by vestibular stimulation (*I unit type*). The

responses were elicited by a 5-shock train at 3T ($120\ \mu\text{A}$) intensity and by a single shock at 5T ($200\ \mu\text{A}$) intensity delivered to the ipsilateral labyrinth. The poststimulus histograms have 1 msec bins; arrowheads designate the delivery of current pulses to the labyrinth. The number of stimulus repetitions employed for each histogram were: **A**, 37; **B**, 34; **C**, 22; **D**, 37; **E**, 48; **F**, 42; **G**, 46; **H**, 32.

Figure 5.

Latencies of responses to stimulation of the vestibular nerve of RVLM neurons whose axons were antidromically activated from T1 or T10. Latencies were determined from the last shock of the shortest stimulus train that elicited an effect. Latencies for E-short (**A**), E-I (**B**), and I (**C**) response types are designated in separate panels. **D** shows the distribution of response latencies for all units. E-diffuse units were omitted from the histograms, as all of these cells responded to vestibular stimulation at long latency.

Figure 6.

Locations of RVLM neurons that responded to vestibular nerve stimulation and had projections to the thoracic spinal cord. Symbols with different shapes are used to designate the type of response each neuron exhibited during stimulation. Filled symbols designate neurons that could be antidromically activated from T10, whereas open symbols indicate units that were antidromically activated from T1 but not T10. Numbers above each panel indicate the distance (in mm) separating the brainstem section from the obex. Abbreviations: *CD*, dorsal cochlear nucleus; *CI*, inferior central nucleus; *IFT*, infratrigeminal nucleus; *IO*, inferior olivary nucleus; *PH*, prepositus hypoglossi; *PPR*, postpyramidal nucleus of the

raphe; *py*, pyramid; *RB*, restiform body; *RFN*, retrofacial nucleus; *SA*, stria acustica; *Sp5*, spinal trigeminal nucleus; *VIN*, inferior vestibular nucleus; *VMN*, medial vestibular nucleus.

Table 1

Characteristics of responses of neurons to electrical stimulation of vestibular afferents. The data illustrated indicate the spontaneous firing rate of the neurons, the response latency from the last shock of the shortest train that elicited a response, and the response duration when a 5-shock train at 5T intensity was delivered. Data are provided for the total pool of neurons, and are also segregated by the type of response that neurons exhibited during stimulation: early and sharp excitation (*E-short*); long and diffuse excitation (*E-diffuse*); excitation followed by inhibition (*E-I*); or inhibition (*I*). Data for neurons that projected to T1 (but not T10), as well as T10 are indicated in separate rows.

Response Type	Projection Level	Spontaneous Firing Rate (spikes/s)	Latency from Effective Shock (msec)	Response Duration (msec)
E-short (n=26)	T1 (n=12)	17.4 ± 4.9	12.5 ± 1.9	47.4 ± 20.6
	T10 (n=14)	26.6 ± 5.9	14.4 ± 1.5	15.4 ± 5.5
E-diffuse (n=15)	T1 (n=7)	30.8 ± 10.4	50.4 ± 14.0	232.2 ± 41.6
	T10 (n=8)	48.4 ± 10.1	28.3 ± 9.6	309.6 ± 58.2
E-I (n=31)	T1 (n=14)	24.0 ± 7.4	10.9 ± 0.7	113.5 ± 31.8
	T10 (n=17)	25.2 ± 4.7	13.2 ± 0.8	51.4 ± 4.9
I (n=8)	T1 (n=2)	18.3 ± 2.9	19.5 ± 5.5	21.7 ± 2.6
	T10 (n=6)	10.4 ± 2.8	17.9 ± 3.2	39.3 ± 10.1
Total (n=80)	T1 (n=35)	22.7 ± 4.0	19.8 ± 3.8	109.3 ± 20.1
	T10 (n=45)	27.8 ± 3.5	16.9 ± 1.9	84.5 ± 18.9