Sympathetic neural responses to increased osmolality in humans

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Farquhar, William B., Megan M. Wenner, Erin P. Delaney, Allen V. Prettyman, and Michael E. Stillabower. Sympathetic neural responses to increased osmolality in humans. Am J Physiol Heart Circ Physiol 291: H2181–H2186, 2006. First published June 9, 2006; doi:10.1152/ajpheart.00191.2006.—The purpose of this study was to examine the relationship between osmolality and efferent sympathetic outflow in humans. We hypothesized that increased plasma osmolality would be associated with increases in directly measured sympathetic outflow. Muscle sympathetic outflow was successfully recorded in eight healthy subjects during a 60-min intravenous hypertonic saline infusion (HSI; 3% NaCl) on one day and during a 60-min intravenous isotonic saline (ISO) infusion (0.9% NaCl) on a different day. The HSI provides an osmotic and volume stimulus, whereas the ISO infusion provides a volume-only stimulus. Muscle sympathetic nerve activity was quantified using the technique of peroneal microneurography. Plasma osmolality increased during the HSI but not during the ISO infusion (ANOVA, P < 0.05). Sympathetic outflow differed between the trials (ANOVA, P < 0.05); during the HSI burst, frequency initially increased from 14.6 ± 2.5 to 18.1 ± 1.9 bursts/min; during the ISO infusion, burst frequency initially declined from 14.7 ± 2.5 to 12.0 ± 2.1 bursts/min. Plasma norepinephrine concentration was greater at the end of the HSI compared with the end of the ISO infusion (HSI: 297 ± 64 vs. ISO: 202 ± 49 pg/ml; ANOVA, P < 0.05). We conclude that HSI-induced increases in plasma osmolality are associated with increases in sympathetic activity in humans.

ANIMAL MODELS used to examine the control of efferent sympathetic outflow have demonstrated that plasma osmolality is a regulator of sympathetic activity. For example, Scrogin et al. (16) used a water-deprived rat model to demonstrate that elevated plasma osmolality is associated with increased lumbar sympathetic nerve activity. They also demonstrated that lowering osmolality with a 2-h infusion of 5% dextrose in water resulted in a reduction in lumbar sympathetic outflow. Toney et al. (19) reviewed the central neural mechanisms that link changes in osmolality to alterations in sympathetic outflow. Weiss et al. (22) demonstrated that the response to an osmotic stimulus is region specific; that is, lumbar sympathetic activity goes up and splanchnic and renal sympathetic activity go down during an intravenous infusion of hypertonic saline. They (22) also demonstrated that arterial baroreceptors oppose thepressor responses (this was demonstrated by comparing intact with sinoaortic-denervated Sprague-Dawley rats). Others have also reported the sympathoexcitatory nature of an osmotic stimulus in experimental animals (1, 6). These studies, which highlight the relationship between osmolality and sympathetic outflow, have potential clinical relevance. Elevated osmolality is not only seen during dehydration (4), but it has been hypothesized that it may be involved in salt sensitivity of blood pressure (2).

Recently, Charkoudian et al. (3) infused hypertonic saline (3% NaCl) intravenously at two rates for 10 min each in humans and assessed muscle sympathetic outflow from the peroneal nerve, as well as baroreflex control of sympathetic outflow. Plasma osmolality and serum sodium concentration were modestly increased with this infusion protocol (osmolality increased ~3–4 mosmol/kg). They found that baseline sympathetic outflow was not altered, but baroreflex control of sympathetic outflow was enhanced at the slow infusion rate but not the fast infusion rate. The lack of change in baseline sympathetic outflow during an osmotic stimulus in humans is important, because it is in contrast to what has been reported in the aforementioned animal literature, although, as noted above, the sympathetic responses are region specific. It could be that larger changes in osmolality must be elicited to observe the increase in sympathetic outflow.

We hypothesized that directly measured sympathetic outflow (peroneal microneurography) would increase during a progressive osmotic stimulus. To test this hypothesis, we had subjects complete a 60-min hypertonic saline (3% NaCl) trial. We (8) have recently demonstrated that this identical infusion protocol produces a robust ~10 mosmol/kg increase in plasma osmolality, as well as an increase in mean arterial blood pressure. Because infusing hypertonic saline also causes volume expansion, we also had subjects complete an isotonic saline (0.9% NaCl) trial on a different day, permitting the comparison of a combined osmotic and volume stimulus (hypertonic saline infusion trial, HSI) to a volume-only stimulus (isotonic saline infusion trial, ISO).

MATERIALS AND METHODS

Subjects. Eight healthy subjects (7 men and 1 woman, age 24 ± 2 yr old, body mass index 23.7 ± 0.8 kg/m2) completed both an HSI trial and an ISO trial on different days separated by 1 mo (randomized via a coin flip). Thirteen subjects were initially recruited, but it is only in these eight subjects that we have a suitable nerve recording during both infusions. All subjects provided verbal and written consent before study participation. The study was approved by the Human Subjects Review Board at the University of Delaware. Screening visit. All subjects completed a medical history form and a physical activity readiness questionnaire. A baseline blood sample was obtained for a complete blood count, a lipid profile (i.e., total cholesterol, HDL-C, LDL-C, and triglycerides), fasting glucose, liver function (i.e., aspartate transaminase and alanine transaminase), kidney function (i.e., creatinine and blood urea nitrogen), and electrolytes

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(i.e., sodium, potassium, and chloride). Height and weight were measured (Healthometer scale, Continental Scale, Bridgeview, IL), and body mass index was calculated. To ensure that all subjects were healthy and had normal cardiovascular function, a resting and exercise 12-lead ECG was performed (Schiller AT-10, Electra-Med, Flint, MI). The exercise ECG was performed on an electronically braked cycle ergometer (Corival V2 Ergometer, Lode B.V. Medical Technology, The Netherlands). The initial workload was 75 W, with a 25-W increase every 2 min; all subjects achieved at least 85% of their age-predicted maximal heart rate (9). Resting and exercise blood pressures were manually assessed using a mercury sphygmomanometer and stethoscope. None of the subjects used tobacco products or were taking any medications.

Measurements. Heart rate was measured using a single lead electrocardiogram. Beat-by-beat blood pressure was assessed using a Finometer (Finometer, Finapres Medical Systems, The Netherlands). A cuff was placed on the left middle finger. The manufacturer’s recommended calibrations were followed. Blood pressure values from the Finometer correlate very well with directly measured radial artery blood pressure (14). Systolic and diastolic blood pressure were also assessed using an automated oscillometric upper arm blood pressure cuff placed on the right arm (Dinamap Dash 2000, GE Medical Systems, Milwaukee, WI). Respiratory excursions were assessed with bands placed around the chest and stomach (Inductotrace System, Ambulatory Monitoring, Ardsley, NY).

Multiunit, postganglionic muscle sympathetic nerve activity was recorded using a tungsten microelectrode inserted into the peroneal nerve below the fibular head (18, 20). The raw signal was amplified, band-pass filtered, rectified, and integrated using a nerve traffic analyzer (model 662c-3, Nerve Traffic Analyzer, University of Iowa Bioengineering, Iowa City, IA).

Protocol. Subjects were instructed to drink ~1,800 ml of water the day before the experimental day (600 ml 3 times throughout the day). They were also instructed to avoid caffeine, alcohol, and exercise 12 h before the protocol. The woman was tested during the early follicular phase of their menstrual cycle. On the morning of the study, subjects were instructed to drink an additional 600 ml of water. Upon arriving at the lab (~0700 AM), subjects emptied their bladder, and the specific gravity of the urine was determined. A 20-gauge intravenous catheter was placed in a vein in the left arm and right antecubital area (the left intravenous catheter was used to infuse the saline solution, the right catheter for blood sampling). Subjects were randomized to receive either isotonic saline (0.9% NaCl) or hypertonic saline (3% NaCl) on their first experimental visit (LifeCare 5000 infusion pump, Abbott Laboratories, North Chicago, IL). They received the other solution on their subsequent visit, scheduled 1 mo after their first visit. To prevent dehydration, the data presented below are baseline blood work was within clinically acceptable normal limits. Resting and exercise 12-lead ECGs were normal. None of the subjects were hypertensive (7). Urine specific gravity the morning of the study averaged 1.012 ± 0.002 before the HSI trial and 1.019 ± 0.003 before the ISO trial, indicating that the subjects were not dehydrated. The data presented below are from the 16 trials (8 HSI and 8 ISO; paired data) where we were able to successfully record sympathetic outflow from the peroneal nerve.

As shown in Fig. 1, plasma osmolality increased during the HSI and remained stable during the ISO infusion (ANOVA, P < 0.01; post hoc analysis reported in Fig. 1). As expected, serum sodium concentrations paralleled the change in plasma osmolality during both infusions (data not shown). Hematocrit declined during both infusions (Fig. 1; ANOVA, P < 0.01).

An example of the sympathetic neurogram recording during the HSI is displayed in Fig. 2. Sympathetic burst frequency initially increased during the HSI and initially declined during the ISO infusion (ANOVA, P < 0.05; post hoc analysis is reported in Fig. 3, top). Whereas we attempted to record sympathetic outflow for the duration of the 60-min infusion, we lost many of the recordings after 45 min. Thus we present muscle sympathetic nerve activity data only through the 40-min time point (Fig. 3). During the HSI, plasma norepinephrine...
(corrected for the plasma volume expansion, see MATERIALS AND METHODS section) at baseline, 30 min, and 60 min was 217 ± 54, 239 ± 45, and 297 ± 64 pg/ml, respectively; during the ISO infusion it was 173 ± 29, 183 ± 30, 202 ± 49 pg/ml, respectively (ANOVA P < 0.05; post hoc analysis reported in Fig. 3, bottom). There was a correlation between baseline sympathetic outflow (bursts per minute) and baseline plasma norepinephrine (Fig. 4, r = 0.55, P = 0.03). 

Systolic blood pressure during the two infusions is displayed in Fig. 5, top (ANOVA, P = 0.08). Diastolic pressure is displayed in the middle panel of Fig. 5 (ANOVA, P = NS), and pulse pressure is displayed in the bottom panel of Fig. 5 (ANOVA, P < 0.01). Mean arterial blood pressure during the HSI was 76.4 ± 3.0, 78.3 ± 3.3, 82.3 ± 3.5, and 86.8 ± 4.2 mmHg at baseline, 20, 40, and 60 min, respectively, and during the ISO infusion it was 80.2 ± 2.2, 81.0 ± 2.3, 84.1 ± 3.0, and 86.0 ± 3.5 mmHg, respectively, at baseline, 20, 40, and 60 min (ANOVA, P = not significant). The change in heart rate (pre- to postinfusion) during the HSI was 6 ± 3 beats/min and during the ISO infusion it was 1 ± 1 beats/min (ANOVA, P = 0.11).
DISCUSSION

The main study finding was that HSI-induced increases in plasma osmolality were associated with an initial increase in directly measured muscle sympathetic outflow in humans. This increase was consistent with the study hypothesis. However, we did not anticipate the transient nature of the response. Nevertheless, there is a clear, early separation of muscle sympathetic nerve activity responses when comparing an osmotic-volume stimulus to a volume-only stimulus (Fig. 3).

With the infusion of hypertonic fluid, there is an expansion of the plasma volume. We attempted to isolate the effects of changes in peripheral osmolality by having an isotonic saline control condition. Volume expansion alone, acting through the baroreceptors, would be predicted to cause sympathoinhibition, as evidenced in human studies (5, 15) and animal studies (13). However, as observed in Fig. 1, hematocrit declined more during the HSI trial (ANOVA interaction, $P < 0.01$), suggesting that plasma volume expanded more during this trial, a finding that is not unexpected. If the infusion-induced expansion of the plasma volume were the only factor driving the sympathetic response, we would expect modest baroreflex-mediated inhibition of sympathetic outflow during the ISO infusion and robust inhibition of sympathetic outflow during the HSI. This is not the case in the present study, and the initial increase in muscle sympathetic outflow, despite a greater sympatho-inhibitory volume stimulus, suggests that increased osmolality influences sympathetic outflow. These data in humans are consistent with what has been reported in the animal literature (1, 6, 10, 16, 22), although the sympathetic response is region specific. An important unanswered question remains: why doesn’t sympathetic outflow continue to increase in humans because the HSI utilized in the present study is a progressive osmotic stimulus?

We speculate that during an HSI the brain stem is presented with conflicting signals. That is, the volume load associated with this stimulus would act (through the baroreflex arc) to inhibit sympathetic outflow (13), and the osmotic stimulus [based on the supporting animal literature (2, 16, 19)] would act (through centrally located osmoreceptors) to increase sympathetic outflow. In normal healthy adults, these conflicting

Fig. 4. Relationship between MSNA and plasma NE during baseline period ($r = 0.55$, $P = 0.03$).

Fig. 5. Systolic blood pressure (top), diastolic blood pressure (middle), and pulse pressure (bottom) during HSI and ISO infusions (ANOVA, $P = 0.08$ for systolic blood pressure; ANOVA, $P < 0.01$ for pulse pressure). $\dagger P < 0.05$ vs. ISO trial at that time point.
stimuli may result in a variable sympathetic response. That is, the early HSI-induced response is driven by the osmoreceptors, which subsequently become overwhelmed by the volume-induced sympathoinhibitory response. Thus, an intact baroreflex may modify the sympathetic response to an osmotic stimulus, which appears to be consistent with experimental findings in animals where the arterial baroreflex opposes the HSI-induced pressure increase (22). This is clearly speculative.

In a recent review paper, Brooks et al. (2) hypothesized that small dietary-induced increases in sodium chloride concentration may drive sympathetic outflow, and this sympathoexcitation may underlie salt-sensitive hypertension. Our data, along with the recent data by Charkoudian et al. (3) may have some relevance for this hypothesis. Charkoudian et al. (3) reported increased mean arterial pressure, and we report a trend toward differences in the systolic blood pressure response between the infusions (ANOVA, \( P = 0.08 \)) and differences in pulse pressure. As stated, if the baroreflexes were the only operative mechanism, we would expect sympathetic inhibition in both studies. This was not the case in either study, and the failure to decrease sympathetic outflow in both studies [Chardoudian et al. (3) report no change, we report an initial increase in muscle sympathetic nerve activity] provides indirect evidence that another mechanism is operative, thereby supporting an osmoreceptor-sympathetic link in humans (2).

It is noteworthy that both our study and the study by Charkoudian et al. (3) utilized young, healthy adults; a population thought to be at the low end of the salt sensitivity of blood pressure continuum. Charkoudian et al. (3) reported an increase in the sensitivity of baroreflex control of muscle sympathetic outflow. Whereas increased sympathetic baroreflex control is often viewed from the perspective of protecting against hypotension, in the context of an acute salt loading protocol, it may also be important in buffering further sodium-induced increases in blood pressure (greater sympathetic inhibition with further increases in gain). In other words, the acute increase in gain reported by Charkoudian et al. could be an important mechanism defending against further and possibly excessive increases in blood pressure. Collectively, these data in humans support the conclusion that plasma osmolality may be an importantafferent signal to the brain stem that influences both sympathetic baroreflex gain and basal sympathetic outflow.

When considering the issue of salt sensitivity, testing a group that has documented salt sensitivity would be informative. We speculate that true salt-sensitive individuals may demonstrate greater increases in sympathetic outflow for a given osmotic load or, alternatively, may fail to acutely increase sympathetic baroreflex sensitivity. Either, or both, mechanism(s) could be an important factor underlying salt sensitivity of blood pressure in humans.

The plasma norepinephrine response differed between the HSI and ISO infusions (Fig. 3), supporting the conclusion that HSI-induced increases in plasma osmolality lead to sympathetic activation (with the limitation that we are not assessing production vs. clearance of norepinephrine). However, the time course between the muscle sympathetic responses and the plasma norepinephrine responses were different; this suggests that there is a change in circulating norepinephrine takes longer to develop. We examined the relationship between plasma norepinephrine and muscle sympathetic nerve activity at study onset. Others have demonstrated a relationship between these two values (12, 20, 21). We therefore ran a correlation between directly measured muscle sympathetic nerve activity and plasma norepinephrine at baseline (Fig. 4). The statistically significant correlation demonstrates that our two indexes of sympathetic outflow were in agreement at study onset, consistent with experimental data reported by others (12, 20, 21).

There are several limitations that should be mentioned. First, the volume stimulus was not matched precisely between the trials. A stronger study design would have been to more precisely match the volume stimulus between the two infusion trials, such that the decline in hematocrit would be similar. However, had we been able to do this, we can only guess that we would have observed greater sympathetic inhibition during the ISO trial. For example, Pawelczyk et al. (15), using a larger saline load, were able to nearly abolish spontaneous muscle sympathetic nerve activity. Thus, had we used a larger isotonic saline load, we would predict an even greater separation of responses between the HSI and ISO trials. Second, we did not assess arginine vasopressin concentration. It is well known that increasing osmolality in humans causes an increase in circulating arginine vasopressin (17). Importantly though, Scrigan et al. (16) concluded that in rats the relationship between osmolality and lumbar sympathetic activity is independent of vasopressin concentration. Third, it is unfortunate that we were not able to maintain the muscle sympathetic nerve recording for the duration of the study for all subjects. Recording from the peroneal nerve in humans is technically demanding, and slight movements can impair the signal-to-noise ratio.

We conclude that HSI-induced increases in plasma osmolality are associated with increases in efferent sympathetic outflow in humans. As shown with the HSI, there was an initial increase in sympathetic outflow. These data support the hypothesis that there is a relationship between plasma osmolality and sympathetic outflow in humans.

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GRANTS

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