Salt Loading on Plasma Asymmetrical Dimethylarginine and the Protective Role of Potassium Supplement in Normotensive Salt-Sensitive Asians

Yuan Fang, Jian-Jun Mu, Lang-Chong He, Si-Cen Wang, Zhi-Quan Liu

Abstract—Asymmetrical dimethylarginine (ADMA) is an endogenous inhibitor of NO synthase. Because endothelial NO pathway is compromised in patients with salt-sensitive hypertension, we investigated whether the plasma ADMA can be modulated by chronic salt loading in normotensive salt-sensitive persons and its relationship with NO, and we further determined whether or not dietary potassium supplementation can reverse them. Sixty normotensive subjects (aged 20 to 60 years) were selected from a rural community of Northern China. All of the people were sequentially maintained on a low-salt diet for 7 days (3 g/day, NaCl), then a high-salt diet for 7 days (18 g/day), and high-salt diet with potassium supplementation for another 7 days (4.5 g/day, KCl). After salt loading, the plasma ADMA concentrations increased significantly in salt-sensitive subjects (0.89±0.02 μmol/L versus 0.51±0.02 μmol/L; P<0.05), whereas the plasma NOx levels reduced considerably (41.8±2.1 μmol/L versus 63.5±2.1 μmol/L; P<0.01). All of the abnormalities normalized when dietary potassium were supplemented (0.52±0.03 μmol/L versus 0.89±0.02 μmol/L for ADMA and 58.1±0.9 μmol/L versus 41.8±2.1 μmol/L for NOx). Statistically significant correlations were found among plasma ADMA level, the mean blood pressure, and the level of NO after salt loading in normotensive salt sensitive individuals. Our study indicates that high dietary potassium intake reduces blood pressure and ADMA levels while increasing NO bioactivity in normotensive salt-sensitive but not salt-resistant Asian subjects after salt loading. (Hypertension. 2006; 48:724-729.)

Key Words: sodium ■ potassium ■ nitric oxide ■ hypertension, sodium-dependent

N O is a potent endogenous vasodilator produced in the vessel endothelial cells from L-arginine by NO synthase (NOS). It is involved in the regulation of vessel homeostasis by inhibiting vascular smooth muscle tone1,2 and growth3 and platelet aggregation,4 as well as leukocyte adhesion to the endothelium.5 Vascular NO plays an important role in the regulation of blood pressure (BP).6 The abnormal L-arginine–NO pathway has been demonstrated in patients with salt-sensitive (SS) hypertension when the dose of dietary salt was altered.7–12 It was found that salt loading attenuated conversion of L-arginine to NO in the endothelium of the renal vasculature in SS hypertension patients.13 In addition, inhibition of inducible (i)NOS in normotensive salt-resistant (SR) rats led to a significant decrease in urinary NOx, converted the SR rats to SS rats, and increased the mean BP (MBP).14 Barton et al15 found a functional defect of NOS in the kidney of Dahl SS (DS) rats in response to salt loading. Furthermore, Deng and Rapp16 showed that the iNOS locus (Nos2) is one of the candidate genes to influence BP in the DS rats. All of this evidence suggests that SS persons or animals harbor a defect of NOS, which could result in elevated BP when there is high salt intake.

N\textsuperscript{5}, N\textsuperscript{6}-dimethylarginine (asymmetrical dimethylarginine [ADMA]) is an endogenous, competitive inhibitor of NOS and has potent vasoconstrictor and pressor actions.17,18 Plasma level of ADMA has been shown to be elevated in hypertensive rats. It was demonstrated that the elevation of plasma ADMA is associated with reduced activity of NOS and impaired endothelium-dependent vasodilation.19,20 ADMA was found to be involved in the development of high BP in DS hypertensive rats but not in spontaneous hypertensive rats, suggesting that salt might modulate the level of ADMA in SS hypertension.21 Potassium intake helps to downregulate BP by increasing sodium excretion.22–25 The study in normotensive blacks found that salt sensitivity occurs when dietary potassium intake was just marginally deficient and that SS was dose-dependently suppressed when potassium intake was increased within its reference range, indicating that the potassium supplementation might prevent or delay the occurrence of hypertension.26 Previous studies in our laboratory demonstrated that moderate increment of potassium intake in children and adolescents could significantly lessen the rising of
BP with age growth, especially in those subjects with salt sensitivity. In the present study, we tested the hypothesis that chronic salt loading could affect the plasma ADMA in normotensive SS persons, further inhibits NO synthesis, and that dietary potassium supplementation might prevent the salt loading–induced elevation of BP by reversing abnormal levels of ADMA and NO.

Methods

Subjects

Normotensive subjects (n=60, aged 20 to 60 years; by a complete history and physical examination) were enrolled from a rural community of Northern China. They were not on any medication and had no symptoms of coronary or peripheral arterial diseases. Subjects with previous history of liver or renal disease or with diabetes mellitus were excluded. All of the subjects are nonsmokers. The Institutional Ethics Committee of Xi’an Jiaotong University Medical School approved the study protocol, and each subject gave written informed consent. All of the procedures were performed in accordance with institutional guidelines.

Protocol

The protocol consisted of a series of investigations, including baseline history and physical examination (height, weight, and BP) for 3 days, 7 days on a low-salt diet (51.3 mmol or 3 g of NaCl per day), 7 days on a high-salt diet (307.7 mmol or 18 g of NaCl per day), and a high-salt diet with potassium supplementation (60 mmol or 4.5 g of KCl per day) for another 7 days. During the baseline investigation, each subject was given detailed dietary instructions to avoid table salt, cooking salt, high-sodium foods, and food rich in nitrite/nitrate for the subsequent 21 days. All of the meals were prepared in research kitchens and consumed onsite.

BP Measurement

On days 5, 6, and 7 of each week, BP was measured in the morning by Hawkshaw random zero sphygmomanometer after subjects had rested quietly for ≥5 minutes, and the cuff was of adult size. BP was measured 3 times with a 1-minute interval, and the mean value was recorded. Systolic BP (SBP) and diastolic BP (DBP) were determined as the first and fifth phases of the Korotkoff sounds, respectively. Pulse pressure (PP) was defined as: PP = SBP − DBP. Of 60 subjects that we examined, 13 subjects showed an increase in MBP by >10 mm Hg from low- to high-salt diet, and the other 47 showed little or no response. Twenty-four-hour urine samples were collected on day 7 of each week and kept frozen at −40°C until analyzed. Twenty-four–hour urine samples were collected on day 7 of each week and kept frozen at −40°C until they were analyzed.

Reagents

ADMA and monomethylarginine were obtained from Sigma and 25% ammonia from Merck. O-phthaldialdehyde and 3-mercaptopropionic acid were obtained from Fluca. NO assay kits were purchased from Jian-Cheng Biological Medical Engineering Institute.

Determination of Plasma ADMA

The plasma concentration of ADMA was measured by precolumn derivatization with o-phthaldialdehyde and high-performance liquid chromatography by a method published previously. It was performed on the chromatographic system consisting of a Shimadzu SPD-10Avp pump, Model 7125 injector, an Anastar work station, and RF-535 fluorescence detector (all from Shimadzu) set at excitation and emission wavelengths of 340 and 455 nm, respectively. Achiral column was a Diamonsil 5 μm ODS column (150 mm×4.6 mm).

Determination of Nitrite/Nitrate Concentration

The levels of NOx in the plasma and urine were determined as described previously.

24-Hour Urinary Sodium and Potassium Determination

Urinary concentrations of sodium and potassium were measured by flame photometer, and 24-hour urinary excretions of sodium and potassium were calculated by multiplying the concentration by the 24-hour total urine volume.

Statistics

All of the data were presented as mean±SE. Differences of mean values were assessed by a paired or unpaired Student t test for comparison of 2 variables and by ANOVA for comparison of multiple variables. Covariate analyses were performed to control the age factor. Linear regression analyses were performed between 2 continuous variables. P<0.05 was considered statistically significant.

Results

Profiles of Studied Subjects

Of 60 subjects that we examined, 13 subjects showed an increase in MBP by ≥10 mm Hg after salt loading (group SS: 7 men and 6 women), and the other 47 showed little or no response (group SR: 27 men and 20 women). No significant difference was found in age and body mass index between 2 groups, but the SBP of SS subjects was significantly higher than that of SR subjects (Table 1; P<0.05). The 24-hour urinary sodium excretion after salt loading in the SS group was less than that in SR group (Table 1; P<0.05). After potassium supplementation, the 24-hour urinary potassium excretions were increased in both groups, and the urinary sodium excretions in SS subjects were increased compared with that in the salt-loading diet (Table 1; P<0.05).

TABLE 1. Characteristics of SS and SR Subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Age, y</th>
<th>Body Mass Index, kg/m²</th>
<th>SBP, mm Hg</th>
<th>DBP, mm Hg</th>
<th>24-Hour Urinary</th>
<th>Na⁺, mmol</th>
<th>K⁺, mmol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LS</td>
<td>HS</td>
<td>HS + K</td>
</tr>
<tr>
<td>SS</td>
<td>13</td>
<td>43.7±2.0</td>
<td>22.3±0.6</td>
<td>116.6±2.2†</td>
<td>74.5±2.7</td>
<td>47±2.1</td>
<td>305±1.6</td>
<td>317±3.5†</td>
</tr>
<tr>
<td>SR</td>
<td>47</td>
<td>42.3±1.3</td>
<td>23.1±0.3</td>
<td>110.3±1.4</td>
<td>71.5±0.9</td>
<td>51±0.3</td>
<td>313±0.5</td>
<td>314±1.4</td>
</tr>
</tbody>
</table>

LS indicates low salt; HS, high salt; HS + K, high salt with potassium supplement.

*P<0.05 vs SR; †P<0.05 vs HS in SS subjects.
Effects of Salt Loading and Potassium Supplementation on BP

The DBP in SS subjects was significantly lower than that in SR subjects on a low-salt diet (Table 2; \(P<0.05\)). After salt loading, both SBP and DBP in SS subjects were significantly higher than that in SR subjects (Table 2; \(P<0.05\)). Potassium supplementation reduced the BP in SS subjects on a high-salt diet (Table 2; \(P<0.05\)), whereas no significant change was observed in the BP of SR subjects with the same treatment (Figure 1).

The Effects of High Salt Intake and Potassium Supplementation on Plasma ADMA

We found that plasma ADMA concentration in SS subjects was higher on a high-salt diet than on a low-salt diet (0.89 ± 0.02 \(\mu\)mol/L versus 0.51 ± 0.02 \(\mu\)mol/L; \(P<0.01\); Figure 1), but a high-salt diet–induced increase in plasma ADMA was abrogated by potassium supplementation (0.52 ± 0.03 \(\mu\)mol/L versus 0.89 ± 0.02 \(\mu\)mol/L; \(P<0.01\); Figure 1). Plasma ADMA level was not affected in SR subjects on a low-salt, high-salt, or high-salt diet with potassium supplementation (Figure 1).

Further analyses showed that in SS subjects, after adjusting for the age factor, there were significantly positive correlations between MBP and plasma ADMA in a low-salt diet, in a high-salt diet (\(r=0.80; P<0.01\)), or in a -alt diet with potassium supplementation (\(r=0.78; P<0.01\); Figure 2). However, no significant correlation between plasma ADMA and MBP was found in SR subjects (Figure 2). Furthermore, the plasma ADMA level was negatively correlated with NOx level in the urine of SS subjects (\(r=−0.85; P<0.01\); Figure 3).

The Effects of High Salt Intake on Plasma NOx and the Protective Role of Potassium Supplementation

The plasma NOx levels in SS subjects were lower than that in SR subjects after low-salt diet (63.5 ± 2.1 \(\mu\)mol/L versus 79.7 ± 1.4 \(\mu\)mol/L; \(P<0.01\); Figure 3) and after high-salt diet (41.8 ± 2.1 \(\mu\)mol/L versus 87.6 ± 0.8 \(\mu\)mol/L; \(P<0.01\); Figure 3). In SS subjects, the plasma NOx levels were decreased significantly after salt loading (41.8 ± 2.1 \(\mu\)mol/L versus 63.5 ± 2.1 \(\mu\)mol/L; \(P<0.01\); Figure 3), which potassium supplementation could reverse (58.1 ± 0.9 \(\mu\)mol/L versus 41.8 ± 2.1 \(\mu\)mol/L; \(P<0.01\); Figure 3). Salt loading signifi-

### Table 2. Effects of Salt Loading and Potassium Supplementation on BP (mm Hg)

<table>
<thead>
<tr>
<th>Gr</th>
<th>N</th>
<th>Low SBP</th>
<th>Salt DBP</th>
<th>High SBP</th>
<th>Salt DBP</th>
<th>Supplement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SBP</td>
</tr>
<tr>
<td>SS</td>
<td>13</td>
<td>107.1±1.5</td>
<td>62.6±0.8*</td>
<td>119.7±1.7*</td>
<td>76.5±1.1*</td>
<td>108.2±1.6†</td>
</tr>
<tr>
<td>SR</td>
<td>47</td>
<td>106.1±1.4</td>
<td>66.7±0.7</td>
<td>109.5±1.7</td>
<td>68.1±0.8</td>
<td>104.6±1.2</td>
</tr>
</tbody>
</table>

*\(P<0.05\) vs SR, †\(P<0.05\) vs HS in SS subjects.
significantly increased the plasma NOx levels in SR subjects (87.6 ± 0.8 μmol/L versus 79.7 ± 1.4 μmol/L; P < 0.01; Figure 3). The plasma NOx level correlated negatively with MBP on a low-salt diet, on a high-salt diet (r = −0.68; P < 0.05), or on a high-salt diet with potassium supplementation in SS subjects (r = −0.63; P < 0.05) after the age factor was adjusted.

The Effects of High Salt Intake and Potassium Supplementation on Urinary NOx
The SS subjects on low-salt diet had higher urinary NOx excretion than those on high-salt diet (137.6 ± 1.7 μmol/L versus 107.4 ± 2.1 μmol/L; P < 0.01; Figure 3), and potassium supplementation could prevent the reduction of urinary NOx excretion induced by high-salt diet (141.6 ± 1.6 μmol/L versus 107.4 ± 2.1 μmol/L; P < 0.01; Figure 3). However, in SR subjects, no significant change was found in urinary NOx excretion when they were on a low-salt diet or on a high-salt diet with or without potassium supplementation.

Discussion
Our study demonstrates that high salt intake induces not only the increases in BP and plasma ADMA significantly but also the decreases in plasma NO synthesis and urinary NOx excretion in normotensive SS Asians, indicating that salt loading may inhibit NO synthesis by increasing the production of ADMA. We could not exclude the possibility that plasma ADMA may also be influenced by the alteration of BP.

The defect in the NO synthesis is one of the mechanisms that may contribute to SS hypertension.32,33 It has been shown that medullary iNOS protein concentration increased remarkably in the kidney of Sprague–Dawley rats on a high-salt diet34 and that a 6-day intravenous infusion of aminoguanidine, a selective inhibitor of iNOS, increased MBP to hypertensive levels.35 It was believed that decreased neural-type NOS activity in DS rat kidney might be involved in the SS hypertension through alterations in renal sympathetic nervous activity and sodium handling.36 Another study in normotensive DR rats showed that iNOS inhibition decreased urinary NOx significantly, caused salt sensitivity, and increased MBP.14 Therefore, salt sensitivity of hypertension may relate to changes in NO activity.

The slight elevation in plasma concentration of ADMA can inhibit the synthesis of NO in the endothelium drastically.37 Osanai et al38 found that the increased shear stress enhances ADMA release in endothelium, indicating that the change in BP might influence the ADMA level. High-salt diet raises BP and increases urinary ADMA excretion in the DS rat, whereas high BP in the spontaneous hypertensive rat was associated with increased urinary NOx excretion and decreased ADMA excretion.21 High-salt intake has been found to increase plasma ADMA levels and BP and to reduce urinary NOx, whereas a low-salt diet reverses these abnormalities in patients with SS hypertension.39 It has been shown that MBP correlates negatively with plasma NOx concentration but positively with plasma ADMA after essential hypertensive patients were loaded with salt.40 In addition, salt sensitivity of BP in normotensive postmenopausal women was found to link to increased ADMA after salt loading.41 All of these studies strongly indicate that salt may be an important influential factor of ADMA and that the change of plasma ADMA and NO may be involved in the pathophysiologic process of SS hypertension. In the present study, we found that plasma ADMA concentration increased, and the ADMA level was correlated with MBP only in the normotensive SS group but not in the SR group after salt loading. The results suggest that there may be some genetic defects or alterations in ADMA modulation when SS develops and that endothelial dysfunction already exists, although the BP of a SS person is within the reference range.

Our study clearly demonstrates that potassium supplementation could block the effects of high-salt diet on plasma ADMA, NOx level, urinary NOx excretion, and BP in
normotensive SS Asians. These observations indicate that potassium supplementation in normotensive SS subjects may influence NOx synthesis by inhibiting the ADMA production and preventing the BP elevation resulting from high salt loading. However, it is not clear how potassium supplementation decreases plasma ADMA and whether or not this effect is through the downmodulation of BP. Because all of the subjects were recruited from the Chinese population, whether or not our observation could be generalized to other racial populations is unknown. Further studies are required to validate our findings in a larger and more diverse sample and to elucidate the mechanisms by which salt loading and potassium supplementation affect plasma ADMA in SS subjects.

Perspectives
This study found that the plasma ADMA could be affected by chronic salt loading and that potassium supplementation reduces BP and ADMA levels and increases NO bioactivity in normotensive SS but not SR Asian subjects after salt loading. Our findings indicates that ADMA might mediate the effect of high salt intake on BP in SS subjects and that potassium supplementation plays a protective role in BP control probably by reversing these processes. Our study sheds some new light on understanding how salt influences BP and how potassium supplementation prevents endothelial dysfunction.

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Disclosures
None.

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