Salt Sensitivity

Hemodynamics and Salt-and-Water Balance Link Sodium Storage and Vascular Dysfunction in Salt-Sensitive Subjects

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Abstract—We investigated 24-hour hemodynamic changes produced by salt loading and depletion in 8 salt-sensitive (SS) and 13 salt-resistant (SR) normotensive volunteers. After salt loading, mean arterial pressure was higher in SS (96.5±2.8 mm Hg) than in SR (84.2±2.7 mm Hg), P<0.01, owing to higher total peripheral resistance in SS (1791±148 dyn·cm⁻⁵·s⁻¹) versus SR 1549±66 dyn·cm⁻⁵·s⁻¹, P=0.05, whereas cardiac output was not different between groups (SS 4.5±0.3 versus SR 4.4±0.2 L/min, not significant). Following salt depletion, cardiac output was equally reduced in both groups. Total peripheral resistance increased 24±6% (P<0.001) in SR, whose mean arterial pressure remained unchanged. In contrast, total peripheral resistance did not change in SS (1±6%, not significant). Thus, their mean arterial pressure was reduced, abolishing the mean arterial pressure difference between groups. SS had higher E/e' ratios than SR in both phases of the protocol. In these 21 subjects and in 32 hypertensive patients, Na+ balance was similar in SR and SS during salt loading or depletion. However, SR did not gain weight during salt retention (−158±250 g), whereas SS did (819±204), commensurate to iso-osmolar Na+ retention. During salt depletion, SR lost the expected amount of weight for iso-osmolar Na+ excretion, whereas SS lost a greater amount that failed to fully correct the fluid retention from the previous day. We conclude that SS are unable to modulate total peripheral resistance in response to salt depletion, mirroring their inability to vasodilate in response to salt loading. We suggest that differences in water balance between SS and SR indicate differences in salt-and-water storage in the interstitial compartment that may relate to vascular dysfunction in SS. (Hypertension. 2016;68:195-203. DOI: 10.1161/HYPERTENSIONAHA.116.07289.)

Key Words: blood pressure ■ body water ■ hemodynamics ■ salt sensitivity hypertension ■ sodium

Salt sensitivity (SS) of blood pressure (BP) is a trait observed in humans and animals, characterized by BP increases after salt loading and BP decreases after salt depletion. SS normotensive or hypertensive humans have a poorer prognosis than their salt-resistant (SR) counterparts.1,2 Thus, SS is a cardiovascular risk factor, independent of BP. SS affects about one quarter of the normotensive adult population and more than half of all hypertensive patients.3 The prevailing viewpoint is that the increase in BP produced by salt loading in SS is a compensatory response to maintain salt balance via pressure natriuresis, owing to an underlying defect in a single or multiple physiological natriuretic systems. This interpretation would explain the nonparallel, rightward-tilted shift in pressure–natriuresis curves observed in SS models of hypertension. However, despite the fact that genetic and physiological research has unraveled multiple possible natriuretic defects in SS, the possibility that the shift in pressure–natriuresis depends on defective renal sodium (Na+) excretion has not been proved with certainty. Other mechanisms could also account for a pressor effect of salt.

In contrast to the extensive research on alterations in natriuretic systems, the mechanism by which they might lead to an increase in BP remains obscure. An intuitive explanation would be that renal salt retention leads to plasma volume expansion, increasing preload and cardiac output (CO) and producing a hyperkinetic form of hypertension. However, studies of SS versus SR subjects have failed to show abnormally increased sodium retention or CO in the former. Actually, the hemodynamic pattern of SS hypertension is one of relative vasoconstriction, or impaired vasodilation in response to a normal salt-induced increase in CO.4-7 Therefore, the question of how increased salt intake translates into increased vascular smooth muscle contractility and higher total peripheral resistance (TPR) remains unanswered.

The concept of total-body autoregulation has been invoked as an explanation,8,9 although the original idea of Guyton's group10 pertained to adjustments in resistance in response to increases in local blood flow, not BP. According to this theory, increases in TPR may be the result of secondary adaptation to undetected increases in CO, occurring at stages earlier than the one at which the hemodynamic studies were conducted. The total-body autoregulation explanation was supported in an early study of young essential hypertensive patients who had initial increases in CO but transformed their hemodynamic pattern to one of increased TPR over a decade.11 However, if a decade must elapse before autoregulation is established,
this explanation would not be applicable to the understanding of vasoconstriction of SS humans, which has been observed after giving a salt load over a few days to weeks.4–7 Therefore, the first aim of our study was to determine whether different responses in TPR occur between SS and SR subjects in response to salt depletion after 24 hours. If so, the differences would constitute further evidence against the autoregulatory hypothesis.

Also, Na+ compartmentalization involves a component better studied in skin and striated muscle, in which Na+ is not stored iso-osmotically with water.12 An increase in accumulation of Na+ in skin and striated muscle has been associated with aging and hypertension.13 Hypothetically, altered accumulation of Na+ and water in the interstitium around vascular smooth muscle might directly affect contractility and explain rapid changes in TPR in SS subjects after a salt load. Therefore, the second aim of our study was to investigate differences between SS and SR subjects in terms of body weight, urine output, or Na+ excretion in responses to salt loading and salt depletion.

Methods

Subjects and Protocol

For the hemodynamic studies in the first aim of this project, we prospectively recruited 21 normotensive volunteers for an Institutional Review Board–approved (Scott and White Hospital) protocol to assess their SS of BP (SBSP) and to obtain echocardiographic measurements of CO after salt loading and salt depletion. For the studies on body weight, salt, and water handling, we pooled the data of these 21 normotensive subjects with those of 32 hypertensive patients who had been previously studied with an identical IRB–approved protocol at the University of Texas Medical Branch.

Subjects were classified as hypertensive if BP was >140/90 mm Hg or if they were receiving antihypertensive treatment. All subjects (normotensive and hypertensive) maintained their habitual salt intake for 2 weeks before study. Hypertensive patients discontinued all medications during this period, with monitoring of BP for safety. Medical histories with demographic and clinical characteristics, physical examinations and routine laboratory data were obtained to assess for specified exclusion criteria.

After about 2 weeks from the consent date, subjects were admitted to the research ward the night before starting the acute protocol for SSBP. This protocol was adapted from Grim et al14 as previously reported. Briefly, after awakening and ambulating at 6:00 am and after obtaining all clinical and baseline laboratory data, subjects started an isocaloric 160 mEq Na+/d diet (metabolic kitchen) at 8:00 AM and were given 2 L of normal saline as an intravenous infusion from 8:00 AM to 12:00 PM for a total 24-hour Na+ intake of 460 mEq (salt loading or HiNa day). After awakening the next morning at 6:00 AM, all clinical and laboratory data representing the effect of salt loading were obtained. Starting at 8:00 AM, salt depletion was initiated with an isocaloric 10 mEq Na+/d diet and 3 oral 40 mg doses of furosemide given at 8:00 AM, 12:00 PM, and 4:00 PM (LoNa day). The following morning, clinical and laboratory data representing the effect of salt depletion were obtained, before the subject was discharged from the hospital. During both diet periods, potassium intake was maintained constant at 70 mEq/d. Oral fluid intake was kept ad libitum and was not monitored throughout the study.

BP was recorded throughout the entire hospitalization with an ambulatory, automated, noninvasive oscillometric device (SpaceLabs 90207). Readings were recorded every 15 minutes from 6:00 AM to 10:00 PM and every 30 minutes overnight. Baseline BP was the average of all readings (7.2±0.4) obtained from awakening on HiNa until starting the saline infusion. BP of HiNa was the average of all readings (51.4±0.8) from 12:00 PM (after the end of the saline infusion) until 10:00 PM (time to retire to bed) and BP of LoNa was the average of all readings (35.0±0.8) from 12:00 PM (after the second dose of furosemide) until 10:00 PM (time to retire to bed). An average fall in systolic BP ≥10 mm Hg from HiNa to LoNa was used to classify a subject as SS.

Body weight at baseline, after salt loading and after salt depletion was measured with the patient in a hospital gown, at ≥6 to 7 AM, before breakfast and using the same scale. Laboratory data included blood counts, chemistries with electrolytes and creatinine, plasma renin activity, aldosterone and insulin (radioimmunoassay), and plasma catecholamines (radioenzymatic assay). Serum osmolality was calculated as BUN (mg/dL)/2.8+glucose (mg/dL)/18+serum Na+ (mEq/L)×2. Insulin sensitivity was calculated as the HOMA2-S index, using the HOMA2 calculator v2.2 of the Diabetes Trials Unit, University of Oxford (www.dtu.ox.ac.uk).15

All urine specimens were collected on ice throughout the study and kept refrigerated. They were divided into a 24-hour sample for the HiNa day, a 12-hour sample for the period of furosemide-induced diuresis and a 12-hour sample starting 4 hours after the last dose of furosemide (salt-depleted state). Volumes for each period were recorded. Creatinine and electrolytes were measured in fresh aliquots obtained at the end of each period. No attempt was made to measure extrarenal (skin or fecal) Na+ losses but the research wards were clinical and laboratory data representing the effect of salt loading and salt depletion.

Echocardiographic Measurements

Echocardiograms (GE Vivid I, GE Healthcare, Chicago, IL) in the normotensive subjects were obtained at ≥5 PM (≥7.4 hours) on both, the HiNa and LoNa days. That is, they were conducted 5 hours after finalizing the saline infusion on HiNa and 1 hour after the final dose of furosemide on LoNa. All subjects were in normal sinus rhythm without ectopy during the study. A measurement of the left ventricular outflow tract (LVOT) diameter was obtained in the parasternal long-axis view in systole for calculation of the LVOT area. The LVOT velocity time integral was measured using pulsed-wave Doppler in the LVOT at the level of the annulus, in the apical 5-chamber view. Stroke volume (SV) was calculated as LVOT area×LVOT velocity time integral and CO as SV×heart rate. Three consecutive measurements were obtained and averaged in each study. These Doppler-based CO measurements correlate well with invasively measured CO.17 TPR was calculated as the mean arterial pressure (from the monitors, within 1.00±1.85 minutes of the echocardiographic measurements) divided by the CO. Measured parameters of LV filling included the peak early (E wave), and late diastolic (A wave) filling velocities of the mitral inflow in an apical view, the E/A ratio, the deceleration time of E velocity, and the ratio between E velocity and e′ (tissue Doppler e annular velocity ratio). They were assessed using pulsed-wave Doppler in the apical 4-chamber view, according to the American Society of Echocardiography’s guideline for evaluation of LV diastolic function.18 E-wave velocity primarily reflects the left atrium-LV pressure gradient during early diastole and depends on preload and LV relaxation, whereas the E/e′ ratio offers a good estimate of LV filling pressures.

Statistical Analyses

Data are presented as means±SEM. Comparisons between SS and SR groups were made with unpaired Student t tests. Differences between periods in the same subjects were analyzed with paired t tests. Correlation coefficients were calculated with Pearson method. All previous tests and single-linear regression analyses were performed with JMP software (SAS Institute). A probability <5% was used to reject the null hypothesis.

Results

The baseline characteristics of the participants are shown in Table, comparing SS versus SR subgroups within normoten-
normotensive versus hypertensive subjects and females versus males was not different between the total SS and SR groups. Eight of 21 normotensive subjects (38%) and 16 of 32 hypertensive patients (50%) were SS. SS subjects were older, with a larger percentage of black subjects, higher BPs, increased aldosterone/renin ratios, and lower insulin sensitivity than their SR counterparts (particularly in normotensive subjects). All of these findings are consistent with usual features of the SS state. Plasma renin activity was somewhat lower, and body mass index (BMI) was somewhat higher in SS than in SR; however, these differences did not reach statistical significance. The higher baseline BPs of all SS subjects were not only because of a slightly higher percentage of hypertensive patients in this group compared with SR but also because of a higher BP in normotensive SS compared with normotensive SR. The average BP fall from the salt replete to the salt-depleted state was significantly greater in SS than in SR, as expected from the cutoff used for classification of these 2 groups. Nonetheless, these responses were normally distributed in the entire population, as described in other studies. 1

The left bars of Figure 1 show the amount of Na+ retained by SR and SS subjects during the 24 hours of salt loading (ie, 460 mEq of Na+ intake minus the Na+ excreted in the 24-hour urine specimen). The middle bars show the calculated theoretical amount of weight that the subjects should have gained if they would have retained an iso-osmolar amount of water (calculated as Na+ retained/serum Na+×1000). The right bars show the actual change of body weight from baseline to the morning after salt loading was completed. In the left panel, we show that SR subjects retained 98±22 mEq Na+, predicting a weight gain of 697±157 g. However, the observed body weight change was −158±250 g, indicating that the entire amount of Na+ retained was somehow stored without accompanying water. The small symbols next to the bars show that this pattern of Na+ retention without concomitant weight gain was observed in both, normotensive and hypertensive SR subjects.

The right panel shows that the Na+ retention of SS subjects was 107±21 mEq, not significantly different from that of SR. This finding predicted a weight gain of 762±151 g. In contrast to SR, the actual body weight gain was 819±204 g, indicating that SS retained an iso-osmolar amount of water approximately concomitant to their Na+ retention, with a similar pattern in normotensive and hypertensive SS subjects. Consistent with this observation, SS subjects sustained a decrease in calculated serum osmolality (−1.7±0.9 mOsm/L, P<0.03), not observed in SR (+0.05±0.9, not significant), not shown.

The left bars of Figure 2 show the amount of Na+ lost by SR and SS subjects during the 24 hours of salt depletion (ie, 10 mEq of Na+ intake minus the Na+ excreted in the 24-hour urine). The middle bars show the calculated theoretical amount of weight that the subjects should have lost if they would have excreted an iso-osmolar amount of water (Na+ lost/serum Na+×1000). The right bars show the actual body weight change from the salt replete to the salt-depleted mornings. In the left panel, we show that SR subjects lost 320±17 mEq Na+, predicting a weight loss of −2327±121 g. The observed
body weight loss was −2222±311 g, not significantly different from expected. Again, the small symbols show that this pattern occurred in both, normotensive and hypertensive SR subjects. Hence, despite the fact that SR subjects had retained Na⁺ without accompanying water, they lost an approximately iso-osmolar amount of water when salt depletion was induced by diet and furosemide treatment.

The right panel shows that the Na⁺ loss of SS subjects was 318±22 mEq, not significantly different from that of SR and predicting a weight loss of 2300±162 g. The actual body weight loss was 2820±227 g, that is, greater (borderline statistical significance) than that of SR and significantly greater (by 519±203 g, P<0.01) than expected. This actual weight loss in excess of predicted weight loss represented only 63.5% of the weight gain produced by salt loading. This pattern of Na⁺ excretion in excess of that expected, but not sufficient to reverse the weight gain of the previous day, was observed in both normotensive and hypertensive SS subjects, as illustrated by the small symbols. Taken together, the data in Figures 1 and 2 suggest that although SR subjects were able to store Na⁺ without accompanying water when given a salt load, SS subjects were unable to do so and retained water commensurate to their Na⁺ retention. This water retention of SS was not fully corrected when they were subjected to salt depletion by a low-salt intake and furosemide.

Results of the echocardiographic hemodynamic studies in 21 normotensive volunteers are given in Figure 3. The data on the second (salt depletion) day of the experiment are presented first (the left of each pair of bars) to emphasize the observations that hemodynamics (mean arterial pressure, CO, and TPR) were not different between SR and SS subjects during Na⁺ depletion. The left top panel shows that although mean arterial pressure of SR was not significantly different between LoNa (85.4±1.8 mm Hg) and HiNa (84.2±2.7 mm Hg), ∆−1.2±2.9, not significant, that of SS was significantly higher on HiNa (96.5±2.8) than on LoNa (82.6±3.3), ∆13.9±3.0, P<0.001, as expected from their classification into the SR and SS subgroups. As a consequence of this, on the HiNa day, mean arterial pressure of SS was significantly higher than that of SR, P<0.01.

The top right panel shows that CO was higher during HiNa than during LoNa in both groups; SR 4.4±0.2 L/min versus 3.7±0.2, P<0.001, and SS 4.5±0.3 L/min versus 4.0±0.5, ∆0.5±0.2, P<0.02. Neither the absolute COs during LoNa or HiNa nor the magnitude of the changes between these 2 days was significantly different between SR and SS subjects. Higher COs during HiNa were a reflection of

**Figure 1.** Columns show data in all salt-resistant (SR, white fill) or salt-sensitive (SS, black fill) subjects analyzed together. The small symbols with dashed error lines that surround each column show the same data in the respective normotensive (squares) or hypertensive (triangles) subgroups of each group. The leftmost columns of both panels show the amount of sodium retained during the sodium loading day of the experiment (see text). The middle columns show the estimated (Est) amount of weight gain to retain the sodium in iso-osmolar manner. The right columns show the actual observed (Obs) weight change in the 2 groups of patients. Symbols inside the black columns are for the difference for each parameter between the SS and SR groups. ns indicates not significant; †P<0.001.

**Figure 2.** Columns show data in all salt-resistant (SR, white fill) or salt-sensitive (SS, black fill) subjects analyzed together. The small symbols with dashed error lines that surround each column show the same data in the respective normotensive (squares) or hypertensive (triangles) subgroups of each group. The leftmost columns of both panels show the amount of sodium lost during the sodium-depleting day of the experiment (see text). The middle columns show the estimated (Est) amount of weight loss needed to lose the sodium with iso-osmolar water. The right columns show the actual observed (Obs) weight change in the 2 groups of patients. Symbols inside the black columns are for the difference for each parameter between the SS and SR groups. Brackets are for the comparison of expected vs actual weight loss in the SR and in the SS subjects. ns indicates not significant. †P<0.06.
changes in SV because heart rates were actually lower during HiNa than during LoNa in both groups (SR 68±2 bpm versus 75±2, \(\Delta -6.5\pm1.8\), \(P<0.001\), and SS 68±2 bpm versus 78±3, \(\Delta -10.6\pm3.3\), \(P<0.001\)), not shown. Therefore, calculated TPR (bottom left panel) was lower in SR during HiNa (1549±66 dyn*cm\(^{-5}\)*s) than during LoNa (1896±92 dyn*cm\(^{-5}\)*s), \(\Delta -347\pm84\), \(P<0.001\), whereas it was not different between the 2 days in SS (1791±148 versus 1767±163, \(\Delta 24\pm76\), not significant). As a consequence of this, during HiNa, TPR of SS was borderline higher than that of SR, \(P=0.05\).

In contrast to the similar hemodynamics between SR and SS during LoNa, left ventricular filling pressures (E/e\(^{\prime}\) ratio, bottom right panel) were higher in SS than in SR on both days of the experiment (LoNa 9.0±0.9 versus 5.7±0.4, \(P<0.002\) and HiNa 9.8±0.7 versus 7.7±0.3, \(P<0.01\)). These filling pressures were higher during HiNa than during LoNa \(\Delta 2.5\pm0.8\), \(P<0.002\) in SR, whereas in SS the overall higher E/e\(^{\prime}\) ratios were not different between the 2 days, \(\Delta 0.8\pm0.6\), not significant. Two SS subjects had E/e\(^{\prime}\) ratios >10 on the HiNa day, a value usually considered as the cutoff between normal and abnormal left ventricular filling. The E/A ratios were not significantly different between SS and SR subjects on either LoNa or HiNa. However, they were higher on HiNa than on LoNa in each group (SR 1.68±0.10 versus 1.22±0.07, \(\Delta 0.48\pm0.13\) \(P<0.001\) and SS 1.40±0.08 versus 1.07±0.13, \(\Delta 0.33\pm0.16\), \(P<0.02\), not shown.

Figure 4 depicts significant relationships between the individual magnitude of SSBP in all subjects (\(\Delta\) systolic BP between LoNa and HiNa), the decrease in TPR between those 2 days (left) and the absolute E/e\(^{\prime}\) ratio on the HiNa day (right). As can be seen, the greater the degree of SS the lesser was the vasodilation during salt loading compared with salt depletion and the greater the left ventricular filling pressures when salt loaded. The respective regression equations were: \(\Delta\)TPR\(_{\text{LoNa–HiNa}}=\Delta\) systolic BP\(_{\text{LoNa–HiNa}}\) and E/e\(^{\prime}\)\(_{\text{HiNa}}=7.501-0.157 \times \) systolic BP\(_{\text{LoNa–HiNa}}\). Thus, for each mmHg increase in SSBP there was a loss in the ability to vasodilate during a salt load of 27.3 dyn*cm\(^{-5}\)*s of TPR and an increase in left ventricular filling pressures during salt loading of 0.157 U of E/e\(^{\prime}\) ratio. The relationship between SSBP and E/e\(^{\prime}\) ratio was also present on the LoNa day of the experiment (\(r=0.57\), \(P<0.02\), not shown).

**Discussion**

The issue of how a salt load initiates an increase in BP in SS animals or humans has been a matter of great controversy. The total-body autoregulatory theory proposes that because of an ill-defined deficit in salt excretion, salt retention leads to increases in plasma volume, cardiac preload, and an initially increased CO without significant change in TPR. Within this theoretical framework, later increases in TPR occur as an autoregulatory response to tissue hyperperfusion. Increased TPR maintains or exaggerates the BP elevation and resets CO back to normal, via pressure natriuresis. Three types of observations render support for this theory. First, young labile hypertensive subjects, whose early hemodynamic pattern was one of increased CO, converted such a pattern into one of normal CO and increased TPR a decade later. Second, most Mendelian forms of hypertension involve mechanisms that could initiate the cascade above by ostensibly causing renal Na\(^{+}\) retention. Third, dogs given aldosterone and salt developed hypertension mediated by increases in CO with
lates endothelial function, vasoconstriction, and renal arterial myogenic responses. Therefore, renal hemodynamic investigations of how an increase in salt intake mediates the increase in aldosterone-salt hypertensive dogs was there a direct investigation of autoregulation of blood flow took place at the time the elevation of BP had reached a steady state.

However, we cannot exclude the possibility that changes in human hemodynamic patterns over a decade represent structural remodeling of the vasculature secondary to hypertension itself, rather than a functional change in vascular tone. Most Mendelian forms of hypertension are unquestionably linked to salt-transport mechanisms in the kidney. However, they have not been tested for SSBP with a formal protocol. In fact, a recently discovered Mendelian form of hypertension, studied with the same protocol applied here, has been ascribed to a sole increase in TPR, rather than to aldosterone reabsorption. Furthermore, in the assumed salt-dependent forms of Mendelian hypertension, the possibility that the primary mechanism for salt-induced increases in BP might be extrarenal is not excluded because many of the involved genes are ubiquitously expressed in vascular tissues, brain, and elsewhere. For example, expression of the epithelial Na⁺ channel in endothelial and vascular smooth muscle cells regulates endothelial function, vasoconstriction, and renal arteriolar myogenic responses. Therefore, renal hemodynamic changes could be responsible for BP responses to salt when this channel is mutated. Furthermore, in the experiment in aldosterone-salt hypertensive dogs, the authors did not prove that normal values of TPR represented a normal response to salt, because there was no control group. Finally, neither in Mendelian forms of hypertension linked to Na⁺ transport nor in aldosterone-salt hypertensive dogs was there a direct investigation of how an increase in salt intake mediates the increase in BP.

In controlled experiments solely assessing the effect of salt, that is, without mineralocorticoid administration, it has been established that the normal vascular response to salt loading in SR animals or subjects was a significant vasodilation for maintenance of normal BP. A few years later, Sullivan et al first studied 10 young normal subjects who were given a 10 mEq/d Na⁺ diet for 4 days, followed by 4 to 6 days of a 200 mEq/d Na⁺ diet. They performed hemodynamic studies by echocardiography at the end of both periods and at 6 and 12 months later, when the subjects were on ad libitum diets containing 144±52 mEq Na⁺/d on average. BP remained at the baseline level throughout the year, whereas CO increased by 9% by the end of the high-salt diet and by 33% to 34% at 6 months and 1 year, corresponding to 19% and 28% to 29% decreases in TPR. This observation was the first demonstration in humans that the normal hemodynamic adaptation to salt-induced increases in CO was a concomitant vasodilation for maintenance of normal BP. A few years later, the same group reported similar studies in 109 subjects, about half of whom were normotensive and half hypertensive. One third of the subjects were SS on the basis of their change in BP when going from a 10 to a 200 mEq Na⁺/d diet both given for 4 days. The hemodynamic responses of SS subjects were similar to those of the normal young volunteers in the previous study. In contrast, SS subjects exhibited salt-induced increases in TPR, with or without concomitant increases in CO, suggesting an abnormal vascular response to salt. The latter was confirmed by demonstrating that SS subjects on high salt had increased forearm vascular resistance and decreased
forearm blood flow compared with SR. Analogous results were obtained by Schmidlin et al. They studied 23 normotensive black subjects in whom repeated hemodynamic measurements were made by impedance cardiography during the last 3 days of a 1-week 30 mEq Na+/d diet and during the 7 days of a 250 mEq Na+/d diet. They demonstrated that their SR and SS subjects did not differ in terms of their Na+ balance, plasma volume, or CO responses to high salt. Although BP remained normal in SR owing to vasodilation, it increased in SS owing to an inability to decrease TPR. In another study by the same investigators involving 37 normotensive blacks nearly equally distributed in SS and SR groups, they reproduced the earlier hemodynamic observations and showed that perhaps the inability of SS subjects to vasodilate in response to salt might be because of an abnormal salt-induced increase in asymmetrical dimethylarginine, not observed in SR.

The hemodynamic results of our studies are entirely consistent with the view that salt-induced increases in BP are the result of abnormal vascular function in SS. However, there are 4 aspects of our study that are novel and important. First, the majority of our normotensive volunteers were white subjects. Thus, despite differences in the prevalence of SSBP between blacks and whites, the underlying hemodynamic mechanisms were indistinguishable. Second, because of our acute, inpatient protocol design, we actually assessed the hemodynamic effects of salt depletion, rather than that of salt loading as in the dietary studies above. Our SS subjects had higher BP than SR during salt loading, because their TPR values were higher and their CO measurements equal to those of their salt-loaded SR counterparts. Salt depletion led to a reduction in CO and increase in TPR in SR, with maintenance of a normal BP. In contrast, salt depletion did not change TPR in SS. Their BP values normalized because of the reduction in CO. Therefore, combined with the lack of normal vasodilation in response to salt, demonstrated in the dietary studies above, our data suggest that there is an impairment in the regulation of vascular tone in SS, in response to either salt loading or salt depletion. Third, we show that otherwise normal SS subjects have increased left ventricular filling pressures compared with SR, regardless of salt balance and not associated with differences in LV relaxation or preload (equal E/A ratios). This finding may indicate that concomitant to their vascular dysfunction, SS subjects exhibit early, subtle changes in myocardial contractility. Finally, perhaps the most striking feature of our studies is that the hemodynamic changes occurred within 24 hours (as opposed to days in the dietary studies) making it highly unlikely that changes in TPR or the lack thereof can be attributed to total-body autoregulation.

Higher but equal CO values in SS and SR after the salt load, compared with those after salt depletion, could theoretically be because of higher heart rates or SVs. In our subjects, they were attributable to SV because heart rates were actually lower on the high-salt day than on the low-salt day, an observation identical to that in normotensive blacks. Increases in SV may be because of increases in preload or cardiac contractility. Lower heart rates and equal levels of plasma catecholamines after salt loading in our study (not shown) make the latter possibility unlikely. In terms of preload, it is interesting that our balance studies show that SR subjects retained Na+ without water, whereas SS ones retained concomitant water. If equal increases in CO by salt reflect equal increases in preload, it follows that the differential retention of salt and water in SS and SR did not differentially affect their effective intravascular volume. In the case of SR, the percent of total retained Na+ that remained in the intravascular compartment must have required a shift of water from the extravascular to the intravascular compartment, to maintain their normal osmolality after the salt load. In contrast, for the total Na+ and water retained by SS to produce an equal increase in preload to that in SR, there are 2 possible explanations. Either blood was pooled in a venous capacitance vascular bed, which is highly unlikely because SS is characterized by increased sympathetic tone, or alternatively, some water must have shifted in the opposite direction. Overall, our data suggest that the differential retention of Na+ and water in SR and SS subjects must have differentially affected their storage in the interstitium.

We estimated Na+ balance from intake minus urinary excretion, and the latter was in the range of 250 to 450 mEq on both days of the experiment (salt loading and furosemide effects). Under the conditions of our experiment (climate-controlled hospital rooms, no gastrointestinal symptoms, and no exercise), total skin, fecal, and respiratory losses may have been in the 20 to 30 mEq/d range. Therefore, our results on Na+ balance could not have been significantly affected by extrarenal losses. Furthermore, we showed almost identical Na+ balances after salt loading and depletion in SR and SS subjects, which is consistent with observations by others.

In contrast, we did not measure fluid intake. Therefore, we could not rely on urine volumes to make assumptions about water balance. The urine volumes were actually not different between SR and SS on either experimental day (not shown). We used 24-hour body weight changes as a surrogate because in such a short period and with isocaloric diets, weight changes most likely reflect acute changes in fluid balance, not in dry weights. It is from these data that we conclude that SS subjects retained more water than SR during salt loading and excreted less water than SR (compared with that retained during the salt load) during the period of salt depletion. Although based on a surrogate measure, this conclusion is consistent with previous observations with thorough measurements of fluid compartments and water balance (food content, fluid intake, urine and fecal water) in normal volunteers. In this study, large increases in salt intake from a habitual salt consumption (similar to the situation in our SR patients) did not change body mass and were associated with unexpected total-body fluid losses. This is opposite to traditional beliefs on salt-induced water retention, derived from previous studies in subjects who were salt-depleted at baseline. Furthermore, these authors detected paradoxical increases in plasma volume, despite lack of increase in extracellular fluid volume. They attributed this to shift of water toward the circulation, as we do for our findings in SR.

The reason for the apparent difference in water handling between SS and SR subjects cannot be ascertained from our studies. Theoretical possibilities include that SS
have increased hypothalamic sensitivity to serum osmolality, reduced osmolar threshold for release of vasopressin, increased sensitivity of the renal tubular response to vasopressin, or increased expression and activity of aquaporins. There is no information in humans about these topics. However, equal expression of the mRNA for the vasopressin type II receptor in the kidney of Dahl SS and SR rats, and markedly reduced expression of that for aquaporin-2 in a mouse model of renal dysgenesis with SS hypertension do not support these explanations.

An intriguing possibility is raised by the recent characterization of subcompartments of the interstitial space in which Na⁺ is stored without commensurate water accumulation. In mouse skin, regulation of the efflux of Na⁺ from such compartment involves hypertonicity-induced lymphangiogenesis. The latter is mediated by a pathway that begins with increased expression of the osmosensitive transcription factor toxicity enhancer–binding protein in skin interstitial macrophages. Toxicity enhancer–binding protein increases macropage production of vascular endothelial growth factor-type C, which activates the vascular endothelial growth factor receptor-3 with ensuing lymphatic vessel proliferation. Interestingly, blockade of this pathway by a toxicity enhancer-binding protein–specific siRNA, by vascular endothelial growth factor-type C trapping with soluble vascular endothelial growth factor receptor-3, or by macropage depletion impairs lymphangiogenesis, thereby reducing lymphatic Na⁺ efflux and producing SS hypertension by mechanisms that may involve plasma endothelin-1. Conceivably, a reduced capacity for Na⁺ storage in this compartment might have led to obligatory iso-osmolar water reabsorption during a salt load in our SS subjects. This idea is supported by the observation that Dahl rats actually have such reduced capacity for storage of osmotically inactive Na⁺. Finally, accumulation of water-free Na⁺ in striated muscle of humans, detected by magnetic resonance imaging techniques, is associated with aging and hypertension, which are both characterized by an increased prevalence of SSBP.

Other investigators have proposed that disturbed nitric oxide activity, aberrant sympathetic activity, or other pathways regulating TPR may underlie the impaired vasodilation exhibited by SS subjects after a salt load. We now propose that altered Na⁺ storage in SS individuals may play a role. Signaling between glycosaminoglycan-rich Na⁺ storage sites and the vasculature is not yet understood. However, there is emerging evidence that hyperosmolar Na⁺ activates and induces oxidative stress in dendritic cells with production of antigenic isoketal adducts that stimulate T-cell proliferation (Annet Kirabo, DVM, MSc, PhD, personal communication), similar to observations in angiotensin and deoxycorticosterone-salt hypertension. Thus, interstitial, hyperosmolar salt-induced vascular dysfunction may be because of the inflammatory state that has been implicated in the genesis of other forms of hypertension.

In conclusion, we provide supporting evidence for a major role of vascular dysfunction in SSBP. We also find that altered smooth muscle contractility may have a counterpart in altered cardiomyocyte contractility, affecting left ventricular filling pressures even in otherwise normal young individuals. Finally, we show that although Na⁺ handling does not differ between SS and SR subjects during salt loading or depletion (as shown previously by others), water handling does. Because our data suggest dissimilar storage of iso-osmolar versus hyperosmolar Na⁺ in the interstitium of SS and SR subjects and such storage has been linked to hypertension, we speculate that this difference may play a role in generating the SS state.

Perspectives

SS of BP is a risk factor for cardiovascular morbidity and mortality in normotensive and hypertensive subjects (ie, independent of BP) that does not have a specific therapy because its ultimate mechanisms and causation are not fully understood. Opposite to a traditional view of this phenotype, based on putative abnormalities in Na⁺ retention, evidence has accumulated indicating that primary defects in vascular function may also play a role. Our data support this contention and allow for speculation about a link between such vascular dysfunction and abnormalities in interstitial sodium storage. This speculation requires proof in prospective studies of compartmentalized sodium storage in humans with and without the SS phenotype, which are now possible owing to emerging magnetic resonance imaging–based techniques that directly detect Na⁺ in tissues. Unraveling of the mechanisms of SS of BP may facilitate development of targeted therapeutic interventions.

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Disclosures

None.

References

Novelty and Significance

What Is New?

- Abnormal vascular responses to salt deprivation occur in salt-sensitive normotensive subjects within 24 hours of the change in salt balance, supporting vascular dysfunction, as opposed to total-body autoregulation, as the underlying mechanism.
- Otherwise normal salt-sensitive subjects have subtle abnormalities in cardiomyocyte function, leading to increased left ventricular filling pressures independent of the status of salt balance.
- Balance data suggest that salt-resistant normotensive subjects exposed to a salt load store sodium without iso-osmolar water in the interstitial fluid compartment; in contrast, salt-sensitive subjects retain the same amount of sodium during the salt load, but require iso-osmolar water retention.

What Is Relevant?

- The combination of the differential hemodynamics and differential sodium and water handling in salt-sensitive versus salt-resistant normotensive subjects supports the view that differential interstitial sodium storage relates to the vascular dysfunction of the former.

Summary

This study contributes to the body of knowledge supporting a role for vascular dysfunction in the salt-sensitive phenotype of humans, a cardiovascular risk factor without specific treatment. Unraveling of its mechanisms is required to allow for the development of therapeutic strategies.

Hemodynamics and Salt-and-Water Balance Link Sodium Storage and Vascular Dysfunction in Salt-Sensitive Subjects
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