Hume, Keith M., and Chester A. Ray. Sympathetic responses to head-down rotations in humans. J. Appl. Physiol. 86(6): 1971–1976, 1999.—Muscle sympathetic nerve activity (MSNA) increases with head-down neck flexion (HDNF). The present study had three aims: 1) to examine sympathetic and vascular responses to two different magnitudes of HDNF; 2) to examine these same responses during prolonged HDNF; and 3) to determine the influence of nonspecific pressure receptors in the head on MSNA. The first experiment tested responses to two static head positions in the vertical axis [HDNF and intermediate HDNF (I-HDNF; ∼50% of HDNF)]. MSNA increased above baseline during both I-HDNF and HDNF (from 219 ± 36 to 301 ± 47 and from 230 ± 42 to 356 ± 59 units/min, respectively; P < 0.01). Calf blood flow (CBF) decreased and calf vascular resistance increased during both I-HDNF and HDNF (P < 0.01). Both the increase in MSNA and the decrease in CBF were linearly related to the magnitude of the downward head rotations (P < 0.01). The second experiment tested responses during prolonged HDNF. MSNA increased (from 223 ± 63 to 315 ± 79 units/min; P < 0.01) and CBF decreased (from 3.2 ± 0.4 to 2.6 ± 0.04 ml·100 ml⁻¹·min⁻¹; P < 0.01) at the onset of HDNF. These responses were maintained throughout the 30-min period. Mean arterial blood pressure gradually increased during the 30 min of HDNF (from 94 ± 4 to 105 ± 3 mmHg; P < 0.01). In a third experiment, head-down neck extension was performed with subjects in the supine position. Unlike HDNF, head-down neck extension did not affect MSNA. The results from these studies demonstrate that MSNA: 1) increases in magnitude as the degree of HDNF increases; 2) remains elevated above baseline during prolonged HDNF; and 3) responses during HDNF are not associated with nonspecific receptors in the head activated by increases in cerebral pressure.

autonomic nervous system; cardiovascular control; orthostasis; vascular resistance; vestibulosympathetic reflex; otolith organs; semicircular canals

NEUROANATOMIC LINKS have been shown to exist between the vestibular and cardiovascular systems of animals (1, 12, 16). Therefore, it is not surprising that stimulation of the vestibular system can affect arterial pressure and blood flow. Direct stimulation of the vestibular nerves in animals results in increased sympathetic activity to a number of vascular beds (2, 4, 6, 15). Similarly, during natural vestibular stimulation (i.e., nose-up tilt in the cat) splanchnic sympathetic nerve activity has been demonstrated to increase (15). It was observed that, as the magnitude of the nose-up tilt increased, splanchnic nerve activity increased. In an earlier study, Doba and Reis (3) reported that both blood flow and vascular resistance in the femoral artery responded incrementally to nose-up tilt performed at two different magnitudes.

In humans, head-down neck flexion (HDNF) has been used as a model to study the vestibulosympathetic reflex because static HDNF stimulates the otolith organs. Muscle sympathetic nerve activity (MSNA) and calf vascular resistance (CVR) increase immediately with HDNF (7, 9, 11). However, sympathetic nerve activity to skin does not increase with the same maneuver (9). These data show that, when HDNF is performed in humans, there is differential sympathetic outflow. Because other inputs during head rotations have been systematically shown not to influence sympathetic nerve activity, the MSNA and vascular responses observed with HDNF have been attributed to stimulation of the vestibular system (8, 11). As with nose-up tilt in cats, static HDNF in humans stimulates the otolith organs.

Unlike static HDNF, dynamic head yaw rotation, which stimulates the semicircular canals, does not elicit marked sympathetic effects. This has been shown during sinusoidal horizontal semicircular canal stimulation at various frequencies (10). Dynamic head yaw rotation did not alter either MSNA or SSNA from stationary baseline levels. Thus it appears that, in humans, the otolith organs and not the horizontal semicircular canals mediate MSNA during head movements.

The purpose of the present study was to determine whether 1) MSNA increases in a graded fashion to HDNF performed at two different magnitudes of head rotation; 2) MSNA remains elevated above baseline levels during prolonged periods of HDNF; and 3) nonspecific receptors in the head also contribute to increases in MSNA during HDNF. It was hypothesized that MSNA would increase in proportion to the change in downward head rotation, because of greater otolith stimulation, and that MSNA would remain elevated during prolonged HDNF, because otolith stimulation is being maintained. Finally, it was hypothesized that MSNA would not change during head-down neck extension (HDNE), because the otolith stimulation would be opposite to that of HDNF. The results from this study demonstrate that MSNA increases as function of downward head rotation and the increase in MSNA during HDNF is maintained during prolonged periods. Also, nonspecific receptors in the head do not mediate the increase in MSNA during HDNF.

METHODS

Subjects

Thirty-seven volunteers (18 men and 19 women) [age, 23 ± 2 (SE) yr; height, 172 ± 2 cm; weight, 68 ± 3 kg] who were normotensive, nonsmokers, and not on medication were...
studied. After verbal explanation of the testing procedures, written informed consent was obtained from all of the subjects. The experiments were approved by the Institutional Review Board of the University of Georgia and Pennsylvania State University College of Medicine.

Experimental Design

Experiment 1 (n = 20). Studies were performed in subjects in the prone position. The subjects were positioned on a table such that the neck could be maximally flexed without interference from the end of the table. Each study began with the neck in the baseline position for 3 min. While in the baseline position, the neck was maximally extended and the chin was supported. This position approximates gravitational orientation of the head when an individual is in the upright posture (9, 11). After the 3-min baseline, one of three different head positions [control (no head movement), intermediate HDNF (I-HDNF), or HDNF] was performed for 3 min. During I-HDNF, the forehead rested comfortably on a supporting device while the neck was straight. The face was directed downward, and the neck was parallel to the floor. During HDNF, the head was maximally lowered over the edge of the table (i.e., chin to chest). After 3 min in the new position, the head was returned to the baseline position for a 3-min recovery period. Each subject performed a 9-min time-control trial with the head in the baseline position. An investigator moved the subject’s head to all positions. The control, I-HDNF, and HDNF trials were performed in random order.

Experiment 2 (n = 22). Studies were performed in subjects in the prone position. The head was oriented in the baseline position for 3 min (see Experiment 1). Subsequently, the head was moved by an investigator into HDNF, as previously described (n = 11), or the head remained in the baseline position (n = 11) for a 30-min time control. This was followed by 3 min of recovery in the baseline position. Because of the length of the trials, different subjects performed the HDNF and time-control studies.

Experiment 3 (n = 14). Studies were performed in subjects in the supine position. The subjects were positioned on a table so that the neck could be maximally extended downward without interference from the table. Each study began with the subject’s neck in the baseline position for 3 min. While in the baseline position, the neck was maximally flexed in a chin-to-chest manner. This position approximates head orientation when an individual is in the baseline position of experiments 1 and 2. After the 3-min baseline period, the neck was extended backward, lowering the head below the level of the heart. While in head-down neck extension (HDNE) the head was supported to allow normal respiration and swallowing. HDNE was performed for either 3 min (n = 9) or 30 min (n = 5). HDNE was followed by 3 min of recovery in the baseline position.

MSNA, calf blood flow (CBF), mean arterial pressure (MAP), and heart rate were measured during all trials for experiments 1 and 2. MSNA, MAP, and heart rate were measured during experiment 3. The ambient temperature of the laboratory during these experiments ranged from 21 to 23°C.

Measurements

Multifiber recordings of MSNA were made by inserting a tungsten microelectrode into a peripheral nerve located behind or lateral to the right knee. A reference electrode was positioned subcutaneously 2–3 cm away from the recording electrode. To ensure that an adequate recording site for MSNA was obtained, all of the following criteria were met: 1) weak electrical stimulation through the electrode elicited involuntary muscle contractions of the appropriate muscles but not paresthesia; 2) tapping of muscles or tendons innervated by the impaled nerve fascicle evoked afferent mechano-receptor discharges, but stroking the skin did not elicit afferent activity; 3) sympathetic impulses occurred as spontaneous bursts within the cardiac rhythm; 4) held expiration (apnea) resulted in increased sympathetic nerve activity; and 5) a sudden arousal stimulus (yell) did not elicit an increase in sympathetic nerve activity. The nerve signal was amplified (50,000–90,000 times) and filtered with a bandwidth of 700–2,000 Hz. The filtered signal was rectified and integrated (time constant, 0.1 s) to obtain a mean voltage display of the nerve activity. Sympathetic recordings that indicated possible electrode-site shifts or electromyogram artifact during the experimental interventions were excluded.

Continuous measurements of arterial blood pressure and heart rate were made by using a Finapres blood pressure monitoring unit (Ohmeda, Englewood, CO). CBF was measured in the contralateral left leg at 15-s intervals by venous occlusion plethysmography (Hokanson EC 4 plethysmograph, D. E. Hokanson, Bellevue, WA) by using a mercury-in-Silastic tube strain gauge placed around the largest diameter of the calf muscles. Circulation to the foot was arrested by a sphygmonanometer cuff, placed around the ankle, that was inflated to 200 mmHg. A venous congesting cuff was placed around the upper leg at a pressure of 50 mmHg. The mean voltage neurogram, heart rate, blood pressure tracing, and CBFs were collected (MacLab 8e, ADInstruments, Milford, MA) and routed to an on-line computer (Macintosh Quadra 840AV) for monitoring and data collection purposes throughout the study.

Angular rotation of the head during neck flexion or extension was measured by a custom-built electrogoniometer. This device comprises a flat aluminum bar, with a 10-turn, 10-kΩ linear potentiometer fitted to one end and a 9-V battery and 100-g brass weight attached to the other end. The battery powered the potentiometer to provide a variable voltage-divider circuit where an angular change in the potentiometer shaft was proportional to a change the output voltage. The potentiometer shaft was attached to an adjustable headgear at a point near the right ear, so that the device hangs vertically without impediment. As the subject performs neck flexion or extension, the headgear rotates, turning the potentiometer shaft while the device itself, owing to the mass of the battery and brass weight, maintains its vertical orientation. The electrogoniometer was calibrated by recording a signal at a designated reference position and again after rotating the potentiometer shaft to a predetermined angle. Head rotation was measured during the prolonged HDNE trials. Head rotation for I-HDNF and HDNF was also determined in these subjects. Two additional subjects performed HDNE, I-HDNF, and HDNF for head rotation measurements.

Data Analysis

MSNA was expressed as bursts per minute and total MSNA. Sympathetic bursts were identified from inspection of the mean voltage neurogram, and the sum of the area of those bursts per minute was measured by a computer program (Peaks, ADInstruments) and was reported as total MSNA, expressed in arbitrary units. CVR was determined as MAP divided by CBF for each minute. An analysis of variance for repeated measures was used to determine the significance of head position on the dependent variables in experiments 1, 2, and 3. A significance level of P < 0.05 was used for all
statistical tests. Trend analysis was performed on the dependent variables in experiment 1 to determine linearity (5). All values are means ± SE.

RESULTS

MSNA responses during the first minute of the time control, I-HDNF, and HDNF trials are shown in Fig. 1. MSNA did not increase during the control trial. However, MSNA significantly increased during the I-HDNF and the HDNF trials (P < 0.02). MSNA increased from 17 ± 2 to 21 ± 2 bursts/min and from 219 ± 36 to 301 ± 47 units/min for I-HDNF; and from 17 ± 1 to 22 ± 2 bursts/min and 238 ± 42 to 356 ± 59 units/min for HDNF. Trend analysis revealed that MSNA increased in a linear fashion as the magnitude of downward head rotation increased (P < 0.01).

Heart rate, MAP, CBF, and CVR responses for experiment 1 are presented in Table 1. CBF did not change during the control trial (Table 1). CBF decreased from 2.6 ± 0.2 to 2.3 ± 0.2 ml·100 ml−1·min−1 during I-HDNF and from 2.7 ± 0.2 to 2.1 ± 0.2 ml·100 ml−1·min−1 during HDNF. As with MSNA (Fig. 1), trend analysis showed that a linear relationship existed between the change in head position and the corresponding change in CBF (P < 0.01). CVR markedly increased from 43 ± 4 to 53 ± 6 units for I-HDNF (P < 0.01) and from 43 ± 4 to 54 ± 5 units for HDNF (P < 0.01). Neither MAP nor heart rate was affected significantly by changing head position.

MSNA responses to prolonged HDNF are shown in Fig. 2. MSNA increased at the onset of HDNF (13 ± 6 significantly by changing head position. During the I-HDNF and from 2.7 ± 1.0 ml·100 ml−1·min−1 and from 35 ± 4 to 43 ± 6 units; P < 0.01, respectively). Both of these responses were maintained for the entire 30 min of HDNF (P < 0.01). CBF was not changed during the 30-min time-control trial, whereas CVR was unchanged initially and then showed a gradual increase from 36 ± 3 to 42 ± 5 units at the later time points (P < 0.05). MAP increased the by the same magnitude during both the prolonged HDNF trial and 30-min time-control trial (from 94 ± 4 to 104 ± 5 and from 97 ± 4 to 105 ± 3 mmHg, control and HDNF, respectively; P < 0.01). However, the increase in MAP was gradual during the time-control trial and only elevated at the later stages during the HDNF trial. Heart rate was not different from baseline during either the prolonged HDNF trial or the 30-min time-control trial.

MSNA, heart rate, and MAP responses to the 3-min and 30-min trials of HDNE are shown in Table 2 and in Fig. 4, respectively. MSNA did not change from baseline levels during the 3-min or the prolonged 30-min trial of HDNE. MAP and heart rate were both decreased during HDNE for the 3-min and the 30-min trials (P < 0.05). The degree of head rotation in these subjects during HDNE was 108 ± 5°. Head rotations were 64 ± 3 and 128 ± 5° for I-HDNF and HDNF, respectively.

DISCUSSION

Increases in MSNA and CVR at the onset of HDNF have been previously reported (8, 9, 11). However, in all of these previous studies only the effect of maximal HDNF was tested. The current study was designed to

Table 1. Cardiovascular responses to acute head rotations (experiment 1)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>I-HDNF</th>
<th>HDNF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Intervention</td>
<td>Baseline</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>66 ± 2</td>
<td>67 ± 2</td>
<td>65 ± 2</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>99 ± 3</td>
<td>99 ± 3</td>
<td>99 ± 3</td>
</tr>
<tr>
<td>CBF, ml·min−1·100 ml−1</td>
<td>2.55 ± 0.20</td>
<td>2.67 ± 0.23</td>
<td>2.64 ± 0.24</td>
</tr>
<tr>
<td>CVR, mmHg·ml−1·100 ml−1</td>
<td>44 ± 4</td>
<td>43 ± 4</td>
<td>43 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 20. HDNF, head-down neck flexion; I-HDNF, intermediate HDNF; MAP, mean arterial pressure; CVR, calf vascular resistance. *Significantly different from baseline, P < 0.05.
examine MSNA and CVRs to graded static downward head rotation performed in the vertical axis and these same responses during a prolonged period of HDNF. The major new findings from the present experiments are 1) MSNA increases in a linear fashion as the magnitude of downward head rotation increases, 2) MSNA remains elevated above baseline levels during prolonged HDNF, and 3) increases in MSNA with HDNF are not mediated by activation of nonspecific receptors in the head that are related to head congestion or to cerebral pressure.

MSNA immediately increased with downward rotation; this is consistent with our previous findings (8, 9, 11). However, a new finding of this study is that the magnitude of the MSNA response was dependent on the degree of head rotation. Specifically, MSNA increased 37% during I-HDNF vs. 50% during HDNF. Head rotations measured with an electrogoniometer in a group of similar subjects showed that the I-HDNF position corresponds to 50 ± 2% of HDNF. The degree of head rotation in these subjects during HDNF was 128 ± 5°. On the basis of these head rotations, MSNA increased −0.5% for each degree of downward head rotation in this current study. Similarly, CBF decreased, in a linear fashion, −0.005 ml·100 ml−1·min−1 for each rotational degree of downward head movement. However, these generalizations are applicable only for these two magnitudes of head rotation. In this study, the incremental magnitude of the head rotation between I-HDNF and HDNF was large. Thus it is possible that a threshold stimulus, as well as a saturation point, exists for MSNA and CBF responses during downward head rotations. CVR increased above baseline levels during both I-HDNF and HDNF. However, the total CVR response was not very different between the I-HDNF and HDNF trials. The absence of a linear CVR response, despite a linear MSNA response, suggests that the absolute change in MSNA that occurred from I-HDNF to HDNF was not large enough to elicit a greater change in the end organ response. It is likely

Table 2. Sympathetic and cardiovascular response to 3 min of head-down neck extension (experiment 3)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1 min</th>
<th>2 min</th>
<th>3 min</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSNA, bursts/min</td>
<td>19 ± 4</td>
<td>16 ± 5</td>
<td>17 ± 5</td>
<td>17 ± 5</td>
<td>20 ± 5</td>
</tr>
<tr>
<td>MSNA, units</td>
<td>285 ± 78</td>
<td>240 ± 88</td>
<td>279 ± 95</td>
<td>254 ± 91</td>
<td>311 ± 89</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>71 ± 3</td>
<td>67 ± 3*</td>
<td>68 ± 3*</td>
<td>69 ± 3</td>
<td>71 ± 4</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>109 ± 5</td>
<td>99 ± 5*</td>
<td>102 ± 5*</td>
<td>103 ± 4*</td>
<td>109 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = MSNA, muscle sympathetic nerve activity. *Significantly different from baseline, P < 0.05.
that CVR is high at I-HDNF, thus making further increases difficult to measure in the calf.

We attribute the linear increase in the MSNA response to changes in the activation of the otolith organs. Inside the utricle and saccule are numerous hair cells that are oriented in all directions. These hair cells bend in accordance to the earth's constant gravitational pull (13). At any given head position, some hair cells are active while others are inactive. Thus, when the head is rotated to a new position, the combination of active and inactive hair cells changes. In this study, as the magnitude of downward head rotation increased, more hair cells were likely activated, with the result of greater afferent input from the otolith organs. The concept that the otolith organs mediate MSNA is strengthened by the observation that MSNA remained elevated during 10 min of HDNF (11).

During HDNF, the head is below the level of the heart; thus changes may occur in cerebral blood flow, intracranial pressure, and intracranial volume. It is not uncommon for subjects to report a congestive feeling in the head during HDNF. Increased cerebral pressure might result in stimulation of nonspecific pressure receptors. We addressed the role of nonspecific pressure receptors activated by increased cerebral pressure during HDNF by measurement of MSNA during HDNE in the supine position. HDNE allows the head to fall below the level of the heart, like HDNF, but stimulates the otolith organs in the opposite direction. Unlike the increase in MSNA observed with HDNF, MSNA did not change during HDNE. These results indicate that nonspecific pressure receptors in the head do not mediate increases in MSNA.

Because otolith stimulation is opposite during HDNF and HDNE, it might be expected that MSNA would decrease during HDNE compared with the increase in MSNA during HDNF. However, this was not observed in this study. We propose two possible explanations for this observation. First, the marked decrease in MAP with HDNE caused a baroreflex-mediated increase in MSNA that obscured our ability to detect a reduction in MSNA. However, the lack of an increase in heart rate during HDNE argues against this possible mechanism. Second, central integration of otolith afferent input is different between HDNE and HDNF.

MAP increased during both the time-control and the prolonged HDNF trials. Subject discomfort may have been responsible for the increase in MAP. Some subjects reported feeling uncomfortable, mainly because of neck extension and chest compression while lying prone for the duration of the prolonged trials. Although not measured in the current study, increases in circulating catecholamines or hormones (i.e., angiotensin II, vasopressin) may have influenced increases in MAP (14). A possible role for circulating agents is also indicated because MAP remained elevated during recovery.

MAP did not change during HDNF but decreased during HDNE. Why MAP was reduced during HDNE is unclear. However, the lack of an increase in sympathetic outflow during HDNE may partly be why MAP was decreased. In addition, a decrease in cardiac output may also contribute to the decrease in MAP with HDNE, as reflected by a reduction in heart rate.

The present study adds strength to the concept that the vestibular apparatus (i.e., otolith organs) mediates increases in MSNA with HDNF. First, MSNA immediately increases and remains elevated during HDNF. Second, MSNA appears to increase in a linear fashion as the head moves from the upright position through 128° of downward head rotation. Third, MSNA does not increase during HDNE performed in the supine position; this indicates that nonspecific receptors in the head are not mediating increases in MSNA during HDNF. These findings, along with our earlier results demonstrating that increases in MSNA during HDNF are not mediated by the cardiopulmonary and arterial baroreceptors (11), visual inputs (11), neck muscle afferents (8), and central command (9), provide strong support that the vestibular system is mediating the increase in MSNA. Additionally, increased muscle tension generated by vestibulospinal reflexes during
HDNF are also unlikely to have any effect on MSNA, because MSNA failed to increase during HDNE, when vestibulospinal reflexes should also be activated, and the forces generated would be well below levels (more than ~15% maximum voluntary contraction) needed to elicit increases in MSNA. Each of the above findings alone does not preclude a role for the vestibular system, but, taken together, they suggest that increases in MSNA with HDNF are mediated by vestibular activation in healthy humans.

In summary, the present study used two magnitudes of downward head rotation to alter the gravitational stimulus to the otolith organs. It was found that MSNA increases in a linear fashion as the magnitude of downward head rotation increases and that increases in MSNA are maintained during prolonged HDNF. Also, nonspecific pressure receptors in the head do not mediate increases in MSNA. These data, as well as our previous reports, provide support for the influence of the vestibular otolith organs on sympathetic outflow in humans.

The authors appreciate the technical assistance of Heidi Harris, Lindsey Steele, and Dr. Michael Herr, who built the electrogoniometer.

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