Blood pressure and hemodynamic responses to an acute sodium load in humans

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Farquhar, William B., Erin E. Paul, Allen V. Prettyman, and Michael E. Stillabower. Blood pressure and hemodynamic responses to an acute sodium load in humans. J Appl Physiol 99: 1545–1551, 2005.—The purpose of this study was to investigate the acute blood pressure (BP) and hemodynamic effects of sodium chloride (3% intravenous solution). Although many studies link a change in dietary sodium to a change in BP, few consider the effects of sodium concentration in the blood on BP. We hypothesized that an intravenous sodium load would increase BP, and we quantified alterations in cardiac output (Qc) and peripheral vascular resistance (PVR). Thirteen subjects (age 27 ± 2 yr) underwent a 60-min 3% saline infusion (0.15 ml·kg⁻¹·min⁻¹). BP was assessed on a beat-to-beat basis with a Finometer, Qc was assessed via the CO₂ rebreathing technique, and PVR was derived. Serum sodium and osmolality increased, and hematocrit declined during the infusion (ANOVA, P < 0.01). Mean arterial pressure (MAP) increased continuously during the infusion from 81.8 ± 3.4 to 91.6 ± 3.6 mmHg (ANOVA, P < 0.01). BP responsiveness to sodium was expressed as the slope of the serum sodium-MAP relationship and averaged 1.75 ± 0.34 mmHg·mmol⁻¹·L⁻¹. BP responsiveness to the volume change was expressed as the slope of the hematocrit-MAP relationship and averaged −2.2 ± 0.35 mmHg%/%. The early change in MAP was mediated by an increase in Qc and the late change by an increase in PVR (P < 0.05), corresponding to a 30% increase in plasma norepinephrine. In conclusion, an acute infusion of hypertonic saline was effective in increasing BP, and both sodium and volume appear to be involved in this increase: acute BP responsiveness to serum sodium can be quantified using a MAP-sodium plot.

THE LINK BETWEEN CHRONIC SODIUM INTAKE and blood pressure (BP) has been well established in large-scale epidemiological studies (6, 9, 22). However, individual responses to dietary sodium vary, with some individuals exhibiting a sodium-sensitive phenotype and others a sodium-resistant phenotype (17, 28). Both neural (8) and hormonal (18) responses may contribute to the sodium-induced change in BP, with the kidney playing a prominent role in long-term pressure homeostasis (13). Presumably, a maladaptation of one of these mechanisms contributes to the sodium sensitivity exhibited in some individuals. It has been demonstrated that black hypertensive adults have a higher degree of sodium sensitivity than white hypertensive adults (30, 32) and that sodium sensitivity increases with age (31). These data are important, since sodium sensitivity has not only been associated with increased mortal-
resting, seated BP (systolic BP 113 ± 3 mmHg, diastolic BP 67 ± 2 mmHg). 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. A baseline blood sample was obtained for a complete blood count, a lipid profile (i.e., total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides), fasting glucose, liver function (i.e., aspartate transaminase and alanine transaminase), kidney function (i.e., creatinine and blood urea nitrogen), and electrolytes (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, resting and exercise 12-lead ECGs were performed (Schiller AT-10, Electra-Med, Flint, MI). The exercise ECG was performed on an electronically braked cycle ergometer (Corival V2 Ergometer, Lode Medical Technology, Groningen, The Netherlands). The initial workload was set at 75 W, with a 25-W increase every 2 min; all subjects achieved at least 85% of their age-predicted maximal heart rate with a rating of perceived exertion of ≥18 on the 6–20 category Borg scale (10). Resting and exercise BP values were manually assessed using a mercury sphygmomanometer and stethoscope. None of the subjects used tobacco products or were taking any medications. Subjects practiced the rebreathing maneuver (see Protocol) during the screening session.

Experimental visit. Subjects were instructed to drink 15 ml/kg of water in the 24 h before the experimental day. They were also instructed to avoid caffeine, alcohol, and exercise 12 h before the protocol. Women were tested during the early follicular phase of their menstrual cycle. On the morning of the study, subjects were instructed to drink an additional 5 ml/kg of water. On arriving at the laboratory (−0700), subjects emptied their bladders (the start of a timed urine-collection period), and specific gravity of the urine was determined. Subjects assumed a semirecumbent position, ECG electrodes were placed on the chest, and an automated oscillometric upper arm BP cuff was placed on the right arm (Dinamap Dash 2000, GE Medical Systems, Milwaukee, WI). Respiratory bands were placed around the abdomen and chest (Inductotrace System, Ambulatory Monitoring, Ardsley, NY). A 20-gauge intravenous catheter was placed in a vein in the left and right antecubital area (the left intravenous catheter was used to infuse the hypertonic solution, the right for blood sampling). A cuff was placed on the left middle finger for beat-by-beat BP assessment (Finometer, Finapres Medical Systems). The manufacturer’s recommended calibrations were followed. BP values from the Finometer correlate very well with directly measured radial artery BP (20). Qc was assessed via the indirect Fick method of CO2 rebreathing (5). During steady-state breathing, CO2 production and end-tidal CO2 were measured (TrueOne 2400 Metabolic Measurement System, ParvoMedics, Sandy, UT). Standard volume and gas calibration procedures were followed before each trial. End-tidal CO2 provided an estimate of arterial CO2. To estimate venous CO2, subjects rebreathed a high CO2–O2 gas mixture from a rebreathing bag until the level of CO2 in the bag and lung reached equilibrium (this was confirmed visually by a plateau in CO2 concentration). This equilibrium value for CO2 provided an estimate of venous CO2. The following equation was then used to calculate Qc: Qc = VCO2[CvCO2] – CAco2 – 1, where VCO2 is CO2 production, [CvCO2] is venous CO2 concentration, and CAco2 is arterial CO2 concentration. PVR was derived from mean arterial pressure (MAP) and Qc; stroke volume was derived from Qc and heart rate.

Protocol. Five minutes of baseline data were collected and included the assessment of respiration, heart rate, beat-to-beat BP, as well as a venous blood draw. To stabilize BP, a paced breathing protocol was utilized; all subjects were verbally cued from a recording to breathe at 0.25 Hz (15 breaths/min) for the 5 min of baseline data. Compliance to the paced breathing protocol was visually confirmed. Whole blood was transferred into the appropriate vacutainer tubes and spun for 15 min at 3,500 rpm in a centrifuge (Allegra X-22R, Beckman Coulter, Fullerton, CA). The serum or plasma was pipetted off and used to determine serum sodium, potassium, and chloride (EasyElectrolyte Analyser, Medica, Bedford, MA) and plasma osmolality (model 3D3 Osmometer, Advanced Instruments, Norwood, MA). Quality control standards were run. For the determination of hematocrit, whole blood was transferred into precalibrated capillary tubes and spun rapidly on a Readacrit Centrifuge (Clay Adams Brand, Becton Dickinson, Parsippany, NJ). All samples were run in either duplicate or triplicate. The total volume of blood drawn during the protocol was 150 ml. Baseline Qc was also determined at this time.

After baseline data collection, a 60-min infusion of 3% sodium chloride (0.15 ml·kg⁻¹·min⁻¹) was started. Hemodynamic variables were assessed continuously. Venous blood samples were obtained at 15, 30, 45, and 60 min. Five minutes of paced breathing commenced at 18, 33, and 50 min. Qc was assessed at 23, 38, and 58 min. On completion of the infusion, the subjects emptied their bladders into a urine-collection container. Urine volume, sodium, osmolality, and specific gravity were determined.

For the determination of plasma norepinephrine (NE) at baseline and at the end of the infusion, whole blood was transferred into specially prepared and chilled EDTA-sodium metabisulfite vacutainers. These samples were spun at 3,500 rpm in a refrigerated centrifuge. The plasma was then pipetted off and promptly frozen in a −70°C freezer for future analysis by high-performance liquid chromatography at the Mayo Medical Laboratories; interassay coefficient of variation for the NE control was 5.5%.

Five additional subjects (all men) were recruited for a direct comparison of the BP change with 0.9 vs. 3% saline infusion, with assessment of serum sodium, plasma osmolality, and hematocrit. These 10 trials were done on separate days, with 1 mo separating the experiments for each individual subject. The same infusion volume and rate were used for this comparison.

Data analysis. The respiratory, ECG, and BP signals were collected at 500 Hz using Windaq recording software (DATAQ Instruments, Akron, OH). The ECG was peak detected, and BP waveform peak and valley were detected (Windaq waveform browser, Advanced CODAS software). Each time point reported for HR and BP represents an average of 5 min of data. The percent change in plasma volume was calculated using the following formula (12):

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\frac{100}{(100 - \text{Hct}_{\text{B}})} \times \frac{\text{Hct}_{\text{A}} - \text{Hct}_{\text{B}}}{\text{Hct}_{\text{B}} - \text{Hct}_{\text{B}}}
\]

where \(\text{Hct}_{\text{B}}\) is the hematocrit at baseline and \(\text{Hct}_{\text{A}}\) is the hematocrit at a given time point during the infusion. Urine production (ml/min) and sodium excretion (mM/min) were assessed. Free water clearance was \(V - (U_{\text{osm, V}} \cdot P_{\text{osm}})^{-1}\), where \(U_{\text{osm}}\) and \(P_{\text{osm}}\) are equal to urine and plasma osmolality, respectively (mosmol/kgH2O), and V is equal to urine flow rate (ml/min).

Statistics. Data are expressed as means ± SE. A repeated-measures ANOVA was used to determine whether there was a change in a particular variable (e.g., MAP) during the infusion (SPSS 12.0). Post hoc pairwise comparisons were performed using Fishers least significant difference procedure (Statistica for windows, release 5.1). Linear regression analysis (least squares method, SigmaPlot 8.0) was used to examine the serum sodium-MAP relationship, the osmolality-MAP relationship, and the hematocrit-MAP relationship. A single line was applied to all of the data (e.g., see Fig. 3, top) and then applied to the data on an individual basis (Fig. 3, middle). The slopes of each individual were used as indexes of MAP responsiveness to acute changes in serum sodium (Fig. 3, bottom), plasma osmolality, and plasma volume, respectively. A stepwise linear regression was also applied to all of the data (SPSS 12.0; independent variables: serum sodium and hematocrit; dependent variable: MAP). For the 0.9 vs. 3% comparison, a two-way ANOVA was utilized (time and treatment), and regression analysis was used to examine the hematocrit-MAP relationship. A \(P\) value of ≤0.05 was considered significant for all statistical tests.
RESULTS

Baseline blood work was within clinically acceptable normal limits. Resting and exercise 12-lead ECGs and BPs were normal. None of the subjects was hypertensive (as defined in Ref. 4). Urine specific gravity the morning of the study was 1.013 ± 0.002.

As shown in Fig. 1, the infusion was successful in acutely increasing serum sodium and plasma osmolality ($P < 0.01$ via ANOVA; post hoc comparisons). Hematocrit declined pre- to postinfusion (0.382 ± 0.013 vs. 0.344 ± 0.010; $P < 0.01$ via ANOVA), corresponding to a plasma volume expansion of 19.7 ± 2.0%. There was a significant increase in finometer-derived MAP over the course of the infusion (Fig. 2; $P < 0.01$ via ANOVA). This increase in MAP was confirmed with the automated oscillometric upper arm cuff ($P < 0.05$ via ANOVA).

The mechanisms underlying this increase in MAP varied from the early phase of the infusion to the late phase of the infusion, with changes in $Q_c$ (Fig. 2, middle) contributing to the early rise in MAP and changes in PVR (Fig. 2, bottom) contributing to the late rise in MAP, as shown in Fig. 2, top ($P < 0.05$ for both via ANOVA). This late change in PVR corresponded with a 30% increase in plasma NE ($n = 8, 175 ± 13.5$ to $227 ± 19.9$ pg/ml; $P < 0.05$). There was no significant change in heart rate during the infusion ($P = 0.09$ via ANOVA). The change in stroke volume paralleled the change in $Q_c$ ($P < 0.05$ via ANOVA).

There was no significant correlation between baseline MAP and baseline serum sodium ($r = 0.03, P = 0.92$). However, there was a significant relationship between the change in serum sodium and the change in MAP across all subjects during the infusion (Fig. 3, top). MAP was regressed against serum sodium on an individual basis (Fig. 3, middle and bottom). All data are shown. Three of the 13 subjects had $r$ values of <0.80 (arbitrarily defined). The change in serum sodium, plasma osmolality, and hematocrit for these 3 subjects was similar to the other 10 subjects, although the infusion-induced change in MAP varied considerably [MAP change: 2.1, −1.1, and 13.2 mmHg; see online data supplement table for additional information (http://jap.physiology.org/cgi/content/full/00262.2005/DC1)].

MAP was also regressed against plasma osmolality. Across all subjects, there was a significant relationship between these variables ($r = 0.56, P = 0.0002$). When plotted on an individual basis, the mean slope was $1.06 ± 0.19$ mmHg/mosmol·kgH$_2$O$^{-1}$ and the mean $r$ value was $0.84 ± 0.04$. See online data supplement for individual data.

There was also a significant relationship between the decline in hematocrit and the increase in MAP during the infusion across all subjects ($r = 0.42, P = 0.008$). MAP was also regressed against hematocrit on an individual basis, and the mean slope was $−2.2 ± 0.35$ mmHg/%, and the mean $r$ value was $0.84 ± 0.04$. See online data supplement for individual data.

In the stepwise linear regression where MAP was the dependent variable, the strong colinearity between serum sodium and hematocrit (i.e., serum sodium and hematocrit; $r = −0.717$) prevented the overall equation from being improved by including both independent variables in the model, and of the two independent variables, serum sodium was the slightly better predictor of MAP. Because the software excluded hematocrit from this model, the final result of the stepwise linear regression equation is identical to the single linear regression result presented in the Fig. 3 legend.

Urine flow rate during the infusion was $2.6 ± 0.5$ ml/min, and free water clearance was $−0.49 ± 0.62$ ml/min. Sodium excretion was $78 ± 12$ mM/min. Sodium excretion as
determined at the end of the test was not correlated with the change in MAP at the end of the test ($r = 0.23$, $P = 0.46$).

Figure 4 depicts the change in MAP with a 0.9% saline infusion compared with a 3% saline infusion. During the 0.9%
trial, from pre- to postinfusion, there was no significant change in serum sodium (137.4 ± 1.1 to 137.5 ± 0.6 mM; *P > 0.40) or plasma osmolality (291.3 ± 1.1 to 290.9 ± 1.3 mosmol/kgH2O; *P > 0.40); hematocrit declined significantly (0.412 ± 0.012 to 0.397 ± 0.012; *P < 0.05). During the 3% trial, there was the typical robust increase in serum sodium (137.0 ± 1.4 to 140.9 ± 0.8 mM; *P < 0.05) and plasma osmolality (289.0 ± 0.8 to 298.3 ± 0.8 mosmol/kgH2O; *P < 0.05), and a decline in hematocrit (0.415 ± 0.018 to 0.373 ± 0.018; *P < 0.05). There was a significantly greater increase in MAP (ANOVA time and treatment effect, *P < 0.05 for both), and plasma volume was expanded more during the 3% saline infusion (19.6 ± 3.6 vs. 6.8 ± 1.7%; *P < 0.05). The results of the regression analysis (hematocrit-MAP relationship) for these 5 subjects demonstrated that the slopes and fits were comparable during the hypertonic infusion (average slope -3.2 ± 0.69 mmHg/%, *r = 0.92 ± 0.01) to the other 13 subjects, but the results of the isotonic infusion were much more variable (average slope -1.95 ± 1.20 mmHg/%, *r = 0.61 ± 0.18). In particular, two of the five subjects had *r values of <0.30, preventing any meaningful comparison of the isotonic vs. hypertonic slopes.

DISCUSSION

The major findings from the present investigation include 1) an acute intravenous hypertonic saline infusion was effective in increasing BP in a group of young, normotensive adults; 2) the mechanism underlying this increase varied from the early to late phase of the infusion, that is, the early rise in BP was mediated by an increase in Qc and the late rise due to an increase in PVR; and 3) the responsiveness of BP to sodium and volume can be estimated using this protocol.

Although we hypothesized that an acute intravenous sodium load would increase BP, we anticipated a modest response due to the young age of the subjects. Nevertheless, the present protocol resulted in an ~10-mmHg rise in MAP from pre- to postinfusion. Twelve of the 13 subjects demonstrated an increase, and 10 of the 13 subjects had an increase of >5 mmHg. This increase was noted with both a Finometer and an automated oscillometric device. Previously published data, using a variety of infusion protocols, have found mixed results. For example, Peskind et al. (discussed below; Ref. 21) reported a BP increase during a hypertonic saline infusion, whereas Stachenfeld et al. (3, 25) and Anderson et al. (1, 2) did not.

Different infusion rates and volumes, as well as individual differences in the subjects recruited, may explain these disparate findings. We conclude that a robust sodium stimulus like that observed in the present study is effective in increasing BP.

With regard to the mechanisms underlying salt-induced increases in BP, much of the literature focuses on the extracellular volume expansion associated with salt ingestion (indeed, this is often referred to as volume-loading hypertension) (14). In experiments that range from days to weeks, the initial rise in BP is attributed to a volume-induced increase in Qc, followed by a secondary increase in PVR [via an autoregulatory mechanism related to increased flow occurring at the local tissues (14)], which also contributes to the elevation in BP. This sequence has been emphasized by Guyton and Hall (14). However, although it is possible that these mechanisms are also operative in the present acute protocol (extracellular volume was not assessed, but plasma volume, a part of the extracellular space, was expanded ~20% with this model), we speculate that sympathetically mediated vasoconstriction also was involved. Specifically, the late increase in PVR was associated with a 30% increase in plasma NE from the pre- to postinfusion period. This finding is consistent with that of Peskind et al. (16, 21). In the context of studying panic disorder, they found that hypertonic saline increased NE concentration (and MAP). This increase is impressive when one considers that the volume stimulus, acting through the baroreflex arc, would be expected to inhibit sympathetic outflow (19, 21). However, Scrögin et al. (23), using a water-deprived Sprague-Dawley rat model, have demonstrated that elevated baseline osmolality is associated with elevated lumbar sympathetic nerve activity. Furthermore, an infusion protocol that acutely lowered plasma osmolality in these rats also lowered lumbar sympathetic nerve activity. Additional support for this osmotic-sympathetic link comes from other animal-based studies that have documented the sympathoexcitatory effects of an osmotic stimulus (11, 33). The central neural mechanisms that link changes in osmolality to alterations in sympathetic outflow have been reviewed by Toney et al. (27). Briefly, neurons within the brain stem that detect peripheral alterations in osmolality project to brain stem areas known to receive baroreceptor and cardiopulmonary input (27). Collectively, it appears that plasma osmolality is a regulator of sympathetic outflow in animal models, and the increase in plasma NE in the present study is consistent with this view. Furthermore, this increase in plasma NE suggests that the sodium stimulus is distinct from that of the volume stimulus.

As part of an exploratory analysis, we regressed MAP against serum sodium and, separately, MAP against plasma osmolality in an attempt to assess MAP responsiveness to sodium and osmolality. We plotted all of the data in Fig. 3, top, but our emphasis is on the individual plots, as demonstrated in Fig. 3, middle. Although the utility of expressing sodium and BP this way clearly must be confirmed in a larger group of subjects, perhaps including those with a statistically higher incidence of salt sensitivity to BP, our data suggest that there is a relationship between these variables in a group of subjects predicted (based on age and race) (32) to have a fairly low
incidence of sodium sensitivity. We speculate that those predicted to have a higher degree of salt sensitivity will demonstrate a steeper sodium-MAP slope. This relationship (observed in the acute setting) does not imply causation, but, in light of the abundance of information on the link between dietary salt and BP and the recent emphasis on circulating sodium and BP (15), this issue merits additional study.

It is of physiological interest to tease out the possible separate effects of sodium and volume, but in normal daily living these stimuli are intertwined. Nevertheless, Fig. 4 represents a comparison of BP responses to an infusion of 0.9% saline (where volume was expanded but serum sodium did not change) and 3% saline (where volume and serum sodium increased, as observed in the 13 other subjects). It is clear with these five additional subjects that BP increases more during a 3% saline infusion than during a 0.9% saline infusion. It is tempting to conclude that the difference between the two represents the “sodium” component of the BP increase. However, the intravascular volume stimulus is not matched with this simple comparison (experimentally, it is difficult to match the plasma volume expansion). Although we used the same infusion rate and volume for the 0.9 and 3% trials in these five subjects, plasma volume was expanded more during the 3% infusion. Presumably, with the hypertonic saline infusion, there was a greater shift in fluid from the intracellular to extracellular space. This might lead one to conclude that mainly volume is causing BP to increase (consistent with the “Guyton” explanation offered above). Consistent with this view, there was a significant relationship between the decline in hematocrit (used as a way to track the plasma volume expansion) and the increase in MAP. And, similar to the serum sodium-MAP plots discussed above, it is possible, within the confines of the current experimental protocol, to create hematocrit-MAP plots on an individual basis. We examined the data on an individual basis to see whether those with poor sodium-MAP fits had better hematocrit-MAP fits as a way to determine whether BP was related more to sodium in some and volume in others. However, this was not the case, and it is difficult to separate these two stimuli since both are changing during the infusion. Indeed, the results of the stepwise linear regression indicated a high degree of colinearity between the change in serum sodium and hematocrit. Alternate experimental paradigms will be needed to truly tease out the possibly separate effects of sodium and volume on the BP increase in humans. But, consistent with the recent hypothesis put forth by He et al. (15) and de Wardener et al. (7), we believe the statistically significant relationship between circulating sodium and MAP provides preliminary support for the concept that circulating sodium contributes to the increase in BP. This view does not discount the volume contribution to the increase in BP but rather supports the conclusion that both serum sodium and volume may be involved in the BP increase.

There are several limitations that should be mentioned. First, the present cohort contained both men and women, and with a total sample size of 13 subjects, it is not possible to fully explore possible sex differences in BP and hemodynamic responses to a sodium and volume load. There were no statistical differences or trends in any of the variables assessed, but we hesitate to make any firm conclusions regarding the lack of sex differences with the present cohort. Second, we are not able to link what occurs in the acute setting (using the infusion protocol) to what occurs in the chronic setting (using dietary manipulations of sodium). Therefore, any discussion on the similarities of these two distinct perturbations is speculative in nature. Third, we did not assess baseline extracellular volume or the change in the extracellular volume in response to the infusion. Including an assessment of the extracellular volume would have improved our ability to discern sodium- vs. volume-induced alterations in BP.

We conclude that a short-term infusion of hypertonic saline in humans permits the assessment of stimulus-response characteristics of serum sodium and volume and BP. Both sodium and volume appear to be related to the increase in BP. The early hypertonic saline-induced increase in BP is mediated by an increase in Qc and the late increase via changes in PVR. The increase in plasma NE provides indirect evidence for the involvement of the sympathetic nervous system. The novelty of this model for studying BP regulation is that some of these mechanisms can be examined during a very short-term infusion protocol. Future studies will need to relate what occurs in the acute setting using intravascular fluids to what occurs in the chronic setting using dietary manipulations of salt.

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