Human cerebrovascular and autonomic rhythms during vestibular activation

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Subjects. We studied eight healthy volunteers (2 men and 6 women; age 19 ± 3.0 yr, height 167 ± 10 cm, weight 60 ± 17 kg; mean ± SE). Subjects were nonsmokers with no history of autonomic dysfunction and abstained from caffeine and exercise for at least 12 h before the study. The experimental protocol was approved by the Human Research Committee of Michigan Technological University, and all subjects provided written informed consent before participating.

Experimental design. Five minutes of baseline data were collected with subjects in the prone position with the neck extended and chin supported (to approximate the gravitational orientation of the head during standing). The head was then passively lowered in the vertical plane by one of the investigators to activate otolith organs of the vestibular system. This procedure has been outlined in detail elsewhere (27). After 5 min of data collection in the head-down position, the head was passively lifted to the original chin-supported position for an additional 5 min (recovery). During each of the three conditions, subjects controlled their respiratory rate by breathing in time to a metronome set at a pace of 15 breaths/min (0.25 Hz).

Measurements. Subjects were instrumented with a three-lead ECG, a finger cuff to record beat-by-beat arterial pressure with finger photoplethysmography (model 2300, Finapres, Ohmeda, Englewood CO), a pneumobelt for respiratory excursions (uncalibrated strain-gauge pressure transducer), a mouthpiece housing a sensor for infrared measurement of end-tidal CO2 concentrations (Gambro, Engström, Sweden), and a 2-MHz Doppler probe positioned at a constant angle over the temporal window to record cerebral blood flow velocity.

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from the left middle cerebral artery (DWL Elektronische Systeme, Sipplingen, Germany). To ensure that isonation angle remained constant during changes in head position, specialized head gear allowing the probe to be both adjusted and fixed in position was placed on the subject’s head and secured with an elastic band around the skull. We also measured MSNA by inserting a tungsten microelectrode (Haer, Bowdingam, ME) into the peroneal nerve in the popliteal region behind the left knee as previously described (3). We were satisfied that our nerve recordings represented efferent sympathetic traffic when bursts were pulse synchronous, increased in frequency and amplitude with Valsalva straining, and did not respond to tactile or auditory stimulation.

Data acquisition and analysis. Data were sampled at 500 Hz and recorded directly to computer with commercial hardware and software (WINDAQ, Dataq Instruments, Akron, OH). Data were then imported into a commercial analysis program (WinCPRS, Absolute Aliens, Turku, Finland). R waves generated from the ECG signal were detected and marked at their occurrence in time. Diastolic and systolic pressures were subsequently marked on the Finapres and Doppler tracings. Mean arterial pressure was calculated by computer on a beat-by-beat basis as diastolic pressure plus 1/3 pulse pressure. Using the arterial pressure waveform as an input, stroke volume and cardiac output were estimated by WinCPRS using the method outlined by Jansen et al. (17). Total peripheral resistance was also estimated online by dividing mean arterial pressure by cardiac output.

Mean cerebral blood flow velocity was calculated as a true average of each integrated waveform. However, each waveform was manually edited to correct spurious detection of maximal (systolic) and minimal (diastolic) velocities occurring as a result of noise spikes at the tops and bottoms of Doppler tracings. For this reason, mean velocity was derived as

\[ V_{\text{mean}} = V_{\text{max}} + 0.4(V_{\text{max}} - V_{\text{min}}) \]

where \( V_{\text{max}} \) is maximal velocity (systolic), \( V_{\text{min}} \) is minimal velocity (diastolic), and \( V_{\text{mean}} \) is mean velocity. This equation has been previously published (8). We derived Gosling pulsatility as an index of cerebral vascular resistance by subtracting diastolic from systolic velocity and then dividing by mean velocity (31). Both \( V_{\text{mean}} \) and Gosling pulsatility were calculated on a beat-by-beat basis by computer after manual editing of the Doppler signal. We also calculated a pulsatility ratio to account for potential influences of systemic pulse pressure on brain pulsatility (29) by dividing the cerebral velocity pulsatility by arterial pressure pulsatility. Increases and decreases in pulsatility index and ratio are associated with cerebral vessel constriction and dilation, respectively (13, 31).

Bursts of MSNA were detected and marked by computer. The computer identified potential bursts based on amplitude (>3:1 signal-to-noise ratio) and latency from the preceding (one removed) R wave to burst peak at 1.3 ± 0.5 s (12). Bursts were then displayed for visual confirmation, and the automated burst detection results were manually checked based on the above criteria. To control for variation in burst amplitudes between subjects (which relate to electrode placement within the nerve fascicle), we normalized sympathetic bursts by calculating the average area of all bursts recorded during baseline control. This average area was assigned a score of 1.0, such that subsequent bursts that were larger or smaller than baseline bursts were either greater than or less than 1.0. MSNA was expressed as bursts per minute, bursts per 100 heart beats, and total activity. Total activity was derived by multiplying the number of bursts occurring during a given phase (baseline, head down, and recovery) by the normalized area of each burst.

Although true stationarity may never be achieved in physiological systems, a certain degree of stationarity is assumed as long as steps are taken to ensure a steady, unchanging state. Transient changes induced on immediate assumption of the head-down position would violate our requirement for steady-state measurements. In addition, such head movement would activate semicircular canals, and our intent was to study otoh activation specifically (26, 32). Therefore, the last 3 min from each condition were considered to be steady state (30) and were used for both time- and frequency-domain analyses. Data were submitted to fast Fourier power spectral analysis and cross-spectral analysis to reveal their oscillatory frequencies and frequency-dependent linear associations. R-R intervals, arterial pressures, cerebral blood flow velocities, and MSNA were made equidistant by spline interpolating and resampling at 5 Hz. Data were then passed through a low-pass impulse response filter with a cutoff frequency of 0.5 Hz. Three-minute datasets were fast Fourier transformed with a Hanning window to obtain power spectra. Spectral power was expressed as the integrated areas within the low-frequency (0.05–0.15 Hz) and high-frequency (0.15–0.4 Hz) ranges. To more accurately compare power spectra from different individuals who may vary widely in total power, we also normalized our data in the low- and high-frequency ranges by dividing integrated low- and high-frequency spectra by the total power (minus oscillations occurring below 0.05 Hz) and then multiplied this value by 100 (28).

The strength of the linear association between two signals, the squared coherence, was calculated by dividing the cross-spectral densities of the two signals by the product of the individual autospectra. The transfer function magnitude was calculated by dividing the cross-spectrum of the two signals by the autospectrum of the first signal (5, 7, 8, 34). Transfer function magnitude was averaged in the low- and high-frequency ranges only where the squared coherence between the two signals was at least 0.5 (9).

Statistical analysis. We analyzed our data with commercial statistical software (SAS Institute, Cary, NC). Differences between the means of each dependent variable were tested with a one-way repeated-measures ANOVA with repeated measures on condition (baseline vs. head down vs. recovery). Significant differences were probed further with Duncan’s post hoc analysis. All data are expressed as means ± SE unless otherwise specified. We accepted differences as being significant if \( P \leq 0.05 \).

RESULTS

Time-domain analyses. Activation of vestibular otoh with head-down rotation did not significantly affect cardiovascular hemodynamics. R-R intervals, arterial pressures, and estimates of stroke volume, cardiac output, and peripheral vascular resistance were similar during all three conditions, although R-R intervals tended to increase during recovery (\( P = 0.06 \)). Controlled frequency breathing attenuated adequately maintained constant end-tidal CO₂ concentrations. Vestibular activation did not affect \( V_{\text{mean}} \), pulsatility index, or pulsatility ratio, although \( V_{\text{mean}} \) tended to decrease during recovery (\( P = 0.06 \)). As expected, head-down rotation caused dramatic and significant increases of MSNA. These descriptive time series data are displayed in Table 1. Figure 1 shows the effects of head-down rotation on \( V_{\text{mean}} \) and MSNA in one subject. In this subject, MSNA was doubled (approximately) with no change in \( V_{\text{mean}} \) during head-down rotation.

Frequency-domain analyses. Data presented in Table 2 show that head-down rotation had no effect on low- or high-frequency R-R interval, arterial pressure, or cerebral vascular oscillations. Results were the same regardless of whether data were expressed as actual integrated areas within each frequency band or as transformed, normalized areas. Representative power spectra from one subject during baseline are shown in Fig. 2.

Associations between pairs of signals were probed with cross-spectral analyses, and these results are shown in Table 3. Values for transfer function magnitude were considered valid only if the two variables displayed significant (i.e. >0.5)
We used the head-down rotation model as a means to activate otolith organs of the vestibule and increase MSNA to better understand associations among various autonomic oscillations and their frequency-dependent relations in humans. This is the first such study, and we report two primary new findings: 1) head-down rotation independently activates the sympathetic nervous system with no effect on parasympathetic activity or cerebral blood flow velocity; and 2) frequency-dependent associations between arterial pressures and cerebral blood flow velocities are independent of vestibular activation. These findings support the concept that vestibular-autonomic interactions independently and redundantly serve to maintain steady-state hemodynamics.

**Time-domain analyses.** Head-down rotation did not affect cardiovascular hemodynamics (Table 1). Others have confirmed that head-down rotation does not affect heart rate or arterial pressure (3, 16, 25) or only induces small increases (27). Head-down rotation has been shown to dramatically decrease calf blood flow and increase calf vascular resistance, and these changes have been attributed directly to vestibulomediated increases of MSNA (27). We documented significant increases in MSNA with head-down rotation without significant changes in estimated cardiac output, stroke volume, or peripheral resistance. Such responses suggest that regional vascular compensations (which we did not measure) were appropriate for the increased sympathetic neural traffic.
Table 2. Frequency domain data during controlled-freQUENCY breathing in the prone (baseline), head-down rotation (head down), and prone (recovery) positions

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Head Down</th>
<th>Recovery</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RRI-HF, ms$^2$</td>
<td>597±245</td>
<td>837±545</td>
<td>819±345</td>
<td>0.76</td>
</tr>
<tr>
<td>RRI-LF, ms$^2$</td>
<td>531±198</td>
<td>599±211</td>
<td>445±113</td>
<td>0.79</td>
</tr>
<tr>
<td>SAP-HF, mmHg$^2$</td>
<td>48.1±8.4</td>
<td>39.8±9.1</td>
<td>48.3±8.5</td>
<td>0.28</td>
</tr>
<tr>
<td>SAP-LF, mmHg$^2$</td>
<td>51.9±8.4</td>
<td>60.1±9.2</td>
<td>51.6±8.5</td>
<td>0.29</td>
</tr>
<tr>
<td>LF/HF</td>
<td>1.8±0.78</td>
<td>2.49±0.66</td>
<td>1.8±0.65</td>
<td>0.61</td>
</tr>
<tr>
<td>SAP-HF, mmHg$^2$</td>
<td>3.4±1.5</td>
<td>3.5±1.8</td>
<td>2.1±0.53</td>
<td>0.42</td>
</tr>
<tr>
<td>SAP-LF, mmHg$^2$</td>
<td>12.3±8.1</td>
<td>14.6±10.9</td>
<td>3.6±1.0</td>
<td>0.39</td>
</tr>
<tr>
<td>SAP-HF, ms</td>
<td>39.9±9.1</td>
<td>37.3±7.3</td>
<td>45.1±8.7</td>
<td>0.58</td>
</tr>
<tr>
<td>SAP-HF, ms</td>
<td>61.9±9.1</td>
<td>62.7±7.3</td>
<td>54.9±8.7</td>
<td>0.57</td>
</tr>
<tr>
<td>Vmean-HF, cm/s$^2$</td>
<td>4.4±1.8</td>
<td>6.7±4.0</td>
<td>4.2±1.8</td>
<td>0.31</td>
</tr>
<tr>
<td>Vmean-LF, cm/s$^2$</td>
<td>4.5±2.5</td>
<td>3.4±0.9</td>
<td>2.7±0.6</td>
<td>0.61</td>
</tr>
<tr>
<td>Vmean-HF, ms</td>
<td>53.3±6.7</td>
<td>52.4±4.7</td>
<td>52.2±5.4</td>
<td>0.98</td>
</tr>
<tr>
<td>Vmean-LF, ms</td>
<td>46.7±6.7</td>
<td>47.5±4.7</td>
<td>47.7±5.5</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Values are means ± SE for 8 subjects. RRI-HF and RRI-LF, RRI spectral powers at the high and low frequencies; RRI-HF and RRI-LF, RRI normalized spectral powers at the high and low frequencies; SAP-HF and SAP-LF, SAP spectral powers at the high and low frequencies; SAP-HF and SAP-LF, SAP normalized SAP spectral powers at the high and low frequencies; Vmean-HF and Vmean-LF, Vmean spectral powers at the high and low frequencies; Vmean-HF and Vmean-LF, Vmean normalized Vmean spectral powers at the high and low frequencies.

With CO$_2$ constant, we anticipated that any changes observed in cerebral blood flow velocity would relate directly to subjects being in the head-down position and to direct vestibular stimulation on MSNA. To our knowledge, only one other study has investigated interactions between head-down rotation and cerebrovascular responses (30). Wilson et al. (30) found head-down rotation had no effect on cerebral blood flow velocity but increased estimates of cerebral vascular resistance. In the present study, head-down rotation did not affect cerebral blood flow velocity or estimates of cerebral vascular resistance despite an approximate doubling of MSNA. [We used the method outlined by Levine et al. (20) to estimate cerebral vascular resistance whereas Wilson et al. (30) used a different estimate.] Although complete ganglion blockade increases transfer function magnitude between arterial pressure and cerebral flow velocity (35), others have reported minimal importance for the autonomic nervous system on cerebral autoregulation. Jordan et al. (18) found only a moderate influence of sympathetic activation with upright tilt or deactivation with ganglionic blockade on cerebral blood flow velocity. Levine et al. (20) reported that sympathetic activation via lower body suction dramatically increases peripheral vascular but only minimally increases cerebral vascular resistance. Our laboratory has recently shown that acute head-down tilt, a maneuver that decreases MSNA (6), has no effect on cerebral blood flow velocity (8). Taken together, these results suggest that peripheral sympathetic activity impacts cerebral vascular responses minimally.

**Frequency-domain analyses.** Frequency-domain analysis of autonomic rhythms has at least one advantage over time-domain measures of signal variability, and that is separation of oscillations into frequency bands that may be attributed to autonomic neural activity. Spontaneous fluctuations in heart period intervals are abolished by atropine administration, and therefore respiratory sinus arrhythmia is thought to be mediated entirely by parasympathetic neural activity (1). Arterial pressures, cerebral blood flow velocities, and MSNA also fluctuate around the respiratory frequency due to direct modulation by respiration (2) and/or through mechanical influences of respiration translating through arterial baroreflexes (33). Autonomic oscillations occurring at frequencies lower than respiration probably have several origins, including parasympathetic neural activity (1), sympathetic modulation of vascular resistance mediated by arterial baroreflexes (14), and/or intrinsic oscillations from a central pacemaker (15). From the subject depicted in Fig. 2, it is apparent that maintaining a strictly controlled respiratory rate allows for the separation of low- and high-frequency rhythms without one or the other...
frequency range being confounded by slow deep or fast shallow breaths. We detected clear respiratory peaks in all of our variables and found that these high-frequency rhythms were not affected by vestibular stimulation. Low-frequency rhythms were similarly unaffected. Although these results do not add insight into mechanisms controlling autonomic rhythms, at least two points may be made.

First, our results underscore difficulties in noninvasively estimating sympathetic neural activation from power spectral analysis (22). Head-down rotation did not affect R-R interval or systolic pressure spectral power at the low frequency, nor did it affect the high-frequency:high-frequency ratio, which others have used as an index of sympathetic activation during vestibular stimulation (19). Frequency-domain estimates of sympathetic traffic failed to accurately reflect directly measured MSNA during head-up tilt (7), head-down tilt (6), and now, during head-down rotation (regardless of whether spectral power was expressed in normalized or absolute units). As suggested by others (11), care should be taken in the interpretation of results when using frequency-domain estimates as surrogates for directly measured sympathetic traffic.

Second, our results conflict with those of Lee et al. (19), who recently showed that head-down rotation decreases high-frequency R-R interval variability. The conclusion, that head-down rotation decreases vagal-cardiac control (19), seems reasonable because sympathetic and parasympathetic activities are usually [but not always (11)] reciprocal. In the present study, respiratory rate was strictly controlled, whereas subjects in the study by Lee et al. (19) were allowed to breathe spontaneously. Although these authors report 1-min averages of respiratory rate and document no change during head-down rotation, the possibility that subjects had episodes of slower or faster breathing cannot be ruled out. Based on results of the present study, vestibular stimulation with head-down rotation specifically stimulates sympathetic efferents with no apparent effect on parasympathetic-cardiac control.

Coordination among autonomic, hemodynamic, and cerebrovascular rhythms. Autonomic rhythms that manifest as oscillations of arterial pressures, cerebral blood flow velocities, cardiac interbeat intervals, and neural traffic such as MSNA, may fluctuate at the same frequencies without necessarily being related. To determine whether pairs of signals oscillating at the same frequency are related (share a common origin), we performed coherence calculations. Signals were considered to be synchronized at specific frequencies if coherence values were ≥0.5 (9). Head-down rotation significantly increased diastolic pressure and MSNA transfer function magnitude at the high frequency and tended to increase these responses at the low frequency (Table 3). Transfer function magnitude decreased to baseline levels during recovery, and this decrease was significantly lower than baseline at the low frequencies.

Clear links between vestibular stimulation and arterial pressure control mechanisms have been demonstrated in both animals (10, 32) and humans (24, 27) with implications for maintenance of peripheral resistance and arterial pressure during orthostatic stress. With this construct, increased sympathetic responses to a given change in arterial pressure with vestibular stimulation would therefore seem to be an effective redundant physiological mechanism to ensure orthostatic stability. Unfortunately, in the present study only three subjects at the high frequency and five subjects at the low frequency showed significant coherence between diastolic pressure and MSNA during the baseline condition (Table 3), and therefore lack of a robust sample size limits the interpretation of these results.

To our knowledge, we are the first to submit MSNA and Vmean to cross-spectral analyses to probe frequency-dependent associations between peripheral autonomic sympathetic and cerebral blood flow velocity oscillations in humans. Low-frequency transfer function magnitudes are difficult to interpret because one-half of the subjects did not achieve coherence of at least 0.5. At the high frequency, however, our results revealed a transfer function magnitude during baseline conditions of ~1 cm s⁻¹burst⁻¹·min. Others have suggested that direct vestibular activation of sympathetic efferents is an effective mechanism independent of arterial baroreflexes to maintain peripheral resistance and arterial pressure during an orthostatic challenge (23, 32). Our data do not prove input-output relations between MSNA and Vmean but document unchanged frequency-dependent associations during vestibular activation.

Perspectives

Human autonomic rhythms are necessarily studied in a closed-loop environment, where definitive mechanisms, stimuli, and responses are often difficult to decipher. However, uncovering associations between control variables and putting these associations into some conceptual model that explains an outcome will increase understanding of how these systems are coordinated.
organized and therefore how these systems can sometimes fail. In the context of the variables investigated in this study, our results have implications for the study of orthostatic stability, as maintenance of an upright posture depends critically on adequate brain blood flow. Our results suggest that two systems contributing to maintenance of steady-state hemodynamics function independently and redundantly. Vestibular activation increases peripheral sympathetic nerve activity but has no effect on arterial pressure or cerebrovascular rhythms. Results from this study suggest that the sympathetic arm of the autonomic nervous system and vestibulosympathetic-cerebrovascular reflex loops function independently to maintain steady-state hemodynamics.

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