Effect of baroreflex loading on the responsiveness of the vestibulosympathetic reflex in humans

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Dyckman DJ, Monahan KD, Ray CA. Effect of baroreflex loading on the responsiveness of the vestibulosympathetic reflex in humans. J Appl Physiol 103: 1001–1006, 2007. First published July 5, 2007; doi:10.1152/japplphysiol.00555.2007.—Activation of the vestibular otolith organs with head-down rotation (HDR) increases muscle sympathetic nerve activity (MSNA) in humans. Previously, we demonstrated this vestibulosympathetic reflex (VSR) elicits increases in MSNA during baroreflex unloading (i.e., lower body negative pressure) in humans. Whether such an effect persists during baroreflex loading is unknown. We tested the hypothesis that the ability of the VSR to increase MSNA is preserved during baroreflex unloading and inhibited during baroreflex loading. Ten subjects (26 ± 1 yr) performed three trials of HDR to activate the VSR. These trials were performed after a period of sustained saline (control), nitroprusside (baroreflex unloading: 0.8–1.0 μg·kg⁻¹·min⁻¹), and phenylephrine (baroreflex loading: 0.6–0.8 μg·kg⁻¹·min⁻¹) infusion. Nitroprusside infusion decreased (Δ7 ± 1 mmHg, where Δ is change; P < 0.001) and phenylephrine infusion increased mean arterial pressure (Δ8 ± 1 mmHg; P < 0.001) at rest. HDR performed during the control [Δ3 ± 2 bursts/min, Δ314 ± 54 arbitrary units (au) total activity, Δ41 ± 18% total activity; P < 0.05] and nitroprusside trials [Δ5 ± 2 bursts/min, Δ713 ± 241 au total activity, Δ49 ± 20% total activity; P < 0.05] increased MSNA similarly regardless of levels at rest (13 ± 2 to 26 ± 3 bursts/min) in the latter. In contrast, HDR performed during the phenylephrine trial failed to increase MSNA (Δ0 ± 1 bursts/min, Δ21 ± 33 au total activity, Δ−8 ± 12% total activity). These results confirm previous findings that the ability of the VSR to increase MSNA is preserved during baroreflex unloading. In contrast, the ability of the VSR to increase MSNA is abolished during baroreflex loading. These results provide further support for the concept that the VSR may act primarily to defend against hypotension in humans.

autonomic nervous system; blood pressure; orthostasis; sympathetic nerve activity

ANIMAL STUDIES HAVE ESTABLISHED the existence of a powerful vestibular-mediated reflex that contributes critically to the maintenance of arterial blood pressure (BP) in the upright posture. Doba and Reis (8) first reported that bilateral transection of the vestibular nerve resulted in persistent hypotension during upright tilt in the cat. Subsequent studies demonstrated that direct electrical stimulation of the vestibular nerve elicits pronounced effects on sympathetic nervous system outflow and vascular resistance in the cat (14, 15, 31, 35). Thus it appears that this vestibular-mediated reflex exerts its effect in part via the sympathetic arm of the autonomic nervous system.

Studies in humans have provided further support for the existence of a vestibulosympathetic reflex (VSR) (2, 12, 19, 34). Using head-down rotation (HDR) as a model to activate the vestibular otoliths, direct measurement of sympathetic outflow [muscle sympathetic nerve activity (MSNA)] has been repeatedly demonstrated to increase and elicit peripheral vasoconstriction (10, 18, 20–22, 24). Our laboratory has previously demonstrated that sympathetic activation during HDR is independent of central command, neck muscle afferents, visual inputs or other nonspecific receptors activated during head movements (20–22, 24). Thus it appears that during the transition from the supine to upright posture vestibular activation contributes to BP regulation. Importantly, this integrative response likely involves other powerful neurocardiovascular reflexes such as the baroreflexes. Therefore, orthostatic BP regulation depends on the interaction of various neurocardiovascular reflexes (4, 26).

Previously, our laboratory has established that the ability of the VSR to increase MSNA during vestibular activation (HDR) is well preserved during orthostatic stress imposed using lower body negative pressure (LBNP) (18). These data suggest that baroreflex unloading does not modulate the sensitivity of the VSR. Moreover, as the ability of the VSR to modulate MSNA is maintained during baroreflex unloading these data are consistent with the concept that the VSR is a powerful reflex that defends against hypotension (8, 11, 19). Whether this ability of the VSR to modulate MSNA persists during baroreflex loading is unknown. Previous animal studies suggest that raising BP (baroreceptor loading) attenuates the vestibulosympathetic responses (15). Thus, based on these previous studies, we developed and tested the hypothesis that the ability of the VSR to modulate MSNA would be maintained during conditions where hypertension (cerebral hypoperfusion) risk is elevated (baroreflex unloading) but not when it is decreased (baroreflex loading).

METHODS

Subjects

Ten young healthy volunteers (5 men and 5 women; age 26 ± 1 yr, height 176.0 ± 2.6 cm, weight 68.4 ± 3.8 kg) participated in the study. All subjects were nonsmokers, nonobese, normotensive, and not taking any medications that may influence the results of the study. The Institutional Review Board of the Pennsylvania State University College of Medicine approved the experiment, and written informed consent was obtained from all subjects before testing.

Measurements

Multifiber recordings of MSNA were obtained from a tungsten microelectrode inserted in the peroneal nerve behind or lateral to the
knee, as previously described (18). A reference electrode was placed subcutaneously 2–3 cm from the recording electrode. Previously identified criteria for an adequate MSNA signal were applied to ensure proper recording (27). The nerve signal was amplified (20,000–50,000 times), fed through a band-pass filter with a bandwidth of 700–2,000 Hz, integrated using a 0.1-s time constant (University of Iowa Bioengineering, Iowa City, IA), and recorded digitally (16SP Powerlab, ADInstruments, New Castle, Australia). The mean voltage neurogram was routed to a computer screen and a loudspeaker for monitoring during the study. Sym pathetic recordings that demonstrated possible electrode site shifts, altered respiratory patterns (e.g., breath holding, inspiratory gasp, and hyperventilation), or electromyographic artifact during experimental intervention were excluded from analysis.

Heart rate was derived from an electrocardiogram. BP was measured continuously by a finger photoplethysmograph (Finapres, Ohmeda, Englewood, CO) during each trial. Respiration pattern was measured using impedance plethysmography.

Experimental Design

The purpose of this study was to determine whether vestibular (i.e., oto lith organ) activation elicits increases in MSNA during baroreceptor loading and unloading. Subjects were instrumented for the study (BP, heart rate, and respiration), and a catheter was inserted in an antecubital vein. Then, subjects were placed in the prone position, and an appropriate MSNA recording site was established. The protocol consisted of three individual trials (trials 1, 2, and 3). Each trial was separated by at least 15 min, where resting BP and MSNA returned to resting values. All experiments were performed in a dimly lit, quiet laboratory maintained at 21–23°C.

Trial 1 (saline). During this trial, which served as a control trial, saline was infused intravenously throughout. Subjects performed HDR in the prone position as previously described (24). Briefly, after a 3-min baseline period with the head in the baseline chin-up neck-extended position, the chin support was removed and the head was passively rotated to the point of maximal rotation. This position was maintained for 3 min followed by the subject’s head being returned to the baseline chin-up neck-extended position for 3 min of recovery. This trial was therefore 9 min in duration.

Trial 2 (nitroprusside). Nitroprusside was infused during this trial. It was performed identically to trial 1 except in the 10-min period before the baseline data collection nitroprusside was titrated to induce a sustained decrease in mean arterial pressure of 10 mmHg. To accomplish this, nitroprusside infusion commenced at a dose of 0.2 μg·kg⁻¹·min⁻¹ for 3 min. After this period, the dose was titrated upward by 0.2 μg·kg⁻¹·min⁻¹ every 3 min until the desired effect on BP was obtained (0.8–1.0 μg·kg⁻¹·min⁻¹). Three minutes after a sufficient sustained decrease in BP was obtained, the baseline period began followed by 3 min of HDR and subsequently a 3-min period of recovery. Once the desired dose of nitroprusside was determined that infusion rate continued until the end of the trial (end of the 3 min recovery period). Subsequent trials did not commence until heart rate and BP returned to baseline (minimum of 15 min).

Trial 3 (phenylephrine). Trial 3 was identical to trial 2, except instead of infusing nitroprusside, phenylephrine was infused. Phenylephrine infusion commenced at a rate of 0.2 μg·kg⁻¹·min⁻¹. After 3 min, this dose was increased in 0.2 μg·kg⁻¹·min⁻¹ increments at 3-min intervals until BP increased by ~10 mmHg (0.6–0.8 μg·kg⁻¹·min⁻¹). Once this dose was established, the infusion continued until the HDR protocol was complete (3 min baseline period followed by 3 min of HDR and 3 min of recovery).

Data Analysis

All data were digitally recorded at 100 Hz for later offline analysis. MSNA was expressed as bursts per minute and total activity (sum of area underlying individual bursts per minute). Sym pathetic bursts were identified from the mean voltage neurogram, and the sum of the area under each burst, expressed in arbitrary units (au), was assessed by a computer program (Chart 5, ADInstruments). Each neurogram was normalized by assigning the tallest burst an amplitude of 1,000 au; each burst was identified from the mean voltage neurogram, and the sum of the amplitudes of each burst was calculated to yield total burst activity.

Statistics

To identify possible differences between each trial infusion, a two-within (infusion trial, intervention), repeated-measures ANOVA was used. Tests for simple effects were used to identify whether there were differences in baseline when the interaction term was significant (13). A significance level of P < 0.05 was used for all tests. Values are presented as means ± SE.

RESULTS

Hemodynamic changes are listed in Table 1. Nitroprusside infusion decreased mean arterial pressure (91 ± 2 to 84 ± 3 mmHg; P < 0.001) at rest (baseline). This was associated with a compensatory increase in heart rate (63 ± 2 to 76 ± 3 beats/min; P < 0.001) at rest (baseline). Phenylephrine infusion increased mean arterial pressure (91 ± 2 to 99 ± 3 mmHg; P < 0.001) and decreased heart rate (63 ± 2 to 57 ± 2 beats/min; P < 0.001) at rest (baseline).

Representative neurograms for each trial from a single subject are presented in Fig. 1. Nitroprusside infusion increased MSNA burst frequency (13 ± 2 vs. 26 ± 3 bursts/min before and during infusion, respectively; P < 0.001) and total MSNA (699 ± 129 vs. 1,815 ± 201 au; P < 0.001) at rest (baseline). Phenylephrine infusion decreased MSNA burst frequency (13 ± 2 vs. 2 ± 1 bursts/min; P < 0.001) and total MSNA (699 ± 129 to 112 ± 66 au; P < 0.001) (Fig. 2).

MSNA increased during HDR in the saline (∆3 ± 2 bursts/min, ∆314 ± 154 au total activity, ∆41 ± 18% total activity (where ∆ is change); P < 0.05) and nitroprusside trials (∆5 ±

<table>
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Values are means ± SE. HDR, head-down rotation; SNP, sodium nitroprusside; PE, phenylephrine; MAP, mean arterial pressure; HR, heart rate. *P < 0.001 vs. saline infusion baseline. †P < 0.001 vs. SNP baseline.
2 bursts/min, $\Delta713 \pm 241$ au total activity, $\Delta49 \pm 20\%$ total activity; $P < 0.05$) (Figs. 2 and 3). In contrast, HDR performed during the phenylephrine trial did not result in an increase in MSNA ($\Delta0 \pm 1$ bursts/min, $\Delta-15 \pm 33$ au total activity, $\Delta-8 \pm 21\%$ total activity; Figs. 2 and 3).

DISCUSSION

The major finding from the present study is that the ability of the VSR to elicit increases in MSNA through HDR is abolished during baroreflex loading. These data are consistent with prior animal studies (15) and provide further experimental support for the concept that the VSR is a powerful reflex system capable of defending against acute risks associated with hypotensive challenges (12, 28, 34, 35).

Our study demonstrates that during the steady-state infusion of nitroprusside, MSNA increases further during HDR (similar to the saline infusion), despite elevated MSNA at rest. Previously, our laboratory established that the ability of the VSR to elicit increases in MSNA during HDR was unaltered during baroreflex unloading induced using lower body negative pressure (18). Additionally, the preserved ability of the VSR to elicit increases in MSNA during HDR during baroreflex unloading suggests that the neural pathways mediating increases in MSNA are sufficiently distinct such that unloading of the baroreflex does not influence the magnitude of response of the VSR. Moreover, these data obtained during baroreceptor unloading are consistent with the observations that HDR performed during head-up tilt is associated with increases in systemic vascular resistance in subjects with neurogenic orthostatic hypotension (3).

In contrast to these observed responses during baroreflex unloading, the ability of the VSR to mediate increases in MSNA during baroreflex loading was blunted. In fact, when HDR was performed during phenylephrine infusion, where the resting level of MSNA was reduced, HDR was unable to elicit further increases in MSNA. The mechanism(s) underlying these effects are unknown. However, animal studies have shown that the ability of the VSR to stimulate increases in sympathetic outflow was abolished after BP at rest was increased by infusion of an $\alpha$-agonist (15). These data, in contrast to the data obtained during baroreflex unloading, suggest that a reflex interaction occurs. It seems the integration between the two reflexes, especially during the two different stimuli (i.e., baroreflex unloading and loading), is unclear in humans. According to animal data, the neurons in the rostral ventrolateral medulla appear to be inhibited by baroreceptor...
stimulation and those neurons are needed for relaying the vestibular signals (15). Our results suggest the baroreflex stimulation (loading) causes a large elevated inhibitory signal that impairs downstream vestibular effects on the peripheral vasculature, whereas baroreflex unloading allows the vestibular stimulation to enhance the overall generation of MSNA. Therefore, this interaction between the reflexes (baroreflex and VSR) occurs in a manner as to not compromise BP regulation at times when hypotensive risk is high (baroreflex unloading). This study provides data to suggest this pathway could be present in humans.

Other data derived from animal studies may also be important in regard to the present findings. For instance, it has been demonstrated that the VSR mediates increases in renal sympathetic nerve activity during hypergravity in rats (9). These increases were attributed to a rapidly acting vestibular-mediated feed-forward mechanism to prevent or attenuate decreases in BP when animals were subjected to gravitational stress. When both the baroreflex and VSR are intact, a modest pressor effect occurs. It was suggested that the true magnitude of the vestibular-mediated feed-forward response might be underestimated due to baroreflex restraint, which was similar to the cat data and could provide an explanation for our data in humans.

Collectively, these findings and our discussion are consistent with the suggestion that the VSR is a reflex system designed to respond to acute hypotensive challenges may be used as evidence for significant integration between these reflexes.

Previous studies in our laboratory demonstrated preserved MSNA responses during HDR while mean arterial pressure was elevated during isometric handgrip (17) and mental stress (7). We believe that our present data do not directly contradict those previous findings, although the MSNA responses differed. During exercise the baroreflex is reset around a higher prevailing level of BP (16). In contrast, during phenylephrine infusion we would not expect such an effect. This may help explain why the ability of the VSR to modulate increases in MSNA is preserved during exercise, but not baroreflex loading. Additionally, Anderson et al. (1) demonstrated that mental stress was able to increase MSNA further when MSNA was suppressed and BP was elevated during a steady-state phenylephrine infusion. The previous study in our laboratory also demonstrated an additive interaction between mental stress and the VSR (5, 7). However, mental stress could possibly reset the baroreflex operating point, similar to what could occur during exercise (6). Thus it may be important to consider the nature of the stimulus applied as well as any potential effect of the stimulus on the set point of the baroreflex.

In contrast to the compelling and definitive body of experimental evidence available from animals, there is less evidence that vestibular activation contributes directly to BP control in humans. More definitive evidence for a critical role of the vestibular system in orthostatic BP control in humans may be obtained in patients with altered vestibular inputs, through vestibular damage (30). These patients experience symptoms associated with orthostasis that can result in light-headedness or presyncope (33). Yates et al. (32) demonstrated an attenuated increase in BP in vestibular-deficient patients during linear acceleration compared with healthy controls. These results show that the loss of vestibular inputs can affect the increases in BP observed during gravitational stress. Consistent with this concept, when older adults perform HDR, the resultant increase in MSNA is blunted compared with responses observed in young adults (23). Interestingly, not only is the increase in
MSNA during HDR blunted but also this occurs in the face of a decrease in systemic BP (23). In addition, Wilson et al. (29) demonstrated that HDR attenuated an increase in cerebral vascular resistance only during LBNP suggesting that the VSR acts to redistribute blood flow throughout the body to maintain consciousness especially in times of orthostatic stress.

Several limitations deserve mention. First, using pharmacological substances to load and unload baroreceptors introduces unavoidable criticism that these substances directly or indirectly influence the results. However, we do not believe this is the case because the observed responses to HDR during the nitroprusside infusion provided results that are nearly identical to our previous data in which no pharmacological substances were used to unload the baroreflex (18). We cannot exclude that phenylephrine did not exert some direct effect on responses independent of baroreceptor loading. However, Somers et al. (25) utilizing steady-state phenylephrine infusions to examine the interaction of the baroreflex and chemoreflex was still able to demonstrate increased MSNA during hypercapnia and a cold-pressor test despite the steady-state phenylephrine infusion, demonstrating the sympathetic nerve responses were still intact. In addition, despite decreased MSNA at rest during the phenylephrine infusion, our results show a tendency to decrease in total activity (au) during HDR, an observation consistent with the results by Somers et al. (25) demonstrating the combination of hypoxia and phenylephrine infusion showed a tendency to decrease in total activity. It is noted that the trials were performed in the same order. Repeated HDR has demonstrated a consistent and similar increase in MSNA (10). Also, the drugs used in the present study have a short half-life, and the time between trials allowed MSNA and BP to return to baseline levels. In addition, another limitation could be that with the use of HDR to stimulate the VSR it is difficult to quantify the stimulus to the otolith organs. To minimize this concern, repeated HDR maneuvers were performed to the same degree of head rotation within subjects. Additionally, HDR is a complex stimulus that activates many different sensory receptors. However, because previous studies have demonstrated that other inputs during HDR (such as neck afferents, baroreceptors, central command, visual inputs) do not influence MSNA responses (20–22, 24), it is likely that the changes in MSNA we observed are directly attributable to stimulation of the VSR through HDR. It is possible that some of these other sensory inputs could have an impact on the integration of the baroreflex and VSR. Finally, studying vestibular deficient patients may provide more definitive insight into the interaction between the VSR and the baroreflexes.

In summary, VSR-mediated increases in MSNA during baroreflex unloading are preserved, whereas they are abolished during baroreflex loading. These results are consistent with prior animal studies. Collectively, these data provide further evidence for the concept that the VSR is a powerful neurocardiovascular reflex that is particularly important at times when immediate homeostatic control of the organism is most at risk (i.e., during acute hypotensive challenge).

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