Is there diurnal variation of the vestibulosympathetic reflex: implications for orthostatic hypotension

Chester A. Ray, Charity L. Sauder, Stephanie A. Chin-Sang, and Jonathan S. Cook

Penn State Heart and Vascular Institute, Department of Cellular and Molecular Physiology, Clinical Research Center, Pennsylvania State University College of Medicine, The Milton S. Hershey Medical Center, Hershey, Pennsylvania

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Ray CA, Sauder CL, Chin-Sang SA, Cook JS. Is there diurnal variation of the vestibulosympathetic reflex: implications for orthostatic hypotension. Am J Physiol Heart Circ Physiol 305: H1555–H1559, 2013. First published September 6, 2013; doi:10.1152/ajpheart.00930.2012—Incidences of adverse cardiac events and orthostatic hypotension are associated with diurnal variations. The primary purpose of the present study was to determine if the vestibulosympathetic reflex (VSR) follows a diurnal variation in humans. We hypothesized that the VSR would be attenuated at night based on the relation between melatonin and the VSR. Arterial blood pressure, heart rate, calf blood flow, and muscle sympathetic nerve activity (MSNA) were measured in nine healthy subjects (28 ± 1 yr, 5 men and 4 women) at rest and during head-down rotation. Each subject was tested during the day at 11:34 ± 13 and again at night 22:10 ± 5. MSNA was significantly decreased at night compared with day (8 ± 1 vs. 11 ± 2 bursts/min, respectively, P < 0.02). Heart rate and arterial blood pressure at rest were significantly increased at night compared with day (heart rate: 70 ± 4 vs. 66 ± 4 beats/min and mean arterial blood pressure: 91 ± 2 vs. 87 ± 1 mmHg, respectively). MSNA and hemodynamic responses to head-down rotation were not significantly altered at night compared with day (changes of 3 ± 1 bursts/min and 25 ± 6% for MSNA and calf blood flow, respectively). The data indicate that MSNA at rest decreases during the late evening hours and exhibits a diurnal variation, whereas the VSR does not. In summary, diurnal variation of orthostatic hypotension in humans does not appear to be associated with changes in the VSR and MSNA at rest.

orthostatic tolerance; circadian rhythm; muscle sympathetic nerve activity

The vestibulosympathetic reflex (VSR) is believed to contribute to postural blood pressure regulation in humans (4, 30, 44). Both human and animal studies (11, 28, 29, 36, 42, 43) have demonstrated that activation of the VSR can trigger increases in sympathetic outflow. Our studies in humans, using head-down rotation (HDR), have demonstrated marked and rapid increases in muscle sympathetic nerve activity (MSNA). Moreover, we have observed that the VSR and baroreflexes can work together to augment sympathetic outflow during an orthostatic challenge (26, 32). Orthostatic hypotension has been reported to be an independent risk factor for cardiovascular mortality (16). Thus, the mechanism(s) responsible for orthostatic hypotension is important.

It has been demonstrated that there is a diurnal variation associated with orthostatic tolerance (14, 18, 38, 45). Inability to maintain blood pressure upon standing can be related to a failure of cardiac output and/or systemic peripheral resistance to increase appropriately. This phenomenon has also been documented in patients with autonomic failure (23). The increase in morning orthostatic hypotension may be related to an attenuation of cardiovascular reflexes, which would activate sympathetic outflow and assist in maintaining postural arterial blood pressure. Recent studies (10, 35) have demonstrated that a number of hemodynamic and autonomic function indexes have significant circadian rhythms. Shea et al. (35) reported that the highest arterial blood pressures occur during a circadian time corresponding to 9:00 PM. Currently, it is unknown if the VSR contributes to a diurnal variation in orthostatic tolerance.

Therefore, the primary purpose of the present study was to test if there is a diurnal variation of the VSR in humans. The secondary purpose was to determine if we could detect a diurnal variation in MSNA at rest between midmorning and evening in subjects that are awake. We hypothesized that the VSR would be attenuated in the morning hours compared with night because of reported increases in the incidence of orthostatic hypotension. Second, we hypothesized that MSNA would be lower at night in awake subjects than in midmorning based on hypothesized increases in sympathetic activity during the morning hours.

METHODS

Ethical approval. Written informed consent was obtained from all subjects after verbal explanation of the experimental protocol. The present study conformed with standards set by the Declaration of Helsinki. The Institutional Review Board of The Pennsylvania State University College of Medicine approved this study.

Subjects. Nine healthy subjects (5 men and 4 women, age: 28 ± 1 yr, height: 174 ± 3 cm, weight: 71 ± 6 kg) were tested. All subjects were normotensive, nonsmokers, nonobese, and not taking any medications that would interfere with measurements of the protocol. Subjects had normal sleeping patterns, and their sleep-wake cycle was not markedly different. We excluded those subjects with markedly different sleep-wake cycles (i.e., nightshift workers). Thus, it is unlikely that the circadian profile of melatonin would be profoundly different among the subjects in our group. All subjects received a physical examination before participation.

Experimental design. Eight subjects started the first trial at midmorning (11:34 ± 13 min). Subjects returned 10–12 h after the completion of the midmorning trial in the evening (22:10 ± 5 min) to repeat the procedures. These time points were selected to correspond to reported lowest and peak levels of endogenous melatonin (3). Moreover, these time differences would provide periods where the incidences of syncope would be markedly different because of its diurnal variation. Subjects were instructed not to sleep between the two sessions and not to exercise 12 h before and between the two sessions. Additionally, subjects lasted at least 4 h before each experimental trial. One subject performed the night trial first and the midmorning trial second. MSNA, calf blood flow, heart rate (HR), and mean arterial blood pressure (MAP) were measured continuously in

Address for reprint requests and other correspondence: C. A. Ray, Penn State College of Medicine, Heart and Vascular Institute H047, 500 University Drive, Hershey, PA 17033-2390 (e-mail: caray@psu.edu).
the prone position during 10 min of baseline, 3 min of HDR, and 3 min of recovery. MSNA recordings at rest were repeated in seven subjects on 2 separate days at midmorning to determine reliability of measurement.

During baseline of both trials, the subject’s neck was extended with the chin supported to bring the head upright as close to the vertical plane as possible. This position approximates the gravitational orientation of the head when an individual is in the upright posture (36). For HDR, the head was maximally lowered in the vertical plane over the edge of the table. An investigator moved the head by supporting the forehead and chin, thus producing a passive head movement. Once the head became stationary, only afferent inputs from the otolith organs and not the semicircular canals were engaged.

Measurements. HR and arterial blood pressure were continuously recorded during all trials using a Finometer (FMS, Amsterdam, The Netherlands). Before each trial, brachial artery blood pressure was measured by an automated sphygmomanometer (Dinamap, General Electric, Waukesha, WI).

Multifiber recordings of MSNA were obtained from a tungsten microelectrode inserted in the peroneal nerve behind or lateral to the knee, as previously described (26). A reference electrode was placed subcutaneously 2–3 cm from the recording electrode. The criteria for an adequate MSNA signal included 1) tapping of the muscles or tendons innervated by the nerve produced afferent mechanoreceptor discharges; 2) apnea produced an increase in sympathetic nerve activity; 3) stroking of the skin did not produce any afferent activity; and 4) a sudden, unexpected arousal stimulus (shout or clap) did not produce any increases in sympathetic activity (37). The nerve signal was amplified (20,000–50,000 times), fed through a bandpass filter with a band width 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, AD Instruments, New Castle, NSW, Australia). The mean voltage neurogram was routed to a computer screen and a loudspeaker for monitoring during the study. Sympathetic recordings that demonstrated possible electrode site shifts, altered respiratory patterns (e.g., breath holding, inspiratory gasp, and hyperventilation), or electromyographic artifacts during experimental intervention were excluded from analysis. MSNA was recorded from the same leg during both experimental trials.

Venous occlusion plethysmography was used to measure calf blood flow. A Hokanson EC-4 system (Bellevue, WA) with mercury-in-silastic strain gauges was used. Strain gauges were positioned around the maximal circumference of the calf. An ankle cuff was inflated to 220 mmHg to arrest circulation to the foot. Calf blood flow was recorded every 15 s during the trials. During blood flow measurements, a venous collecting cuff was inflated proximal to the knee to a pressure of 50 mmHg. Calf vascular resistance was calculated by dividing MAP by calf blood flow.

Data analysis. Sympathetic bursts were identified from individual inspection of the mean voltage neurograms and with computer assistance. A signal-to-noise ratio of 2:1 and a latency period of ~1.3 s from the R wave of the ECG was required. MSNA was expressed as burst frequency (in bursts/min) and total MSNA (i.e., sum of burst areas). The area of the bursts was measured by a computer program (Peaks, AD Instruments). Absolute changes from baseline are reported for burst frequency. Relative changes (in %) from baseline are reported for total MSNA.

For all trials, the 10 min of baseline were averaged together and reported as the baseline value for the respective trial. During HDR, the first minute is reported and was used for data analysis of the various measurements. Statistical analyses of the data during HDR trials were performed with two-within factor [time of day × intervention (HDR)] repeated-measures ANOVA ($n = 9$). A paired $t$-test was performed for hemodynamic and MSNA values at baseline. Pearson’s product correlation between the changes during night and day of MAP and MSNA during baseline was performed to determine association. Significance was set at $P < 0.05$. All data are presented as means ± SE.

RESULTS

Rest. Figure 1, left, shows a representative neurogram from one subject during baseline of the day and night trials. MSNA burst frequency was significantly less at night compared with day ($8 \pm 1$ vs. $11 \pm 2$ bursts/min, respectively, $P < 0.05$; Fig. 2). In contrast, there was no difference in MSNA burst frequency when measured at the same time (midmorning) on 2 separate days ($n = 7$, $11 \pm 2$ vs. $12 \pm 1$ bursts/min, $P = 0.82$). HR and arterial blood pressure were elevated at night compared with day ($70 \pm 4$ vs. $66 \pm 4$ beats/min, systolic arterial blood pressure: $123 \pm 3$ vs. $115 \pm 3$ mmHg; diastolic arterial blood pressure: $73 \pm 2$ vs. $70 \pm 2$ mmHg, and MAP: $91 \pm 2$ vs. $87 \pm 1$ mmHg, respectively, $P < 0.05$). Calf blood flow and calf

Fig. 1. Muscle sympathetic nerve activity (MSNA) recordings from one subject at baseline and during 30 s of head-down rotation (HDR) during day and night conditions. MSNA total activity is indicated above each recording. MSNA was lower at rest during the night trial compared with day trial. There was no difference in MSNA responses to HDR between the day and night trials. a.u., Arbitrary units.
vascular resistance were not significantly different between night and day at rest (calf blood flow: 3.2 ± 0.3 vs. 2.8 ± 0.5 ml·100 ml⁻¹·min⁻¹ and calf vascular resistance: 26 ± 4 vs. 29 ± 5 arbitrary units, respectively). The increases in MAP and decreases in MSNA at night at rest were not associated ($R^2 = 0.02$).

**HDR.** Figure 1, right, shows a representative neurogram from one subject during 30 s of HDR during the day and night trials. HDR significantly increased MSNA responses during both trials ($P < 0.05$; Fig. 3). However, time of day had no affect on MSNA responses during HDR (burst frequency: change of 3 ± 1 vs. 3 ± 1 bursts/min and total MSNA: change of 41 ± 24% and 68 ± 21%, night and day, respectively). Additionally, HDR significantly decreased calf blood flow and increased calf vascular resistance during the night and day ($P < 0.05$). As with MSNA, the time of day had no affect on calf blood flow or vascular resistance during HDR (calf blood flow: change of −25 ± 6% vs. −26 ± 7% and calf vascular resistance: change of 34 ± 10% vs. 41 ± 13%, night vs. day, respectively; Fig. 3). HR and MAP responses to HDR were not significantly altered between night and day (HR: change of 0 ± 1 vs. 0 ± 1 beats/min and MAP: change of −3 ± 2 vs. −1 ± 2 mmHg, respectively).

**DISCUSSION**

Two novel findings from the present study are 1) MSNA at rest is less at night compared with day, suggesting a diurnal variation in MSNA in awake humans, and 2) the VSR is not different between morning and night in humans. These two findings suggest that the VSR is unlikely to contribute to diurnal variations in orthostatic tolerance and that increases in MSNA associated during the morning hours might contribute to increased adverse cardiovascular events. However, the elevation of MSNA at rest in the morning also suggests that basal level of MSNA does not contribute to increase incidence of orthostatic hypotension.

It has been reported that there is a diurnal variation associated with orthostatic tolerance (14, 18, 23, 38, 45). In patients with autonomic failure, it has been reported that impaired orthostatic hypotension and tolerance in the morning was related to larger declines in stroke volume and cardiac output in the morning compared with evening (14). This finding is in contrast to normal healthy subjects, which have reduced orthostatic hypotension and tolerance because of declines in systemic vascular resistance and not stroke volume and cardiac output (23). Thus, this latter study implies a defect in sympathetic nervous response to an orthostatic challenge. Baroreflex sensitivity has been reported to be reduced in the morning and may contribute to this response (22). We speculated that an attenuation of the VSR during the morning hours contributes to this slower activation of sympathetic outflow and to the lower orthostatic tolerance previously reported in the morning hours. However, because there was no diurnal variation of the VSR, it is unlikely that this reflex would be associated with the diurnal variation of orthostatic tolerance.

Previous research from our laboratory demonstrated that 3 mg of exogenous melatonin attenuates the VSR in humans (6). However, the VSR was not altered by time of day in the present study (Fig. 3). The expected peak physiological melatonin levels during evening hours is ~60 pg/ml, whereas, the peak in plasma melatonin due to 3 mg oral melatonin ingestion was ~900 pg/ml (6, 27). Therefore, it is plausible that the lower physiological peak levels of plasma melatonin concentration in our subjects were not sufficient to elicit an attenuation of the VSR, as in our previous study with melatonin supplements.

Altering sympathetic activity has been demonstrated to alter adrenergic responsiveness of the vasculature in humans (5, 9). In the morning hours compared with afternoon, Panza et al. (24) observed an increase in α-sympathetic vasomotor tone. This alteration in α-vasoconstrictor activity coincides with our observation of greater MSNA in the morning. Furthermore, Middlekauff and Sontz (17) hypothesized that an increase in tissue responsiveness to norepinephrine increases the incidence of adverse cardiac events. Recently, Scheer et al. (33) observed
a circadian rhythm associated with norepinephrine and epi-
ephrine release, demonstrating that both hormones decrease
during late evening hours. These results combined with our
present results indicate a diurnal variation of MSNA with
activity greater during the morning hours and less during the
night hours between 10:00 PM and 4:30 AM.

Why was a diurnal variation in MSNA observed in the
present study and not by previous studies (17, 22)? First,
Middlekauff and Sontz (17) made their measurements at 6:30
to 8:30 AM and 2:00 to 4:00 PM to coincide with the observed
peak and trough of adverse cardiac events, respectively, and
cortisol was used as their circadian marker. However, in the
present study, our time points were chosen to coincide with the
known trough and zenith in plasma melatonin (3), which would
allow us to make measurements during both day and night.
Additionally, the morning time point coincides with increased
rates of orthostatic hypotension (14, 18, 45). Plasma melatonin
levels would not be expected to be different between the two
time points used by Middlekauff and Sontz (17). Therefore, if
melatonin is important for the diurnal variation of MSNA in
humans, the results of Middlekauff and Sontz (17) would be
expected because of the time points tested. Second, Nakazato
et al. (22) reported no difference in MSNA at rest at 7 AM and
midnight. We cannot explain the difference between our study
and theirs except that our morning time points varied by ~4 h.
However, it would be expected that the increase in MSNA
would be at least the same or greater since they measured
MSNA at 7 AM, when adverse cardiac events are even greater.

Whether arterial blood pressure follows a circadian rhythm
in humans is equivocal. Several studies (12, 39) have demon-
strated that arterial blood pressure does not appear to have a
circadian rhythm. However, recent well-controlled studies by
Scheer et al. (33) and Shea et al. (35) have indicated that
arterial blood pressure has a circadian rhythm with a peak in
the evening and lower pressure in the early morning hours.
This finding is consistent with our observations of an increase
in arterial blood pressure in the evening despite behavioral
activity during the day that was not rigorously controlled.

Baroreflex loading has been demonstrated to decrease
MSNA at rest (7). It is possible that the decrease in MSNA
during the night trial is due to the observed elevated MAP.
However, this effect is not likely because the change from night
to day in MAP and MSNA was not significantly associated.
Therefore, it is probable that the small increase in baroreflex loading did
not affect the results. Additionally, subjects were not instructed to
rest before the night study. Because the subjects engaged in their
daily activities before arriving to the laboratory, we would expect
MSNA to be elevated. Thus, the decrease in MSNA at night
suggests that the body’s circadian rhythm has a powerful influ-
ence on MSNA.

The frequency of adverse cardiac events increases in the
morning hours with decreases in events at night (15, 19, 20,
41). An increase in sympathetic nerve activity, due to a
circadian rhythm or an increase in morning activity, has been
hypothesized to increase the occurrence of early morning
cardiac events (13, 25). Patients who have an increased sus-
ceptibility to cardiovascular incidents (e.g., hypertension and
coronary heart disease) demonstrate an altered circadian
rhythm of HR variability (8, 21), vasodilatory response (34),
and plasma melatonin level (2). Therefore, these findings
suggest that the cyclic variability of cardiac events is related to
the body’s biological clock. Because the subjects in the present
study were awake before the day and night trials, the observed
diurnal variation in MSNA suggests there is a possible intrinsic
circadian rhythm to MSNA in humans despite the limitation of
not controlling behavioral activity between trials.

Epidemiological data suggest that夜shift workers have a
40% increased risk of cardiovascular disease (1). Although the
exact cause is unknown, the underlying mechanism is hypo-
thesized to be related to changes in circadian rhythm, such as
changes in melatonin (31, 40). Because we observed a diurnal
variation in MSNA in young, healthy subjects, future research
on circadian rhythms of MSNA should include populations that
exhibit altered circadian rhythms in addition to increased risk
for adverse cardiac events.

In summary, MSNA at rest decreases during the late evening
hours compared with midmorning and exhibits diurnal varia-
tion, whereas the VSR does not. The diurnal variation of
MSNA is a novel finding that furthers our understanding of the
physiological effects of circadian rhythms in humans. Furth-
more, diurnal variation of MSNA in humans may be important
for understanding the cause of increased adverse cardiac events
in the morning hours. Finally, reported diurnal variations in
orthostatic tolerance do not appear to be associated with the
VSR.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: C.A.R. conception and design of research; C.A.R.,
C.L.S., S.A.C.-S., and J.S.C. performed experiments; C.A.R., C.L.S., and
S.A.C.-S. interpreted results of experiments; C.A.R., C.L.S., and J.S.C.
final version of manuscript; J.S.C. prepared figures.

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