Influence of increased plasma osmolality on sympathetic outflow during apnea

Jody L. Greaney,1 Chester A. Ray,2 Allen V. Prettyman,2 David G. Edwards,1 and William B. Farquhar1

1Department of Kinesiology and Applied Physiology, University of Delaware, Newark, Delaware; 2School of Nursing, University of Delaware, Newark, Delaware; and 3Department of Medicine and Cellular and Molecular Physiology, Pennsylvania State University College of Medicine, Hershey, Pennsylvania

Submitted 25 May 2010; accepted in final form 19 July 2010

Greaney JL, Ray CA, Prettyman AV, Edwards DG, Farquhar WB. Influence of increased plasma osmolality on sympathetic outflow during apnea. Am J Physiol Regul Integr Comp Physiol 299: R1091–R1096, 2010. First published July 21, 2010; doi:10.1152/ajpregu.00341.2010.—Animal models have shown that peripheral chemoreceptors alter their firing patterns in response to changes in plasma osmolality, which, in turn, may modulate sympathetic outflow. The purpose of this study was to test the hypothesis that increases in plasma osmolality augment muscle sympathetic nerve activity (MSNA) responses to chemoreceptor activation. MSNA was recorded from the peroneal nerve (microneurography) during a 23-min intravenous hypertonic saline infusion (3% NaCl; HSI). Chemoreceptor activation was elicited by voluntary end-expiratory apnea. MSNA responses to end-expiratory apnea were calculated as the absolute increase from the preceding baseline period. Plasma osmolality significantly increased from pre- to post-HSI (284 ± 1 to 290 ± 1 mOsm/kg H2O; P < 0.01). There was a significant overall effect of osmolality on sympathetic activity (P < 0.01). Duration of the voluntary end-expiratory apnea was not different after HSI (pre = 40 ± 5 s; post = 41 ± 4 s). MSNA responses to end-expiratory apnea were not different after HSI, expressed as an absolute change in burst frequency (n = 11; pre = 8 ± 2; post = 11 ± 1 burst/min) and as a percent increase in total activity (pre = 51 ± 4% AU; post = 53 ± 4% AU). A second group of subjects (n = 8) participated in 23-min volume/time-control intravenous isotonic saline infusions (0.9% NaCl). Isotonic saline volume-control infusions yielded no change in plasma osmolality or MSNA at rest. Furthermore, MSNA responses to apnea following isotonic saline infusion were not different. In summary, elevated plasma osmolality increased MSNA at rest and during apnea, but contrary to the hypothesis, MSNA responsiveness to apnea was not augmented. Therefore, this study does not support a neural interaction between plasma osmolality and chemoreceptor stimulation.

blood pressure; MSNA; osmoreceptor; chemoreceptor; hypoxia; osmolality

IN HUMANS, CHANGES IN CHEMORECEPTOR stimulation (26, 33) and changes in plasma osmolality (9, 12) have been shown to independently alter sympathetic outflow. Hypocapnia acts primarily on centrally located chemoreceptors, potentially located on the medullary surface (7); hypoxia exerts effects principally through the peripheral receptors located on the carotid bodies (24, 41). Hypocapnia and hypoxia increased muscle sympathetic nerve activity (MSNA) when assessed both during spontaneous breathing and during a voluntary apnea (19, 23, 26, 33).

Experimentally induced changes in plasma osmolality also influence the control of sympathetic outflow in both animals (4, 22, 27, 30, 43) and humans (9, 12, 44). Acute administration of hypertonic saline in anesthetized animals increased splanchic, adrenal, and lumbar sympathetic activity and blunted renal sympathetic activity (4, 22, 27, 43). Increases in plasma osmolality in humans, also induced via hypertonic saline infusion, augmented basal MSNA (12), indicating a potential direct relation between osmolality and sympathetic activity.

Studies using animal models have suggested that peripheral chemoreceptors are sensitive to direct osmotic stimulation, although results are conflicting, with studies reporting hyperosmolality-induced increases (15, 39) or decreases (14) in afferent carotid chemoreceptor firing. These contrasting results may be the consequence of differences in experimental preparation and design. However, these studies indicate that chemoreceptor nerve endings are responsive to changes in plasma osmolality by modifying their firing patterns.

Despite the well-recognized effect of chemoreceptors and, separately, osmoreceptors on sympathetic activity, it is not known whether these two mechanisms interact in modifying the control of sympathetic outflow in humans. Chemoreceptors (7, 16–17), and also osmosensitive neurons (2, 5, 38), have been localized to the ventrolateral medulla, which may be a central site for the potential interaction of these reflex arcs in humans. Therefore, the purpose of this study was to determine whether acute elevations in plasma osmolality alter the sympathetic outflow during chemoreceptor stimulation. On the basis of our previous observations, it was hypothesized that the MSNA responses to apnea would be augmented during the condition of elevated plasma osmolality. To test this hypothesis, we assessed MSNA under normal and elevated plasma osmolality conditions during spontaneous breathing and maximal voluntary end-expiratory apnea.

MATERIALS AND METHODS

Subjects. Fifteen subjects (7 male, 8 female) were recruited to participate in this study. All participants were healthy and normotensive (age: 31 ± 3 yr; body mass index: 24.5 ± 0.7 kg/m2). Women were tested during the early follicular phase of their menstrual cycle. The Human Subjects Review Board at the University of Delaware approved all study procedures before data collection. All participants provided full verbal and written consent before study participation.

Screening visit. All participants completed a medical history form and a physical activity readiness questionnaire. Height and weight were measured (Healthometer Scale, Continental Scale, Bridgeway, IL), and body mass index was calculated. A resting 12-lead electrocardiogram was performed (Schiller AT-10; Electra-Med, Flint, MI). Baseline blood samples were obtained for a complete blood count, a lipid profile (total cholesterol, HDL-C, LDL-C, and triglycerides), fasting blood glucose, liver and kidney function, and electrolytes. Subjects were excluded for any preexisting medical condition, tobacco use, or obesity (defined as a body mass index > 30 kg/m2). In addition, subjects practiced a maximal voluntary end-expiratory apnea several times to ensure a consistent maximal apneic duration during the experimental trials.

Address for reprint requests and other correspondence: W. B. Farquhar, 541 South College Ave., Fred Rust Arena-Office #143/HPL, Newark, DE 19716 USA (e-mail: wfb@udel.edu).
Measurements. Heart rate was measured via a single-lead ECG throughout the experiment (Dinamap Dash 2000, GE Medical Systems, Milwaukee, WI). Blood pressure was monitored on a beat-by-beat basis (Finometer, Finapres Medical Systems, The Netherlands). Blood pressure values obtained from the Finometer correlate well with directly measured radial artery blood pressure (28). Blood pressure values from the Finometer were obtained from a cuff placed on the right middle finger, supported at heart level. Finger pressure was calibrated to brachial artery pressure, according to the manufacturer’s instructions. Systolic and diastolic blood pressures were also obtained using an oscillometric upper arm blood pressure cuff placed on the left arm (Dinamap Dash 2000, GE Medical Systems, Milwaukee, WI). Breathing depth and frequency were monitored using elastic respiratory transducer bands wrapped around the midstitch and upper abdomen (Inductotrace System, Ambulatory Monitoring, Ardsley, NY). Arterial oxygen saturation was measured by pulse oximetry, with a probe placed on a finger of the left hand (Dinamap Dash 2000, GE Medical Systems, Milwaukee, WI).

Multiunit, postganglionic MSNA was recorded using a tungsten microelectrode inserted into the peroneal nerve below the fibular head, as previously described (37, 40). The microelectrode was inserted manually through the skin into the fascicle of the peroneal nerve to record efferent nerve signals. A reference electrode was inserted 2–3 cm away. MSNA was distinguished from other nerve signals using the following characteristics: increased burst activity during voluntary end-expiratory apnea; the presence of bursts with passive muscle stretching, but not with skin stretching in the innervated area or with arousal stimuli, such as unexpected loud noises (40). The raw signal was amplified (70,000-fold), band-pass filtered (700–2,000 Hz), rectified (full), and integrated (time constant: 0.1 s) before recording (model no. 662–4, Nerve Traffic Analyzer, University of Iowa Bioengineering, Iowa City, IA).

Protocol. To avoid wide fluctuations in sodium and fluid intake between subjects, for the 3 days before the experimental visit, subjects were instructed to follow a controlled sodium diet, in which they consumed ≤2,300 mg of sodium per day (3, 29). In addition, subjects were instructed to consume 1,800 ml of water the day before the study and an additional 600 ml of water on the morning of the study. Participants were instructed to be fasted and to avoid caffeine, alcohol, and exercise for the 12 h prior to the experimental visit. On arrival to the laboratory, subjects provided a urine sample. This was used to measure urine specific gravity, and for the women, to confirm menstruation.

The subjects were instrumented in a semirecumbent position, and 20-gauge intravenous catheters were placed in a vein in the left and right antecubital area: one was for infusion of saline and the other was for blood sampling. A 3% NaCl solution was infused (Lifecare 5000 Infusion Pump, Abbott Laboratories, North Chicago, IL) at a rate of 1.2 kg/m² during an infusion of isotonic (0.9%) saline. Similar procedures were followed; however, the rate of infusion was increased to 0.25 ± 0.02 ml/kg⁻¹·min⁻¹ for 23 min to better match fluid volume expansion.

Data analysis. The respiratory, ECG, blood pressure, and MSNA signals were sampled at 500 Hz using Windaq recording software (DATAQ Instruments, Akron, OH). The ECG was peak detected, and the blood pressure waveform was peak and valley detected (Windaq Waveform Browser, Advanced CODAS Software). MSNA was first identified by visual inspection, ensuring a signal-to-noise ratio of greater than 3:1, and then analyzed with custom software written in Labview (MatLab, The MathWorks, Natick, MA) (18). The time delay from the R wave to the corresponding sympathetic burst was measured in Windaq and used in the MatLab program to determine that sympathetic bursts occurred in time with the cardiac cycle (36). The amplitude of the largest burst at baseline, before the start of the protocol, was assigned a voltage value of 1,000 arbitrary units (AU), and a period of neural silence between bursts was assigned 0 AU; all other bursts were normalized with respect to this value. Sympathetic activity was quantified using standard measures, including total activity (product of burst frequency and mean burst area; AU) and burst frequency (bursts/min). For simplicity, total activity is presented as a factor of 10⁻² AU.

MSNA and hemodynamic responses to end-expiratory apnea were compared preinfusion and postinfusion. Baseline arterial blood pressure, heart rate, and MSNA at rest were calculated as mean values over a 5-min time period preceding each apnea, preinfusion and postinfusion. To determine the blood pressure response to maximal end-expiratory apnea, changes in systolic, diastolic, and mean blood pressure were calculated as the difference between the peak pressure during apnea and the average pressure of the preceding baseline. Change in heart rate was calculated as the difference between the average heart rate during the apnea and the average heart rate of the preceding baseline period. MSNA was determined as an average of the entire apnea period. For completeness, to determine whether the response to apnea was augmented during a condition of elevated plasma osmolality, MSNA responses were calculated both as an absolute and percent increase from the preceding baseline. Although calculating a percent change in total activity from baseline will be affected by differences in resting total activity, percentage increases were used as a normalization procedure to account for unexpected shifts in microelectrode position. Absolute delta values were avoided when examining the MSNA responses in total activity because data can be difficult to interpret without knowing exact electrode positions within the nerve fiber.

Statistics. All data are reported as means ± SE. MSNA at rest was analyzed over a 5-min period preceding the apnea, before and after infusion. A two-way, within-factor repeated-measures ANOVA (condition × time) was used to analyze responses to apnea before and after increases in plasma osmolality. Condition represents spontaneous breathing or apnea, and time represents preinfusion and postinfusion of hypertonic saline. Post hoc tests were examined using Tukey pairwise comparisons when necessary. The MSNA responses to apnea were compared using two-tailed, paired Student’s t-tests. P ≤ 0.05 were considered statistically significant for all tests.
RESULTS

Urine specific gravity was 1.019 ± 0.002 before testing, indicating that the subjects were adequately hydrated. Hyperosmotic saline infusion increased plasma osmolality (284 ± 1 to 290 ± 1 mOsm/kg H2O; \( P < 0.001 \)). Increased plasma osmolality was associated with increases in both serum Na⁺ (138.8 ± 0.3 to 141.6 ± 0.4 mmol/l; \( P < 0.001 \)) and serum Cl⁻ (103.2 ± 0.4 to 107.5 ± 0.5 mmol/l; \( P < 0.001 \)). Decreases in both hemoglobin (13.9 ± 0.3 to 13.0 ± 0.3 g/dl; \( P < 0.001 \)) and hematocrit (39.9 ± 1 to 36.8 ± 0.7%; \( P < 0.001 \)) were consistent with modest intravascular volume expansion. Hypertonic saline infusion yielded significant increases in mean arterial pressure (94 ± 4 to 103 ± 4 mmHg; \( P < 0.001 \)) but did not change heart rate (62 ± 3 to 63 ± 3 beats/min).

MSNA responses to apnea were not augmented by increased plasma osmolality. A suitable nerve recording was maintained in 11 subjects during maximal voluntary end-expiratory apnea, both before and after hypertonic saline infusion. Duration of the apnea was not different after infusion (pre = 40 ± 5 s; post = 41 ± 4 s). The arterial oxygen saturation decline during apnea was not different postinfusion (pre = −3.2 ± 1.3%; post = −3.3 ± 2.0%).

The hemodynamic responses to apnea are listed in Table 1. Responses were calculated as a difference between apnea and the preceding baseline. There were no differences in the blood pressure or heart rate response to apnea after infusion of hypertonic saline.

Table 1. Blood pressure and heart rate response to apnea, before and after hypertonic saline infusion

<table>
<thead>
<tr>
<th>Variable</th>
<th>Preinfusion</th>
<th>Postinfusion</th>
</tr>
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<tbody>
<tr>
<td>∆ Systolic BP, mmHg</td>
<td>21 ± 3</td>
<td>17 ± 3</td>
</tr>
<tr>
<td>∆ Diastolic BP, mmHg</td>
<td>18 ± 3</td>
<td>16 ± 3</td>
</tr>
<tr>
<td>∆ MAP, mmHg</td>
<td>23 ± 3</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>∆ HR, beats/min</td>
<td>5 ± 2</td>
<td>2 ± 2</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. ∆, difference between apnea and the preceding baseline; BP, blood pressure; MAP, mean arterial pressure; HR, heart rate.

Representative neurograms for a single subject at baseline and under conditions of increased plasma osmolality, both at rest and during apnea, are presented in Fig. 1. There was a significant overall effect of osmolality (\(* P < 0.01\)) and apnea (\(\# P = 0.02\)) on MSNA but no interaction.

Volume/time-control experiments did not alter baseline MSNA or MSNA responses to apnea. Isotonic saline volume-control infusions yielded no change in plasma osmolality (280 ± 2 to 281 ± 2 mOsm/kg H2O; \( P > 0.05 \)) or MSNA at rest (14 ± 2 to 15 ± 2 bursts/min; \( P = 0.872 \)). Likewise, MSNA responses to apnea were not different following isotonic saline infusion when examined as an absolute change in burst frequency (15 ± 3 bursts/min).
The sympathetic response to end-expiratory apnea appears governed principally by changes in arterial oxygen saturation. End-expiratory apnea presumably stimulates peripheral chemoreceptors, as it is not generally thought that central chemoreceptors respond to modest hypoxia (7, 24, 41). Additionally, on the basis of work of Morgan et al. (26), it is unlikely that hypercapnia, and its influence on central medullary chemoreceptors, contributes to the neurocirculatory responses to apnea, because these responses were abolished with administration of supplemental oxygen, preventing hypoxia but not hypercapnia. Previously reported measures of arterial gases indicate that a 30-s apnea in a healthy subject at functional residual capacity would yield an arterial PO2 of ~65 Torr, corresponding to an arterial oxygen saturation decline of ~4% (13). Although the decline in arterial oxygen saturation measured by pulse oximetry in this study was slight, we estimate that a 40-s apnea would result in a modestly reduced arterial PO2 compared with this reported value. Therefore, we attribute the MSNA response to voluntary end-expiratory apnea in this study to the resulting modest hypoxia.

Despite the additive nature of the response to apnea, plasma osmolality does influence sympathetic outflow. The results of this study demonstrate that an acute elevation of plasma osmolality has an overall effect on MSNA, confirming previous findings in humans (9, 12, 44) and animals (4, 22, 27, 43). Additionally, in humans, increased plasma osmolality enhances sensitivity of baroreflex control of MSNA (9, 44). In animal models, plasma osmolality differentially regulates sympathetic activity to various vascular beds (4, 22, 27, 43). Hypertonic saline-induced peripheral hyperosmolality in anesthetized rats increased lumbar (43) and adrenal (4) sympathetic activity but decreased renal sympathetic outflow (4, 43). Additionally, animal models support a role for chronic changes in plasma osmolality influencing sympathetic activity (30). Water-deprived rats, which have high basal osmolality, demonstrated sustained increases in lumbar
sympathetic nerve activity (LSNA). In addition, an infusion protocol that lowered plasma osmolality also decreased LSNA (30). Taken together, these studies indicate that changes in plasma osmolality alter sympathetic nervous system activity. Animal models also support a direct link between osmosensitive receptors in the forebrain lamina terminals and the sympathetic nervous system, which may represent the underlying central circuitry controlling these responses (2, 38).

Limitations. There are several limitations that deserve mention. First, the hypoxic stimulus in the present study is brief (an ~40-s apnea), and therefore, interpretation of the findings must be limited to relative short periods of hypoxia. Although a study design representative of a more prolonged challenge would have been useful, end-expiratory apneas in awake subjects, such as in the context used in this study, mimic the brief periods of hypoxia that repeatedly occur during sleep in patients with OSA (31). Second, we did not measure arterial blood gases. Measurement of arterial gas tensions would have been more definitive in ensuring that the chemoreceptor stimulus was matched between conditions. However, the mean apnea duration and the decline in arterial oxygen saturation were not different preinfusion and postinfusion, suggesting a comparable chemoreceptor stimulus. Finally, we did not assess arginine vasopressin concentration. It is well known that increasing plasma osmolality in humans causes a reflex increase in circulating arginine vasopressin (34). However, it is important to note that the available animal data (30) suggest that the chemoreceptor stimulus was matched between conditions. However, the mean apnea duration and the decline in arterial oxygen saturation were not different preinfusion and postinfusion, suggesting a comparable chemoreceptor stimulus. Specifically, rats that were pretreated with a V1-receptor antagonist also demonstrated a relation between plasma osmolality and sympathetic activity (30).

Perspectives and Significance

Hypoxic stress elicits exaggerated sympathetic nerve responses in patients with borderline hypertension (32) and obstructive sleep apnea (OSA) (31). Importantly, patients with OSA also demonstrate heightened sympathetic activity during waking hours, in spite of normal arterial oxygen saturation (8, 31). This excessive sympathetic outflow presumably contributes to the development of hypertension in patients with OSA. Evidence suggests that over 50% of hypertensive individuals have salt-sensitive blood pressure (42). Increases in dietary salt consumption cause increases in plasma sodium/osmolality (20). There is also evidence that increases in plasma sodium/osmolality modulate sympathetic outflow (12, 30, 43). In addition, dietary salt intake alters the responsiveness of RVLM neurons in animal models of salt-sensitive hypertension (1, 6, 21). Extending these recent findings to humans suggests that an enhanced sympathoexcitatory nature of the RVLM may contribute to salt-sensitive hypertension and that this may be augmented by increases in dietary salt intake (35). While it is not known whether the hypertension that accompanies OSA is salt sensitive, the interaction of central neural circuitry involved in cardiovascular control is likely altered, contributing to the increases in vascular tone and arterial blood pressure characteristic of these pathologies.

ACKNOWLEDGMENTS

The authors thank the subjects for participation in this study.

GRANTS

This research was supported by the National Heart, Lung, and Blood Institute Grant R15 HL074851-02 and HL077670.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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