Proposition of a Feasible Protocol to Evaluate Salt Sensitivity in a Population-Based Setting

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Although a variety of techniques have been devised to assess salt sensitivity, most have proven cumbersome from a methodological perspective. We therefore attempted to develop a 2-week method by which participants could be tested in an outpatient setting without requirement of a strict dietary regimen. In this method, subjects take 140 mEq of an NaCl supplement per day for 1 week and 25 mg of hydrochlorothiazide daily for another week while maintaining their customary diet. In our first trial, 8 healthy volunteers submitted to this method, as well as to a widely-used rapid volume expansion and contraction protocol. Blood pressure measurements, blood sampling and 24-h urine collection were performed before, in the middle of, and after each intervention. There was a fair correlation ($r = 0.69$) between the two protocols with respect to the changes in mean blood pressure ($\Delta$MBP), a measure of salt sensitivity. In our second trial, we tested the method on 82 Japanese subjects who had never been treated with antihypertensive drugs. $\Delta$MBP was significantly correlated with plasma renin activity (PRA) during salt loading ($r = 0.52, p < 0.0001$) and with the changes in atrial natriuretic peptide ($\Delta$ANP) ($r = -0.34, p = 0.0018$). When total subjects were divided into two subgroups by age, a similar tendency of correlation was observed. Age, PRA during salt loading, $\Delta$ANP, and $\Delta$norepinephrine were proven to be significant predictors of salt sensitivity and accounted for 46% of the $\Delta$MBP variances. Based on these results, the dietary method presented here seems to be applicable for a population-based survey. Our preliminary data also suggest that PRA and ANP would be of predictive value in the salt sensitivity test. (Hypertens Res 2002; 25: 801–809)

Key Words: salt sensitivity, hypertension, plasma renin activity, atrial natriuretic peptide, diet

Introduction

It has long been recognized that environmental factors such as dietary habits play an important role in the development of hypertension. A number of epidemiological studies have shown that the prevalence of hypertension increases with habitual salt intake or urinary sodium chloride excretion in various parts of the world (1), whereas some studies have failed to demonstrate such a significant association between salt and hypertension (2). These conflicting observations may be due, in part, to the substantial heterogeneity that is thought to exist in the individual blood pressure (BP) response to changes in sodium balance, often referred to as salt sensitivity.

Vascular responses to salt can be reproducibly quantified in animal models using standardized protocols, which have therefore provided insight into the physiological characteristics, modulators, and possible mechanisms of salt sensitivity.
In humans, on the other hand, the definition of salt sensitivity itself remains arbitrary (3). Despite the fact that there are no uniform criteria for measuring salt sensitivity as a function of BP change, a variety of techniques have been devised to assess the BP response to changes in sodium balance and extracellular fluid volume. These techniques can be largely separated into two categories. One is a rapid saline infusion followed by low salt diet and diuretic administration, and the other is a short-term dietary manipulation which usually takes 2 weeks or longer (4).

Irrespective of the techniques used, the BP change with salt loading and depletion has mostly yielded a normal distribution, in accordance with the multifactorial nature of the trait. Salt sensitivity is thus presumed to be the result of interactions among a number of modulators, genetic or acquired (5). For example, impairments in the sympathetic nervous system and the renin-angiotensin (R-A) system, biological activity of atrial natriuretic peptide (ANP), and other neuro-hormonal factors are known to affect the natriuretic ability of the kidney (6). Nonetheless, because of the absence of appropriate intermediate phenotypes, an eventual outcome, i.e., the BP change with salt loading and depletion, has been exclusively used to identify individuals as salt-sensitive or salt-resistant. Accordingly, most of the techniques currently available for the assessment of salt sensitivity are cumbersome due to the requirement of strict dietary regimen or hospitalization. They are not suitable for application in a population-based setting, and this limitation has hindered progress in the studies of salt sensitivity to date.

For this reason, we attempted to develop a practicable dietary method for testing salt sensitivity by means of two trials. In the first trial, we tested two techniques—the rapid protocol and a dietary method we devised—on 8 healthy male subjects in order to validate our new method. In the second trial, we further tested our dietary method on 82 Japanese subjects to identify simple biological markers that can predict salt sensitivity.

### Methods

#### Study Population

In the first trial, 8 male volunteers (mean age, 29.3 years; range, 26–36 years) were recruited from the hospital staff of Teikyo University, Tokyo, Japan (Table 1), and in the second trial, a total of 82 Japanese subjects were enrolled in Campo Grande, Brazil and in Kobe, Japan independently (Table 2). The 51 residents from Campo Grande were second- or third-generation Japanese immigrants, and their ethnic origin was confirmed by genealogical information. Because aging is a principal confounding factor for salt sensitivity, 31 elderly males (60–70 years of age) from Kobe were also enrolled. Then, the two populations were combined and categorized into 2 subgroups by age in order to adjust for confounding influences of aging and to investigate the reproducibility of the study results (Table 2). The older subgroup (≥60 years) comprised all the subjects enrolled in Kobe and 10 (5 males and 5 females) subjects enrolled in Campo Grande. All subjects gave their informed written consent to participate. Participants underwent a medical consent to participate. Participants underwent a medical consent to participate. Participants underwent a medical consent to participate.
check-up that included hematological screening tests, urinalysis, and a physical examination to exclude the presence of serious illness. Subjects were considered eligible for the study if they had never been treated with antihypertensive drugs and their BP levels did not exceed 160/100 mmHg at two separate visits. Patients with diabetes mellitus or impaired glucose tolerance were excluded.

### Study Design

All parts of the study were performed on an outpatient basis. The protocols were approved by the ethics committees of the Teikyo University School of Medicine and the Graduate School of Human and Environmental Studies, Kyoto University, and were in conformity with the ethical guidelines of these institutions. Two distinct techniques, the rapid protocol and the dietary method, were employed in the first trial to validate the congruity between the techniques, and then the dietary method was undertaken in the second trial.

The rapid protocol was the one previously reported by Cusi et al. (7), which was a modified version of Weinberger’s method (8). Briefly, normal saline (2L) was infused intravenously over 2 h (0900–1100 h). During this period supine BP was measured by the same physician every 30 min until the last 15 min of the infusion, when it was measured by the same physician every 5 min, and these three BPs were averaged. Hemodynamic changes were carefully observed over a 4-h recovery period. On the next day, participants were advised to eat a low sodium, isocaloric diet and ingested two 40 mg furosemide tablets at 0800 h and 1600 h. At 0900 h of the next morning—i.e., the end of the salt depletion period—supine BP was measured three times at 5 min intervals and these values were averaged.

The dietary method was a modified version of Fujita’s method (9). Three minor modifications were made as follows: 1) the order of salt loading and diuretic treatment was reversed; 2) the amount of NaCl supplement was reduced from 180 mEq/day to 140 mEq/day; and 3) 25 mg/day hydrochlorothiazide was used instead of 25 mg/day mefruside. According to a recent survey, Japanese people consume a relatively large amount of dietary salt (200–220 mEq/day on average), and consequently, the total salt intake (regular meals plus NaCl supplement) can sometimes exceed 350 mEq/day. The procedure involved a 2-week intervention consisting of a high salt diet and a diuretic treatment period of 1 week each. Hemodynamic and biochemical measure-
ments, and a 24-h urine collection were made while subjects maintained their customary diet. Based upon their 24-h sodium and potassium excretions, participants were instructed to adhere to a constant sodium and potassium intake without altering their caloric intake throughout the intervention. Subjects ingested 140 mEq of NaCl supplement as a condensed consommé cube (40 mEq/cube; Ajinomoto Inc., Tokyo, Japan) twice daily and 6 tablets of slow sodium (approx. 10 mEq/tablet; HK Pharma, Ltd., Hitchin, UK) for 1 week salt-loading period, and they took diuretics (25 mg/day hydrochlorothiazide) for another week salt-depletion period. Blood sampling was made on the last day of each period after 10 min of rest in a quiet room. In the first trial, the dietary method was retested in each participant at least 2 months after the rapid protocol.

Measurements

In the first trial, BP was measured using a mercury sphygmomanometer throughout the procedures, while an automated BP recorder (ES-P203; Terumo Inc., Tokyo, Japan) was provided for each participant to monitor hemodynamic changes voluntarily. In the second trial, BP was measured using a standardized automated sphygmomanometer (Khi Machine; VINE Co., Ltd., Tokyo, Japan) (10) with subjects in the seated position after 5 min of rest. For each study period—i.e., baseline, salt-loading, and salt-depletion—BP was calculated as the mean of 3 readings taken at 1- to 2-min intervals. The overall response to each maneuver was assessed by changes in mean BP (ΔMBP) during transition from the salt-loading to the salt-depletion period.

When undergoing the rapid protocol, subjects had an indwelling catheter inserted in a forearm vein. At least 10 min after insertion of the indwelling catheter, blood was drawn for the determination of electrolytes, catecholamines, plasma renin activity (PRA), plasma aldosterone concentration (PAC), angiotensin II (A-II), ANP, and adrenomedullin. Then saline was infused through this catheter. When submitting to the dietary method, subjects had blood samples taken at 0900–1100 h after an overnight fast for each study period. In 11 instances, however, blood samples were taken not after an overnight fast but >3.5 h after breakfast. Dietary compliance and sodium balance were ensured by measurement of urinary sodium and creatinine in a 24-h urine collection.

Serum and urinary electrolytes were measured by an ion-selective electrode method. Serum catecholamines were measured with high-pressure liquid chromatography. Commercially available radioimmunoassay (RIA) kits were used to measure PRA, PAC, A-II, ANP, and BNP (Shionogi Pharmaceuticals, Osaka, Japan), cyclic GMP (cGMP), and adrenomedullin (Peninsula Laboratories, Inc., San Carlos, USA) and insulin. All assays were performed in a single laboratory (SRL, Tokyo, Japan). The HOMA (Homeostasis Model Assessment) index was calculated as fasting plasma glucose (mmol/l) ÷ insulin (µU/ml)/22.5.

Statistical Analysis

The paired Student’s t-test and correlation analysis were carried out to evaluate the concordance of studied variables between the rapid protocol and the dietary method in the first trial. The correlation coefficient was calculated to measure the strength of the linear relationship between two given variables, and Fisher’s Z-transformation was made to assess the statistical significance of the correlation. Multiple regression analysis was also performed to estimate $R^2$ values explained by studied variables. The values were expressed as the means $\pm$ SE unless otherwise indicated. Values of $p < 0.05$ were considered to indicate statistical significance.

Results

Preliminary Evaluation of Hormonal Changes in the Rapid Protocol

The rapid protocol was undertaken in one participant to collect preliminary data on hormonal changes during the salt-loading and -depletion periods. This individual alone under-
went the rapid protocol twice. In the course of saline infusion, PRA, PAC, and plasma A-II levels were gradually decreased and stayed at the lowest levels for 4 h after the infusion. Among the studied parameters, plasma A-II levels became $\leq 3.0$ pg/ml, whereas the RIA used reliably measured concentrations of $\geq 3.0$ pg/ml. Thus we did not include A-II in the subsequent study.

According to the original protocol (8), a participant should take three 40 mg furosemide tablets while eating a low salt diet on the second day of the rapid protocol. However, because of frequent urination and dehydration, we decided to reduce the number of furosemide tablets (two tablets at 0800 h and 1600 h), which still induced a comparable degree of R-A system activation (data not shown).

Concordance between the Rapid Protocol and Our Dietary Method

In the first trial, we tested two techniques on 8 male subjects to validate the dietary method devised in the present study. The average MBP for the 8 subjects tended to decrease over the course of saline infusion, and showed a further slight decrease during the salt depletion period. On the other hand, marginal changes in MBP were found during the salt-loading and salt-depletion periods of the dietary protocol (Fig. 1). There was a good correlation between the two techniques for $\Delta$MBP (correlation coefficient $r = 0.69$, $p = 0.053$), PRA during salt loading ($r = 0.61$, $p = 0.11$), and PRA during salt depletion ($r = 0.86$, $p = 0.004$) (Table 1 and Fig. 2).

To determine whether 25 mg of hydrochlorothiazide per day could produce a salt-depleted state on the 7th day, 24-h sodium excretions were monitored every day during the salt depletion period in 2 of the 8 subjects. Their urinary sodium excretions decreased from the 1st to 5th day and remained lower than the baseline levels thereafter: the mean values were 154, 160, and 152 mmol/day for the 5th, 6th and 7th days vs. a baseline level of 188 mmol/day. In addition, the PRA value during the salt-depletion period of the dietary protocol exceeded that during the rapid protocol (4.69 ± 13.6 vs. 3.35 ± 0.79 ng/ml/h), and this supported a salt-depleted state in patients treated with the dietary method after a period of 7 days with diuretic treatment.

Biochemical Parameters and BP Changes in the Dietary Method

Most of the participants in the dietary method ($n = 71$) were normotensive, while 11 subjects (10 subjects $\geq 60$ years and 1 subject < 60 years) were mildly hypertensive according to the criterion of 140 and/or 90 mmHg. Table 2 shows a series of biochemical parameters studied in the second trial. Salt-sensitive subjects appeared to be more prevalent in the older subgroup ($\geq 60$ years) than in the younger subgroup (< 60 years). Based on the criterion of a $\geq 10\%$ decrease in $\Delta$MBP (3), for example, the percentages of salt-sensitive subjects were $32\%$ (13/41) and $12\%$ (5/41) for the corresponding subgroups. Several baseline parameters significantly differed between the two subgroups, including MBP, BMI, HOMA-index, insulin, norepinephrine, ANP, BNP, and cGMP. Here, the observed differences in BMI and related phenotypes were likely to reflect differences in the origin of the cohorts rather than differences in age: that is, residents in Campo Kato et al: Evaluation of Salt Sensitivity in a General Population 805
Grande tended to be more obese than those in Kobe. On the other hand, it was difficult to explain the subgroup differences observed for some of the other parameters. For example, baseline PRA was higher and PRA changes to salt manipulation were more exaggerated in the older subgroup than in the younger subgroup. This is somewhat contradictory to the notion that a natural age-related decline in renal function may be accompanied by bluntness in the R-A system activity and lead to inability to excrete excess dietary salt (11). When restricted to the older subgroup, no apparent differences in the relevant parameters were observed between the participants in Campo Grande and those in Kobe, but we could not
exclude the potential existence of confounding factors between the two cohorts.

In search of potential correlates with salt sensitivity, we calculated correlation coefficients between the studied variables and ∆MBP in subjects submitting to the dietary method (Table 3). A significant correlation was found for 5 variables—PRA, PAC, PAC/PRA ratio, ANP, and BNP—during salt loading and/or the changes (∆) during transition from the salt-loading to the salt-depletion period. PRA during salt loading was most strongly correlated with ∆MBP when analyzed in the total subjects (p ≤ 0.0001) and separately by subgroup (p = 0.0006 for the subgroup < 60 years and p = 0.0001 for the subgroup ≥ 60 years) or by cohort (data not shown). Also, PAC/PRA and ANP during salt loading, and ∆PAC/PRA and ∆ANP were significantly correlated with ∆MBP, and the correlation was more pronounced in either of the subgroups. While ANP was significantly correlated with a second messenger, cGMP, in both trials (r = 0.49–0.52, p < 0.0001), cGMP itself was not correlated with ∆MBP. In a stepwise logistic regression, 6 variables—age, PRA during salt loading, ∆ANP, ∆norepinephrine, BNP at baseline, and ∆PAC/PRA—turned out to be important covariates in the prediction of salt sensitivity after screening of the 35 variables incorporated in the present study (data not shown). The six covariates accounted for 46% of the variances of ∆MBP (R² = 0.46), among which age (p = 0.00004), PRA during salt loading (p = 0.0013), ∆ANP (p = 0.01), and ∆norepinephrine (p = 0.03) were significant predictors by multiple regression analysis (p < 0.05).

Our results showed that adrenomedullin, a potent vasoactive peptide mediating vasodilatory and natriuretic properties through the cyclic AMP, nitric oxide, and renal prostaglandin systems (12), was almost unaffected by changes in sodium balance, similarly to ACE levels. In addition, the HOMA index, a measure of insulin resistance, significantly increased during transition from the salt-loading to the salt-depletion period in the total subjects (p = 0.0009) and in each subgroup (p = 0.018 and p = 0.013 for subjects < 60 years and ≥ 60 years, respectively). This may have partly resulted from diuretic-induced glucose intolerance (13). Otherwise, we encountered no serious complications among participants in the present study.

Discussion

The present study addressed two important issues regarding salt sensitivity. First, with a view to developing a method of salt-sensitivity measurement with practicability in the general population, we devised a 2-week protocol involving dietary manipulation and diuretic treatment, and made a preliminary evaluation of its correlation with a rapid protocol frequently used as a standard test of salt sensitivity (4, 8). Second, by using the protocol thus devised, we attempted to identify simple biological markers that can predict salt sensitivity. We found that BP responses to changes in sodium balance were reproducible between the two techniques and that age, the R-A system, plasma ANP, and norepinephrine levels were important predictors of salt sensitivity in the Japanese subjects studied.

The dietary method presented here can be regarded as an outpatient- or clinic-based transformation from the rapid volume expansion and contraction protocol, i.e., acute saline infusion followed by a low sodium diet and diuretic treatment. In our dietary method, volume expansion was achieved by 1 week of NaCl supplement and volume contraction was achieved by 1 week of diuretic administration, and participants maintained their customary diet throughout the intervention. From a methodological perspective, some studies have reported the congruity of different approaches for the assessment of salt sensitivity (r = 0.40–0.56) (14, 15) and others have reported the congruity of the repeated rapid protocols (r = 0.56–0.60) (16, 17). Although we tested the two techniques—the rapid protocol and our dietary method—on a limited number of subjects (n = 8), they showed a fair correlation (r = 0.69) almost equivalent to those in the studies above-mentioned. It might be argued that the combination of diuretic administration and dietary regimen does not strictly evaluate the disposition to salt sensitivity in a narrow sense, but rather represents homeostasis to volume expansion and contraction. However, just as in the arguments frequently given for the acute saline infusion protocol (8), detailed characterization of the latter phenotype would constitute a practical strategy under the current circumstances and thus should help delineate the complex nature of salt sensitivity in a broad sense. It should also be noted that BP tended to decline slightly during salt loading in the rapid protocol in our study. One possible explanation for this unexpected observation is that the tested healthy males can be considered “counterregulators,” as proposed by Overlack et al. (18). That is, upon salt loading, factors enhancing natriuresis would predominate as a counterregulatory system and/or factors shifting the pressure-natriuresis relation towards higher values would be over-suppressed. Based on our preliminary findings in 2 subjects (see Results), we used 1 week of diuretic administration to achieve a salt-depleted state as in Fujita et al. (9). Further evaluation is warranted to determine whether 1 week is sufficient to produce a consistent and stable low sodium state in the general population.

Although a number of studies have pointed out the importance of the R-A system in salt sensitivity (19, 20), the metabolic alterations in this system appear to be complex and remain to be clarified. We found the most significant and consistent correlation between ∆MBP and PRA during salt loading in the normotensive to borderline-hypertensive population, and this correlation was exaggerated in the older subgroup. On the other hand, a few previous studies have reported a significant correlation between ∆MBP and PRA during salt depletion or ∆PRA in both hypertensive and normotensive subjects (19, 20), and this finding was not replicated in our study (Fig. 3). Also, we found a significant cor-
relation between \( \Delta \text{MBP} \) and \( \Delta \text{PAC/PRA} \), suggesting that salt-sensitive subjects had a blunted increment in PAC per given PRA value during transition from the salt-loading to the salt-depletion period. A possible explanation is that certain confounding factors may have prevented us from finding the uniform relationship between salt sensitivity (or \( \Delta \text{MBP} \)) and PRA, \( \Delta \text{PRA} \), and other variables related to the R-A system. For example, hypertension-induced or age-related renal damage and resultant hormonal impairment may cause the blunted PRA response to stimulatory factors such as diuretic treatment in some but not all subjects. Thus, the potential confounding factors should be taken into account when we interpret a predictive value of the R-A system for salt sensitivity.

Interestingly, the present study provided significant evidence for an association between plasma ANP levels and \( \Delta \text{MBP} \), as previously implied by Overlack et al. \((18)\). This relation was supported by both correlation analysis and multiple regression analysis. ANP during salt loading and \( \Delta \text{ANP} \) were both significantly correlated with \( \Delta \text{MBP} \) in the total subjects and subjects < 60 years \((p = 0.0018–0.005)\), and \( \Delta \text{ANP} \) was a significant predictor of \( \Delta \text{MBP} \) by multiple regression analysis \((p = 0.01)\). In agreement with a previous study \((21)\), there was a significant inverse correlation between PRA and ANP in our study \((r = -0.35, p < 0.002)\) (Fig. 3). Two explanations can be proposed for the observation that salt-sensitive subjects showed a greater elevation in ANP and a greater suppression in PRA levels during salt loading than salt-resistant subjects. First, these changes may be compensatory or secondary phenomenon to the exaggerated volume expansion induced by salt loading. This hypothesis is appealing, but it remains to be defined whether salt-sensitive subjects show relatively greater volume expansion than their salt-resistant counterparts. Second, salt-sensitive subjects may have impaired natriuretic responses to ANP, which could cause sodium-water retention in the body and lead to suppression of PRA. In this regard, it is worth noting that hypo-responsiveness to ANP has been suggested to be etiologically related to blunted natriuresis and other hemodynamic abnormalities in the Dahl salt-sensitive rat \((22)\). Nonetheless, it remains to be clarified whether hypo-responsiveness to ANP occurs in salt-sensitive subjects, and if so, whether it contributes to the development of salt-induced hypertension.

In addition to two major components, the R-A system (salt-retaining hormones) and ANP (salt-losing hormones), we evaluated several of the other candidate physiology mechanisms for salt sensitivity. The overall responses to changes in sodium balance function to preserve the excretory ability of the kidney: salt loading results in a shift that enhances natriuresis \((e.g., \text{ANP and prostaglandin E2})\) and results in a reduction in factors that shift the pressure-natriuresis relation downward \((e.g., \text{PAC and norepinephrine})\) \((6)\). On the other hand, salt depletion operates in an opposite manner. Considering the closely controlled homeostasis in sodium-water handling, it is plausible that combined defects in more than two separate components in a given pathway or pathways—rather than a defect in a single component—plays a major role in determining salt sensitivity. In this context, the accumulating results for our dietary method would allow for comprehensive analysis using a series of simultaneously studied candidate parameters. Because precise and detailed monitoring of BP is critical, repeated measurements of casual BPs and/or ambulatory BP monitoring can be taken in this setting \((23)\).

Finally, salt-sensitive subjects are assumed to have a higher risk of developing “essential” hypertension, and elucidation of the molecular basis of salt sensitivity will provide novel insights into the pathophysiology of hypertension. Even so, because a substantial percentage of salt-sensitive subjects remain normotensive, the physiological and biochemical determinants of salt sensitivity need to be explored without restricting target subjects to hypertensive patients. It can be speculated that certain factors may promote salt sensitivity in some individuals without predisposing them to essential hypertension. We expect that the dietary method validated in the present study will be a valuable tool to delineate the mechanisms underlying salt sensitivity in a population-based setting.

References


