Protective Effect of Potassium Against the Hypertensive Cardiac Dysfunction
Association With Reactive Oxygen Species Reduction

Hiromitsu Matsui, Tatsuo Shimosawa, Yuzaburo Uetake, Hong Wang, Sayoko Ogura, Tomoyo Kaneko, Jing Liu, Katsuyuki Ando, Toshiro Fujita

Abstract—Potassium supplementation has a potent protective effect against cardiovascular disease, but the precise mechanism of it against left ventricular abnormal relaxation, relatively early functional cardiac alteration in hypertensive subjects, has not been fully elucidated. In the present study, we investigated the effect of potassium against salt-induced cardiac dysfunction and the involved mechanism. Seven- to 8-week–old Dahl salt sensitive rats were fed normal diet (0.3% NaCl) or high-salt diet (8% NaCl) with or without high potassium (8% KCl) for 8 weeks. Left ventricular relaxation was evaluated by the deceleration time of early diastolic filling obtained from Doppler transmitral inflow, the slope of the pressure curve, and the time constant at the isovolumic relaxation phase. High-salt loading induced a significant elevation of blood pressure and impaired left ventricular relaxation, accompanied by augmentation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase activity in the cardiac tissue, measured by the lucigenin chemiluminescence method. Blood pressure lowering by hydralazine could not ameliorate NADPH oxidase activity and resulted in no improvement of left ventricular relaxation. Interestingly, although the blood pressure remained high, potassium supplementation as well as treatment with 4-hydroxy-2,2,6,6-tetramethyl-piperidine-N-oxyl, a superoxide dismutase mimetic, not only reduced the elevated NADPH oxidase activity but also improved the left ventricular relaxation. In conclusion, a high-potassium diet has a potent protective effect on left ventricular active relaxation independent of blood pressure, partly through the inhibition of cardiac NADPH oxidase activity. Sufficient potassium supplementation might be an attractive strategy for cardiac protection, especially in the salt-sensitive hypertensive subjects. (*Hypertension. 2006;48:225-231.*)

Key Words: sodium ■ blood pressure monitoring ■ cardiac function ■ heart failure ■ oxidative stress ■ potassium

High-salt loading on the salt-sensitive subjects results in hypertension, left ventricular (LV) hypertrophy, and hypertensive heart failure.1–4 The hypertensive heart failure is characterized by the LV diastolic dysfunction composed of the LV abnormal relaxation and the increased LV chamber stiffness.5 The impaired LV active relaxation, which is observed in the patients with hypertension or diabetes,6 has been reported recently to predict a poor prognosis,7 thus, the preservation of the LV active relaxation from early stage may be a reasonable concept.

High-salt loading on the salt-sensitive model also induces overproduction of reactive oxygen species (ROS) through activation of NADPH oxidase,8 and ROS level had a significant positive correlation with the severity of heart failure.9,10 In the pressure overload model by aortic banding, the impaired LV relaxation was improved effectively by antioxidant treatment, such as vitamin C or deferoxamine, indicating that excess ROS can induce LV abnormal relaxation.11,12

To reduce ROS for cardioprotection, potassium-rich diet may be one of the candidates, because physiological increase of potassium concentration inhibited ROS formation from endothelium or white blood cells.13 Furthermore, the cardiac hypertrophy was improved by potassium supplementation in deoxycorticosterone acetate/Na mice independent of blood pressure (BP).14 Consistent with these data, modest potassium depletion impaired LV contractile and relaxation responses to epinephrine or to preload alteration in the dog model,15 and potassium repletion improved the LV relaxation, which was enfeebled by the restriction of potassium intake in the normal volunteers.16 But the precise mechanism of cardiac functional improvement by potassium has not been fully elucidated.

Thus, the purpose of this study was to investigate the effect of potassium supplementation against salt-induced LV abnormal relaxation and to elucidate the involved mechanism of potassium effect against ROS-producing systems. We used Dahl salt-sensitive (DS) rats, because high-salt loading on DS rats has been reported to induce isolated diastolic heart failure17 and to augment ROS production in plasma, blood

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From the Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Japan.
Correspondence to Toshiro Fujita, Department of Nephrology and Endocrinology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku Tokyo, 113-8655, Japan. E-mail fujita-dis@h.u-tokyo.ac.jp
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vessels, kidney, or heart. Other than high potassium supplementation, we examined the effect of BP lowering or the effect of antioxidant to clarify the role of potassium against the LV abnormal relaxation induced by high-salt loading.

**Methods**

**Production of the Model**

Seven-to-eight-week-old male DS rats (Eisai, Tokyo, Japan) were randomized into 5 groups for 8 weeks: 1, normal salt (0.3% NaCl) group (NS; n = 5); 2, high-salt (8% NaCl) group (HS; n = 5); 3, high salt with administration of hydralazine (80 mg/L in drinking water) group (HS+HY; n = 5); 4, high salt with supplementation of high-potassium diet (8% KCl) group (HS+K; n = 5); and 5, high salt with administration of 4-hydroxy-2,2,6,6-tetramethyl-piperidine-N-oxide (hydroxy-TEMPO) 1 mmol/L group (HS+TE; n = 5). Animals were maintained at 26±2°C with 12:12-hour light-dark cycle. All of the animals were handled in an accredited institute facility in accordance with the institutional animal care policies, and all of the research protocols conformed to the guiding principles for animal experimentation as enunciated by the Ethics Committee on Animal Research of the University of Tokyo.

**BP Monitoring**

Systolic BP (SBP) was monitored every week by a tail cuff method (BP-98A, Softron).

**Pathological Studies**

Left ventricle (4 hearts for each group) were fixed with 4% paraformaldehyde, embedded in paraffin, and subsequently cut into sections 3 μm in thickness (3 sections at the level of the papillary muscle for each rat). Azan staining was performed for evaluation of peri-vascular and myocardial interstitial fibrosis.

**Echocardiography**

Transthoracic echocardiographic studies were performed with a 12-MHz phased-array ultrasound system (Aplio). Rats were lightly anesthetized with intraperitoneal injection of ketamine HCl (25 to 50 mg/kg) and xylazine (5 to 10 mg/kg) and were held in the decubitus position. Rats were allowed to breathe spontaneously during the procedure. M-mode recordings of the left ventricle were recorded at the papillary muscle level to measure interventricular septal dimension, LV end-diastolic dimension, and posterior wall dimension. LV ejection fraction was calculated the formula related flow (A), and the deceleration time of the mitral early inflow peak velocity of a mitral early inflow (E), an atrial contraction related flow (D), and the deceleration time of the mitral early inflow (EdcT) were recorded. The average of 3 values was calculated.

**Hemodynamic Study**

Rats were anesthetized by ether and allowed to breath spontaneously during the operation. The 1-mm high-fidelity monometer-tipped catheter (AT-601G, Nihon Koden) introduced from the right carotid artery retrograde through the aortic valve to reach the cavity of left ventricle. The LV pressure (LVP) curve, the peak LV systolic pressure, and the LV end-diastolic pressure were recorded. The recorded LVP curve was read into the image soft (Adobe Photoshop) and the average of 4 beats of maximal negative slope at the isovolumic relaxation phase (-dp/dtmax) was calculated. For calculation of the time constant (τ), LVP curve was digitized every 5 ms, and LVP versus time data for 40 ms after peak -dp/dtmax were first fitted into single exponential relationship by the method of least squares. Beginning at the time of -dp/dtmax, LVP was plotted and fit by the method of least squares to the function \( P = e^{(-t/T)} \) \( (P \) is the LVP at the isovolumic relaxation time; \( T \) is the a negative number that represents the slope of \( \ln P \) versus time in \( s^{-1} \); \( B \) is the y intercept for \( \ln P \); and \( T \) was calculated by \(-1/A\)).

**Evaluation of NADPH Oxidase Activity by Lucigenin Chemiluminescence**

\( \text{O}_2^\bullet- \) production by LV tissue homogenate was measured using lucigenin chemiluminescence in a microplate lumimeter (LB 9507, Berthold Technologies). After the hemodynamic studies, rats were euthanized with ether, and blood was removed through the catheter placed at the left ventricle. The heart was taken out and atrium and right ventricle were removed. Left ventricle was washed with the buffer 3 times to immediately homogenized, and then incubated in 1 mL of Krebs buffer containing 10 mmol/L HEPES-NaOH (pH 7.4) for 20 minutes at 37°C. After incubation, lucigenin was added into the sample to final concentration of 10 μmol/L. The chemiluminesence of the sample was measured in a luminometer as relative light units (RLU) emitted, integrated over 30s intervals for 5 minutes. The data of the last 2.5 minutes were used for the reading, because the value of the RLU was stabilized at a relatively late time. The \( \text{O}_2^\bullet- \) level was reported as RLU after background luminescence subtraction and was normalized to milligrams of wet tissue weight. NADPH (100 μmol/L of final concentration), the substance of NAPDH oxidase, was added just before the measurement of luminescence. In some experiments, diphenileniiodonium (100 μmol/L of final concentration), a flavoprotein inhibitor that blocks NADPH oxidase, was used to confirm ROS generation from NADPH oxidase.

**Statistical Analysis**

All of the values were expressed as mean±SEM. Comparisons among groups were made by ANOVA followed by Scheffe’s method, and \( P<0.05 \) was considered to indicate statistical significance.

**Results**

During the study, all of the rats survived without any sign of congestive heart failure.

**BP and Cardiac Weight**

High-salt loading caused the significant elevation of SBP at 8 weeks (NS: 145.8±5.3 mm Hg; HS: 206.3±22.7 mm Hg; \( P<0.01 \)). Hydralazine normalized SBP to the level of NS rats (HS+HY: 155.3±3.3 mm Hg; \( P<0.01 \) versus HS). On the contrary, high-potassium diet or treatment with hydroxy-...
TEMPO had a small effect on SBP (HS+K: 185.2±19.6 mm Hg; HS+TE: 201.8±4.7 mm Hg; P<0.01 versus NS, Figure 1). The heart weight/body weight after 8 weeks of loading was increased with high-salt loading significantly (Table 1).

Pathological Study

In the pathological studies, perivascular fibrosis was observed, but myocardial interstitial fibrosis could not be detected in any group, suggesting the early functional change without apparent cardiac remodeling (Figure 2).

Echocardiographic Analysis

After 8 weeks of salt loading, LV systolic function was preserved in all of the experimental groups because of the same level of LV ejection fraction with symmetrical LV wall motion (Table 2 and Figure 3). The interventricular septal dimension was increased by high-salt loading (Table 2). The significant prolongation of EDcT, which indicates the impaired LV active relaxation, was induced by high-salt loading (NS: 48.5±0.6 ms; HS: 57.7±2.1 ms; P<0.01; Figure 4A and 4B). BP lowering by hydralazine failed to normalize EDcT (HS+HY: 59.2±1.8 ms; P<0.01 versus NS). On the contrary, potassium supplementation shortened the prolonged EDcT by high-salt loading, indicating improvement of the LV relaxation (HS+K: 48.7±1.3 ms; P<0.01 versus HS and HS+HY). Similarly, hydroxy-TEMPO successfully shortened the EDcT (HS+TE: 51.2±1.9 ms; P<0.01 versus HS and HS+HY). During the echocardiography, there was no significant difference of heart rates (NS: 288±7.9 bpm; HS: 306.3±12.3 bpm; HS+HY: 302.7±5.0 bpm; HS+K: 289.9±4.8 bpm; and HS+TE: 300.0±2.8 bpm).

Hemodynamic Analysis

The hemodynamic parameters were directly examined at 8 weeks. Consistent with the data of BP obtained by tail cuff method, LVESP was elevated in HS, HS+K, and HS+TE (P<0.05 versus NS) but reduced to the NS level in the HS+HY (Table 2). There was no significant difference in the LVEDP among all of the experimental groups (Table 2). High-salt loading blunted the −dp/dtmax significantly (NS: −6.4±0.2 mm Hg/ms; HS: −3.9±0.2 mm Hg/ms; P<0.01; Figure 5A and 5B). The blunted maximal negative slope at the isovolumic relaxation phase was not ameliorated by hydralazine (−4.4±0.5 mm Hg/ms; P<0.01 versus NS) but recovered independent of BP by potassium supplementation (−6.6±0.4 mm Hg/ms; P<0.01 versus HS and HS+HY) or by hydroxy-TEMPO treatment (−6.3±0.4 mm Hg/ms; P<0.01 versus HS and HS+HY; Figure 5B). T was significantly prolonged by high-salt loading (P<0.05 versus NS), which was not improved by hydralazine, but potassium supplementation or hydroxyl-TEMPO shortened the time constant (P<0.01 versus HS, respectively; Table 2). No significant difference of heart rate was observed during the measurement procedure (NS: 393.4±5.9 bpm; HS: 393.4±4.7 bpm; HS+HY: 385.8±2.9 bpm; HS+K: 409.1±20.0 bpm; and HS+TE: 405.1±12.4 bpm).

ROS Generation and NADPH Oxidase Activity in the Cardiac Tissue

NADPH oxidase activity in the cardiac tissue, reported to possess a pivotal role in the heart, were measured by lucigenin chemiluminescence method (Figure 6). Addition of NADPH as a substrate increased ROS production, which was inhibited by diphenyleneiodonium, indicating that the increase of ROS reflects NADPH oxidase activity. High-salt loading elevated the NADPH oxidase activity significantly (NS: 5.5±1.7 RLU/mg; HS: 27.8±9.9 RLU/mg; P<0.01). Hydralazine could not ameliorate the NADPH oxidase activity (HS+HY: 23.4±5.7 RLU/mg; P<0.05 versus NS). On the contrary, the augmented NADPH oxidase was reduced to the same level as NS rats by potassium supplementation (HS+K: 3.9±2.1 RLU/mg; P<0.01 versus HS and HS+HY) or hydroxy-TEMPO (HS+TE: 0.5±0.2 RLU/mg; P<0.01 versus HS and HS+HY; Figure 6). ROS generation was not affected by xanthine with or without allopurinol. Also, rotenone, a mitochondrial electron transport chain inhibitor, did not change ROS level, indicating that xanthine oxidase or respiratory chain of mitochondria is not likely to be the major source of ROS in this model (data not shown).

Discussion

High-salt loading on DS rats induced LV abnormal relaxation demonstrated by prolonged EDcT, blunted −dp/dtmax, and increased T,24 accompanied by the augmentation of the NAPDH oxidase activity. There were no apparent signs of the

| Table 1. Body Weight and Cardiac Weight/Body Weight After 8 Weeks of Loading |
|-------------------------|--------------------------|---------|
| Group | Body Weight, g | Cardiac Weight/Body Weight, mg/g |
| NS | 402.0±7.6 | 3.03±0.1 |
| HS | 376.7±14.7 | 3.67±0.1† |
| HS+K | 400.0±7.1 | 3.65±0.1‡ |
| HS+TE | 364±16.0 | 3.88±0.15† |

The body weight and the heart weight/body weight at 8 weeks of the NS or HS with or without HY, K, or TE.

*P<0.05 vs NS; †P<0.01 vs NS.

Figure 2. Representative histopathologic photomicrographs of left ventricle at the point of 8 weeks of DS rats (azan staining: original magnification, ×200).
congestive heart failure or LV remodeling, and LV systolic function was preserved with the same level of LVEDP, which might be attributable to the shorter-term high-salt loading than in the previous study.20

The LV abnormal relaxation could not be ameliorated by BP lowering but was successfully improved by the potassium supplementation with small effect on BP. Similar protective effect of potassium on LV function has been reported in the deoxycorticosterone acetate/Na mice14 or in the human model.16 But the precise mechanism of the cardioprotective effect of potassium has not been shown previously. Interestingly, our results first proved that the improvement of LV active relaxation by potassium supplementation was accompanied by the normalization of the NADPH oxidase activity. NADPH oxidase has been reported to possess a pivotal role as ROS origin in the failing myocardium of the patients with dilated or ischemic cardiomyopathy and the myocardium of the pressure-overloaded guinea pig.25,26 Excess ROS, possibly from NADPH oxidase, can inhibit sarcoplasmic reticulum Ca2+ uptake pump by irreversible direct oxidation of its thiol residue27 or by impairment of the physiological phosphorylation of phospholamban.28 The dual effects of ROS on intracellular Ca2+ concentration might be involved in the mechanisms for ROS-induced cardiac dysfunction. There are reports that mitochondrial electron transport chain29 or xanthine oxidase30 can also induce ROS overproduction. But in our high-salt loading model, we could

<table>
<thead>
<tr>
<th>Group</th>
<th>IVSd, mm</th>
<th>LVDd, mm</th>
<th>LVEF, %</th>
<th>LVEDP, mm Hg</th>
<th>LVESP, mm Hg</th>
<th>T, ms</th>
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<tr>
<td>NS</td>
<td>1.67±0.05</td>
<td>8.13±0.58</td>
<td>70.9±2.6</td>
<td>13.1±1.4</td>
<td>135.3±2.4</td>
<td>12.3±1.1</td>
</tr>
<tr>
<td>HS</td>
<td>1.91±0.06*</td>
<td>7.66±0.26</td>
<td>72.6±3.0</td>
<td>11.9±0.6</td>
<td>158.2±14.9*</td>
<td>25.8±7.4*</td>
</tr>
<tr>
<td>HS+HY</td>
<td>1.83±0.10</td>
<td>8.31±1.25</td>
<td>75.9±8.3</td>
<td>13.3±2.3</td>
<td>127.9±9.2†</td>
<td>18.5±3.1</td>
</tr>
<tr>
<td>HS+K</td>
<td>1.90±0.13</td>
<td>7.79±0.43</td>
<td>70.2±2.5</td>
<td>12.2±2.5</td>
<td>157.5±8.8*</td>
<td>11.6±1.3‡</td>
</tr>
<tr>
<td>HS+TE</td>
<td>1.88±0.03</td>
<td>7.54±0.24</td>
<td>75.2±3.1</td>
<td>13.4±0.7</td>
<td>152.9±5.7*</td>
<td>12.2±2.7†</td>
</tr>
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Table 2. Value of Echocardiography and the LVEDP, LVESP, and Time Constant After 8 Weeks of Loading

The value of echocardiography, LVEDP, LVESP, and T obtained by direct measurement of the DS rats fed NS or HS with or without HY, K, or TE for 8 weeks. IVSd, interventricular septal dimension; LVDd, LV end-diastolic dimension; LVEF, LV ejection fraction.

*P<0.05 vs NS; †P<0.05 vs HS; ‡P<0.01 vs HS.

Figure 3. Representation of the M-mode tracing of the LV chamber at the point of 8 weeks of DS rats. IVSd indicates interventricular septal dimension; LVDd, LV end-diastolic dimension; LVDs, LV end systolic dimension; PW, posterior wall dimension.

Figure 4. A, Representative mitral inflow image obtained by the Doppler echocardiography at the point of 8 weeks of DS rats. B, The deceleration time of the early mitral inflow; the semiquantification of the LV active relaxation, obtained by Doppler echocardiography on DS rats. *P<0.01 vs NS; †P<0.01 vs HS and HS+HY; n.s. indicates not significant.
not obtain any data suggesting mitochondria or xanthine oxidase as a major source of ROS. Taken together, the inhibition of ROS-producible NADPH oxidase activity may be the reasonable target in the therapeutic strategy. In the present study, the expression of NADPH oxidase components p22phox, gp91phox, p47phox, and p67phox were not affected by high-salt loading or by any treatment (data not shown). The activation of NADPH oxidase can be driven by the translocation of the regulatory subunit to myocyte membranes, which can be achieved without enhancement of the enzyme component.31 BP lowering by hydralazine failed to inhibit NADPH oxidase with no improvement of LV relaxation. On the contrary, the inhibition of NADPH oxidase could be achieved by potassium supplementation. The precise mechanism of potassium on the NADPH oxidase inhibition must be further explored, but some clue might be given from our results. Similar to potassium supplementation, hydroxy-TEMPO, a radical scavenger, inhibited further production of ROS from NADPH oxidase. These results can suggest that ROS itself can activate NADPH oxidase possibly in the feed-forward loop in the cardiac tissue, which has been reported previously in the vascular system,32–34 and in turn, reduction of ROS can interrupt NADPH oxidase activity.

The cardiac hypertrophy induced by high-salt loading was not ameliorated by any treatment in the present study, but the functional improvement by potassium or hydroxy-TEMPO treatment was proven, suggesting the mechanism of functional alteration independent of the structural change. According to the antioxidative effect of potassium, it should be noted that potassium could inhibit ROS production in salt-loaded rats with salt-sensitive hypertension. Several investigators have demonstrated that salt loading induced salt retention and the resultant overproduction of oxidative stress in the heart and kidney of salt-sensitive hypertensive animals but not in those of salt-resistant ones.18,19 Therefore, it is a plausible hypothesis that potassium supplementation could attenuate salt retention during the high-salt diet period in DS rats, possibly through the natriuretic effect of potassium.35–37

Perspectives

Many risk factors, such as hypertension,38 smoking,39 diabetes, and obesity,40 may cause chronic ROS loading and may be the onset of the feed-forward loop of NADPH oxidase. Furthermore, in the salt-sensitive hypertensives, ROS level greatly exceeds that of nonsalt-sensitive hypertensives.41 Thus, the counteracting effects of potassium against salt loading might
Figure 6. ROS production in the cardiac tissue of DS rats quantified by the lucigenin (10 μmol/L) chemiluminescence method. RLU/mg indicates the amount of ROS production. A, Evaluation of NADPH oxidase activity of the cardiac tissue (DS rats fed high-salt diet; 8% NaCl), NADPH, a substance of NADPH oxidase, was added to evaluate the oxidative burst originated from the NADPH oxidase. To further confirm the source of ROS production, pretreatment with diphenileneiodonium (DPI), a specific inhibitor of NADPH oxidase, was performed. B, Comparison of oxidative burst from NADPH oxidase among all groups. *P<0.05 vs NS; †P<0.01 vs NS; ‡P<0.01 vs HS and HS-HY; n.s. indicates not significant.

stand out, especially in the case of salt-sensitive hypertensives. In the clinical megatral, the BP-lowering effect by a potassium-rich diet has been proven, but its prevention of the cardiovascular disease must be explored. Our results proved that inhibition of NADPH oxidase is one of the cardioprotective mechanisms by potassium supplementation. Along with those agents that possess both effects of ROS reduction and cardioprotection, such as β-blockers, statins, angiotensin-converting enzyme inhibitors, or angiotensin 2 receptor blockers, potassium supplementation can be the valuable therapeutic strategy even at the initial stage of cardiac dysfunction in the salt-sensitive patients.

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Disclosures
None.

References


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