Potassium supplement upregulates the expression of renal kallikrein and bradykinin B\textsubscript{2} receptor in SHR

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Jin, Lan, Lee Chao, and Julie Chao. Potassium supplement upregulates the expression of renal kallikrein and bradykinin B\textsubscript{2} receptor in SHR. Am. J. Physiol. 276 (Renal Physiol. 45): F476–F484, 1999.—High potassium intake is known to attenuate hypertension, glomerular lesion, ischemic damage, and stroke-associated death. Our recent studies showed that expression of recombinant kallikrein by somatic gene delivery reduced high blood pressure, cardiac hypertrophy, and renal injury in hypertensive animal models. The aim of this study is to explore the potential role of the tissue kallikrein-kinin system in blood pressure reduction and renal protection in spontaneously hypertensive rats (SHR) on a high-potassium diet. Young SHR were given drinking water with or without 1% potassium chloride for 6 wk. Systolic blood pressure was significantly reduced beginning at 1 wk, and the effect lasted for 6 wk in the potassium-supplemented group compared with that in the control group. Potassium supplement induced 70 and 40% increases in urinary kallikrein levels and renal bradykinin B\textsubscript{2} receptor density, respectively (P < 0.05), but did not change serum kininogen levels. Similarly, Northern blot analysis showed that renal kallikrein mRNA levels increased 2.7-fold, whereas hepatic kininogen mRNA levels remained unchanged in rats with high potassium intake. No difference was observed in β-actin mRNA levels in the kidney or liver of either group. Competitive RT-PCR showed a 1.7-fold increase in renal bradykinin B\textsubscript{2} receptor mRNA levels in rats with high potassium intake. Potassium supplement significantly increased water intake, urine excretion, urinary kinin, cAMP, and cGMP levels. This study suggests that upregulation of the tissue kallikrein-kinin system may be attributed, in part, to blood pressure-lowering and diuretic effects of high-potassium intake.

Tissue kallikrein-kinin system; blood pressure; adenosine 3',5'-cyclic monophosphate; guanosine 3',5'-cyclic monophosphate; gene expression; spontaneously hypertensive rat

High potassium intake has been shown as a nonpharmacological intervention for hypertension. An inverse relationship between potassium supplement and blood pressure exists in hypertensive rat models and in human subjects (41, 51). High potassium intake exerts other protective effects, such as attenuation of renal lesions, including glomerular sclerosis and tubular dilation in Dahl salt-sensitive rats, prevention of ischemic damage, reduction of endothelial injury, arterial wall thickening, and stroke-related mortality in hypertensive rats (42, 43). The underlying mechanisms for the multiple effects of a high-potassium diet are still not clear, but some candidates, such as kallikrein-kinin, renin-angiotensin systems, aldosterone, and prostaglandin have been implicated as mediators of the effects (2, 11, 30, 54).

The renal kallikrein-kinin system is involved in the homeostasis of sodium and water balance in the kidney (37). Renal kallikrein is synthesized in the connecting tubule cells in the distal nephron where potassium is secreted (45). Zinner et al. (55) first demonstrated a close relationship between urinary kallikrein and potassium levels. High potassium intake induced hypertension and hyperplasia of the kallikrein-containing cells in the kidney and increased the secretion of renal kallikrein into urine (45). Urinary potassium excretion was found to be more closely correlated with urinary kallikrein excretion than aldosterone and other antidiuretic hormones both in dynamic and static sodium states in the body (28). There was a significant association between urinary potassium excretion and the major gene determining kallikrein levels in individuals in a Utah family pedigree (17). These results suggested a potential role of potassium in the regulation of renal kallikrein in humans and rodents.

Previous studies suggested a role of tissue kallikrein-kinin system in blood pressure regulation. Tissue kallikrein (EC 3.4.21.35) hydrolyzes low-molecular-weight kininogen to produce vasoactive kinin. The binding of kinins to bradykinin B\textsubscript{2} receptor activates second messengers that trigger a broad spectrum of biological effects, such as vasodilation, smooth muscle contraction and relaxation, inflammation, pain, and cell proliferation (5). An inverse relationship between blood pressure and urinary kallikrein levels was reported in genetically hypertensive rat models (13) and in epidemiological studies (23). A large family pedigree study showed that a dominant allele, expressed as high urinary kallikrein excretion, might be associated with a decreased risk of essential hypertension (4). The rat tissue kallikrein gene has been linked with hypertension by restriction fragment length polymorphism and cosegregation studies in hypertensive rats (34, 52). These studies suggest that high renal kallikrein could have a protective effect against the development of high blood pressure. Recently, we showed that transgenic mice overexpressing human tissue kallikrein under the control of metallothionin gene metal response element or albumin promoter/enhancer had lifelong reduction in blood pressure (40, 48). Administration of aprotinin, a tissue kallikrein inhibitor, or icatibant (Hoe-140), a bradykinin B\textsubscript{2} receptor antagonist, restored blood pressures to normal levels. Furthermore, systemic or local delivery of the human tissue kallikrein gene in the form of naked DNA or an adenoviral vector into spontane-

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ously hypertensive rats (SHR) caused sustained reduction of blood pressure for several weeks (7, 19, 46, 53). These findings demonstrated a direct linkage between tissue kallikrein gene expression and blood pressure regulation.

To explore the potential role of the tissue kallikrein-kinin system in blood pressure reduction after potassium supplement, we analyzed the expression of the system components in SHR. The results showed that a high potassium intake induced increases in the expression of renal kallikrein and bradykinin B₂ receptor as well as increases in urine excretion, kinin, cGMP, and cAMP levels. Activation of the renal kallikrein-kinin system may be attributed to the blood pressure-lowering and diuretic effects of high potassium intake.

**METHODS**

Animal treatment. Young SHR (male, 4 wk old, 50–70 g) were purchased (Harlan Sprague Dawley, Indianapolis, IN, USA). Rats were housed at a constant room temperature (25°C) with a 12:12-h light-dark cycle and had free access to rat chow and tap water. SHR were randomly divided into two groups with six animals in each group. The control group was given regular tap water and the experimental group was given 1% KCl in tap water. All procedures were in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD).

Blood pressure measurement. Systolic blood pressure of SHR was measured with a manometer-tachometer (Nastume KN 210; Nastume Seisakusho, Tokyo, Japan) using a tail-cuff method (46). Unanesthetized rats were placed in a plastic holder mounted on a thermostatically controlled warm plate that was maintained at 37°C during measurements. An average of 10 readings was taken for each animal after they became acclimated to their environment. Body weight and heart rate were recorded at the same time as blood pressure was monitored.

Urine collection. Twenty-four-hour urine was collected in metabolic cages 6 wk after potassium supplement. To eliminate the contamination of urine samples during urine collection period, rats were not given food but were given only tap water with or without 1% KCl in tap water. All procedures were in accordance with the Guide for the Care and Use of Laboratory Animals. The urine volume was measured, and the supernatant was analyzed for kininogen levels.

Tissue homogenate preparation and protein determination. Six weeks after receiving tap water or 1% KCl, rats were anesthetized intraperitoneally with pentobarbital sodium (50 mg/kg body wt). Five ml blood was withdrawn directly through the heart. The vena cava was cut and the heparin (100 U/rat) was injected into the left ventricle. The circulation was perfused with 60 ml normal saline until tissues appeared bloodless. The kidney was quickly removed, minced, and homogenized with a Polytron (Brinkmann Instruments, Westbury, NY) in PBS, pH 7.0. The homogenate was centrifuged at 1,000 g for 10 min. The supernatant was incubated in 0.5% sodium deoxycholate and then centrifuged at 10,000 g for 30 min. Protein concentration was determined by the method of Lowry et al. (21). The protein extracts were used to measure intrarenal kallikrein levels, and sera were used to measure kininogen levels.

Membrane protein preparation. Rat kidneys were rinsed in ice-cold saline and minced by scissors. Tissues were suspended in 50 mM Tris-HCl buffer, pH 7.4, with 5 mM EDTA with a hand-held glass homogenizer. The suspension was centrifuged at 500 g for 5 min, and the supernatant was centrifuged again at 40,000 g for 20 min to pellet membranes. Membrane proteins were aliquoted and stored at −80°C.

Kininogen RIA. Urinary and intrarenal kininogen levels were determined by a direct RIA (39). The iodogen method was used to label 5 µg of purified rat tissue kallikrein. A G-5 column (Pharmacia, Uppsala, Sweden) was used to separate the unlabeled and labeled kallikrein. ¹²⁵I-labeled kallikrein (100 µl; 10,000 cpm/100 µl), 100 µl tissue kallikrein antiserum (at a 1:200,000 dilution), 100 µl sample, and 100 µl assay buffer containing 1% BSA in PBS, bringing to a final volume of 400 µl, were incubated at 4°C overnight. Separation of free kallikrein and antibody-bound kallikrein was performed by centrifugation at 3,500 g for 30 min after adding 400 µl of 1% bovine γ-globulin and 800 µl of 25% polyethylene glycol in PBS. The standard kallikrein used ranged from 80 pg to 10 ng.

Determination of kininogen levels. Kininogen levels in rat sera were measured as described previously (8). Sera (50 µl) were added to 450 µl 0.02 M Tris·HCl, pH 8.0, and boiled for 30 min to eliminate kininase activity. Forty micrograms N-tosyl-L-phenylalanine chloromethyl ketone-trypsin (Sigma, St. Louis, MO) in 400 µl of 0.02 M Tris·HCl, pH 8.0, was added to 100 µl supernatant of boiled sera after 5 min of microcentrifugation. Samples were incubated at 37°C for 10 min, and the reaction was stopped by boiling for 10 min. The aliquots were used in a kinin RIA as described (38). Briefly, 100 µl of ¹²⁵I-labeled [Tyr⁰]bradykinin ([Tyr⁰]BK; 10,000 cpm/100 µl), 100 µl rabbit antiserum against bradykinin (at a 1:100,000 dilution), 100 µl diluted sample, and 100 µl 0.1% assay buffer (0.1% egg albumin, 10 mM EDTA, 3 mM 1,10-phenanthroline in PBS, pH 7.0) in a final volume of 400 µl were incubated at 4°C overnight. After addition of 400 µl of 1% bovine γ-globulin and 800 µl of 25% polyethylene glycol in PBS to the reaction mixture, free and antibody-bound bradykinin were separated by centrifugation at 3,500 g for 30 min. The standard bradykinin used ranged from 4 to 500 pg. Kininogen levels were expressed as micrograms kinin equivalents per milliliter serum.

Bradykinin B₂ receptor binding assay. Synthetic [³⁵S]-SB 203580, ¹²⁵I-labeled bradykinin ([Tyr⁰]BK) was used as radioligand for bradykinin B₂ receptor binding studies. [³⁵S]-SB 203580 was labeled as described previously (19). For saturation studies, aliquots of the membrane extract (100 µg protein) were incubated in duplicate for 2 h at 25°C in the binding buffer consisting of 1 mM 1,10-phenanthroline, 140 µg/ml bacitracin, 1 µM I-1252-SQ-44525 (captopril), 1 mM DTT, and 0.1% BSA in 25 mM TES, pH 6.8, in the presence of increasing amounts of [³⁵S]-SB 203580. Specific binding was calculated by subtracting nonspecific binding obtained in the presence of excess unlabeled bradykinin (0.1 mM) from total binding obtained in the absence of unlabeled peptide. The final assay volume was 0.5 ml. At the end of the incubation, 4 ml of washing buffer (0.1% BSA in 25 mM TES buffer, pH 6.8) was added. The reaction mixture was filtered on a Whatman GF/C glass fiber filter (1.2 µm) previously soaked for at least 2 h in 0.1% polyethyleneimine. The filter was washed four additional times with 4 ml of washing buffer. The filter-bound radioactivity was detected in a gamma counter. Results were calculated by Scatchard transformation of binding data using the Kinetic Radioligand computerized program (27) and expressed as means ± SE of three independent experiments conducted with three different membrane preparations from each group of rats.

RIA of urinary cAMP. Urinary cAMP levels were determined by a RIA as previously described (9). cAMP (5 µg) was labeled with 1 mCi of [³¹⁵]iodide and incubated with chlora-
mine-T (Sigma) for 30 s at room temperature followed by addition of 50 µl 25% acetic acid. Iodinated cAMP in 50 mmol/l potassium phosphate buffer, pH 7.0, was separated on a reversed-phase C-18 HPLC column in an acetonitrile gradient (10% solution A containing 0.1% trifluoroacetic acid and 90% solution B containing 100% acetonitrile in 0.1% trifluoroacetic acid). Samples (100 µl) were acetylated with 5 µl acetylation agent of triethylamine and acetic anhydride in 2:1 ratio and were then added to 900 µl 50 mm sodium acetate buffer, pH 6.0. The reaction mixture, containing 100 µl 125I-labeled cAMP (12,000 cpm/100 µl), 100 µl cAMP antiserum (at a 1:20,000 dilution) in assay buffer (1% BSA in 50 mM sodium acetate buffer, pH 6.0), and 100 µl sample in a final volume of 300 µl, was incubated at 4°C overnight. Free and antibody-bound cAMP were separated by centrifugation at 1,500 g for 30 min after incubation for 20 min with 50 µl 1% bovine γ-globulin and 500 µl of 25% polyethylene glycol in PBS. The standard cAMP ranged from 0.8 to 200 pg.

RIA of urinary cGMP. Urinary cGMP levels were determined by a RIA as described (9). cGMP (5 µg) was labeled with 1 mCi of [125I]iodide using the same method as labeling cAMP tracer. The reaction mixture, containing 25 µl 125I-labeled cGMP (15,000 cpm/25 µl), 25 µl cGMP antiserum (at a 1:14,400 dilution), 25 µl sample (1 ml sample was incubated first with 20 µl triethylamine and 10 µl acetic anhydride), and 25 µl assay buffer (1% BSA in 50 mM sodium acetate buffer, pH 4.75) in a final volume of 100 µl was incubated at 4°C overnight. Free and antibody-bound cGMP were separated by centrifugation at 1,400 g for 20 min after incubation for 1 h with 50 µl 5× diluted human plasma (50 mM sodium acetate buffer, pH 4.75) and 1 ml 12% polyethylene glycol (50 mM sodium acetate buffer, pH 6.2). The standard cGMP ranged from 20 pM to 10 nM.

RNA preparation. Total RNA was extracted from fresh tissues by the guanidine isothiocyanate-cesium chloride gradient ultracentrifugation method (35). The extracted RNA was dissolved in diethyl pyrocarbonate-treated water. The concentration of RNA was determined by the absorbency at 260 nm. The RNA was stored at −80°C until use.

Northern blot analysis. Nick-translated cDNA probes of rat tissue kallikrein-kininogen and γ-actin were used for Northern blot analysis. Total kidney RNA (20 µg) and liver RNA (10 µg) from rats were separated by electrophoresis on a 1.5% agarose gel containing 0.66 M formaldehyde. The RNAs were transferred to Immobilon-N membranes in 20× sodium chloride-sodium citrate (SSC) solution overnight. After crosslinking, the membrane was prehybridized in buffer (5× sodium chloride-sodium phosphate-EDTA (SSPE), 10× Denhardt, 0.5% SDS, and 100 µg/ml herring sperm DNA) at 60°C for at least 4 h. Nick translation for labeling the cDNA probes was performed using α-32P dATP (New England Nuclear Research Products, Boston, MA), according to the instructions of the manufacturer (Bethesda Research Laboratories, Bethesda, MD). A G-50 spin column was used to remove unincorporated components. The specific activity of the probe was 2 × 106 cpm/µg DNA. After hybridization at 60°C for 16–18 h, the membrane was washed with 2× SSPE and 0.1% SDS at 60°C and exposed to X-ray film at −80°C. The blot was stripped and reprobed with the β-actin cDNA probe. The films were scanned to Adobe Photoshop 4.0 with a Hewlett Packard Scan Jet 1 II CX/T, and the mean intensity of respective bands was determined by NIH Image 1.47 computer software package. The ratio between the intensities of the competitor and target PCR products was plotted against the concentration of competitor cDNA added to the samples. The quantity of bradykinin B2 receptor mRNA was taken as the absorbance value that corresponded to a ratio of 1 on the ordinate axis.

Statistical analysis. Data were analyzed using standard statistical methods. Repeated blood pressure measurements were taken for comparison between control and experimental groups at each time point with the use of unpaired Student's t-test. Group data are expressed as means ± SE. Values were considered significantly different at a value of p < 0.05.

RESULTS

Effect of high potassium intake on blood pressure in SHR. The effects of high potassium intake (1% KCl in drinking water) on blood pressure of young SHR were monitored weekly from 1 to 6 wk postsupplement. The basal blood pressure was 130 mmHg in both groups before potassium supplement. Potassium supplement caused a significant delay of blood pressure rise beginning on week 1 and the effect lasted for 6 wk (Fig. 1). At 1 wk postsupplement, the rise of systolic blood pressure of SHR given KCl in drinking water was significantly reduced in SHR given 1% KCl in drinking water compared with that in control rats (131.9 ± 2.2 vs. 141.1 ± 2.3 mmHg, n = 6, P < 0.05). A maximal blood pressure reduction was observed 3 wk postsupplement between potassium and control groups (150.1 ± 6.6 vs. 163.5 ± 1.6 mmHg, n = 6, P < 0.001). Significant reduction of blood pressure was observed from 2 to 6 wk in SHR given potassium supplement compared with that of control rats.

Physiological parameters after potassium supplement. Table 1 shows the physiological parameters in SHR 6 wk postsupplement. The systolic blood pressure was significantly reduced in SHR given 1% KCl in drinking water compared with that in control rats given tap water (173.6 ± 1.4 vs. 185.1 ± 2.5 mmHg, n = 6, P < 0.05). No significant differences in body weight or heart rate of both groups were observed. However, there were significant increases in water intake (18.6 ± 1.4 vs. 8.8 ± 0.7 ml/100 g body wt·day−1, n = 6, P < 0.001) and urine excretion...
bradykinin B2 receptor density (81.1 ± 0.8 vs. 59.2 ± 5.2 pM/mg protein, n = 3, P < 0.05) in SHR given potassium in drinking water. Intrarenal tissue kallikrein levels were not altered (27.3 ± 1.9 vs. 28.0 ± 2.7 ng/ml serum, n = 6) between the two groups. Similar kininogen levels in serum (3.3 ± 0.3 vs. 3.4 ± 0.3 µg kinin/ml serum, n = 4) were observed between experimental and control groups.

Fig. 1. Blood pressure profiles of young spontaneously hypertensive rats (SHR) given 1% KCl in drinking water or tap water. Systolic blood pressure is expressed as means ± SE (n = 6). Bars represent SE. *P < 0.05 between control rats given tap water and rats given 1% KCl in drinking water. Statistical analysis was performed by unpaired Student’s t-test.

Table 1. Physiological parameters after potassium supplement

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<tr>
<th>Variables</th>
<th>Control</th>
<th>1% KCl</th>
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<tbody>
<tr>
<td>Blood pressure, mmHg</td>
<td>185.1 ± 2.5</td>
<td>173.6 ± 1.4*</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>274.4 ± 8.6</td>
<td>270.9 ± 5.0</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>370.0 ± 1.0</td>
<td>361.8 ± 2.4</td>
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<tr>
<td>Urine excretion, ml·100 g body</td>
<td>6.3 ± 0.5</td>
<td>15.3 ± 1.7†</td>
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<tr>
<td>Water intake, ml·100 g body</td>
<td>8.8 ± 0.7</td>
<td>18.6 ± 1.4†</td>
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Values for each group are means ± SE (n = 6). Four-week-old spontaneously hypertensive rats (SHR) received 1% KCl in their drinking water, and physiological measurements were performed at 6 wk after potassium supplement compared with control rats receiving tap water. Statistic significance between the 2 groups was determined by unpaired Student’s t-test: *P < 0.05 and †P < 0.001.

Table 2. Immunoreactive tissue kallikrein, kininogen, and renal bradykinin B2 receptor levels in rats with or without potassium supplement

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1% KCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary kallikrein, µg/day</td>
<td>35.4 ± 2.0</td>
<td>58.8 ± 6.3†</td>
</tr>
<tr>
<td>Intrarenal kallikrein, ng/mg protein</td>
<td>28.0 ± 2.7</td>
<td>27.3 ± 1.9</td>
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<tr>
<td>Total kininogen, µg/mg equivalent/ml serum</td>
<td>3.4 ± 0.3</td>
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<tr>
<td>Renal B2 receptor density, pM/mg protein</td>
<td>59.2 ± 5.2</td>
<td>81.1 ± 0.8*</td>
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Values are means ± SE. Immunoreactive tissue kallikrein, kininogen, and renal bradykinin B2 receptor measurements were performed at 6 wk after potassium supplement. Tissue kallikrein levels in the kidney and urine were measured by direct kallikrein RIA. Total kininogen levels expressed as µg kinin equivalent/ml serum were determined by RIA measuring amount of released kinin after tryptic treatment. Renal bradykinin B2 receptor densities were measured by receptor binding assay. *P < 0.05 and †P < 0.001, respectively, between control and potassium group. Statistical analysis was performed by unpaired Student’s t-test.
control group, whereas intrarenal kallikrein levels remained the same after 6 wk of potassium loading. Our results showed that potassium supplement caused increased expression of renal kallikrein. Because urinary kallikrein is mainly originated from the kidney, these results indicate that rapid secretion of renal kallikrein into the urine may be attributed to the unchanged renal kallikrein content. In the liver (Fig. 3B), no significant change of kininogen mRNA was observed between potassium and control groups (115.7 ± 4.7 vs. 102.3 ± 8.3 densitometric units, n = 3). No difference was observed in β-actin mRNA levels in the liver of either group.

Effect of potassium supplement on bradykinin B2 receptor mRNA levels. The efficiency of amplification of the competitor to that of bradykinin B2 was first tested by kinetic analysis. We found that the same molar quantity of a competitor and a target yielded a similar amount of products after various PCR cycles. Figure 4A shows electrophoretic profiles of the bradykinin B2 receptor (target) and its competitor in the kidney of control (Fig. 4A, left) and potassium-loaded (Fig. 4A, right) rats. The competitor concentration used in PCR ranged from 0.01 to 5.00 pM. The ratio between the competitor and target PCR products was plotted against the amount of added competitor cDNA. The quantity of B2 mRNA was taken as the abscissa value that corresponded to a ratio of 1 on the ordinate axis using linear regression analysis to fit the data. Figure 4B shows that bradykinin B2 receptor mRNA levels in the kidney were significantly higher in potassium-supplemented rats than that in control rats (0.46 ± 0.10 vs. 0.27 ± 0.10 pM, n = 3, P < 0.05, respectively).

Effect of potassium supplement on urinary kinin, cAMP, and cGMP levels. Figures 5 and 6 show urinary kinin, cAMP, and cGMP levels measured by their respective RIAs. After 6 wk of potassium supplement there were significant increases in urinary kinin levels (2.7 ± 0.4 vs. 1.5 ± 0.3 ng·100 g body wt−1·day−1, n = 6, P < 0.05) (Fig. 5), cAMP levels (152.5 ± 10.3 vs. 106.6 ± 9.7 nmol·100 g body wt−1·day−1, n = 6, P < 0.05), and cGMP levels (31.6 ± 2.4 vs. 22.7 ± 1.6 nmol·100 g body wt−1·day−1, n = 6, P < 0.05) in rats receiving potassium supplement compared with those in control rats receiving tap water (Fig. 6).

**DISCUSSION**

The present study demonstrated that high potassium intake attenuated the rise of blood pressure in SHR.
that was accompanied by upregulation of the expression of the renal kallikrein-kinin system. Potassium supplement induced increases in water intake, urine excretion, urinary kinin, cAMP, and cGMP levels. Previous studies employing transgenic and somatic gene delivery strategies showed that the expression of the tissue kallikrein transgene could induce prolonged reduction of blood pressure and attenuation of renal injury in various animal models (6, 19, 40, 48, 53). Together, these combined results suggest that the blood pressure-lowering and diuretic effects exerted by high potassium intake could be, in part, due to the activation of renal kallikrein-kinin-bradykinin receptor system components.

It has been reported that dietary KCl supplement blunted blood pressure rise in SHR but had no effect on blood pressure in normotensive Wistar-Kyoto (WKY) rats (24, 45). Furthermore, KCl in drinking fluid has been shown to cause increases in urine excretion and fluid intake, urinary potassium, and sodium excretion as well as urinary kallikrein excretion in normotensive Sprague-Dawley rats (32). These findings indicate that dietary KCl supplement affects urinary excretion and renal kallikrein excretion in both hypertensive and normotensive rats. A previous report showed that both serum K and aldosterone levels were increased after potassium supplement in rats (30, 54). In addition, aldosterone has been shown to stimulate kallikrein release and increase kallikrein protein/activity, without affecting kallikrein mRNA transcription (14, 16). Similarly, acute administration of aldosterone did not induce the synthesis of renal kallikrein (25). Our present study is the first one to demonstrate increases in both renal kallikrein protein and mRNA levels after potassium supplement.

Both potassium and thiazide diuretics induce increased kallikrein excretion and have diuretic and blood pressure-lowering effects (33). However, their mechanisms of action may not be the same. Thiazide diuretics act on the cortical diluting segment of the renal tubule and increase salt and water excretion primarily by inhibition of sodium and water reabsorption, whereas potassium intake produces effects similar to osmotic diuresis to increase potassium excretion (3). One to three weeks of thiazide treatment has minimal effect on blood pressure in SHR (31). Twenty-six weeks of thiazide therapy did decrease blood pressure in SHR (18). Our study showed that potassium supplement for 1 wk had a significant effect on blood pressure in SHR. Therefore, the effect of high potassium intake on early blood pressure reduction in SHR is not, for the most part, due to its diuretic actions but may be due to other related mechanisms. In this study, we did not observe body weight changes after potassium supplement. In agreement with our study, Barden and coworkers (2) also showed no change of body weight in rats with or without supplement with 1% KCl for 5 wk (2). The reason for the lack of body weight loss from marked diuresis with 1% KCl may be due to a similar magnitude of increased water drinking, which may offset the loss of body weight.

A proposed scheme for the action of the tissue kallikrein-kinin system in blood pressure reduction after
Increased renal kallikrein levels after potassium supplement suggest that increased CAMP may be involved in upregulation of the expression of tissue kallikrein and bradykinin B2 receptor genes due to a positive feedback mechanism. CAMP has been shown to enhance the synthesis and expression of bradykinin B2 receptor in cultured arterial smooth muscle cells (10). We also found that the mRNA levels of both bradykinin B2 receptor and tissue kallikrein were significantly increased by adding cAMP to primary cultured human renal proximal tubule cells (unpublished results). cAMP response elements were identified in the 5' flanking region of the bradykinin B2 receptor and tissue kallikrein genes (29, 47). Collectively, these results support the notion that CAMP may upregulate the expression of renal kallikrein and bradykinin B2 receptor genes via positive feedback mechanisms.

Alternatively, activation of bradykinin B2 receptor may stimulate phospholipase C, which triggers nitric oxide formation (Fig. 7). Increased nitric oxide formation may result in stimulation of guanylate cyclase and increased cGMP levels (20). It has been shown that bradykinin stimulates the release of endothelium-derived relaxing factor, which in turn induces the production of cGMP via activation of bradykinin B2 receptors in cultured porcine aortic endothelial cells (36). Nitric oxide has been shown to be the epithelium-derived relaxing factor released by bradykinin in the guinea pig trachea (15). Bradykinin and the angiotensin-converting enzyme inhibitor (ramiprilat) enhance the levels of cytosolic calcium, prostacyclin, and nitric oxide in porcine brain capillary endothelial cells (49). Activation of B1 and B2 bradykinin receptors produces cGMP in cultured bovine aortic endothelial cells (50). Our results suggest that elevated kallikrein levels after potassium supplement may result in an increase in urinary cGMP formation. Elevated cGMP and cAMP levels have been shown to correlate with relaxation and antiproliferation of smooth muscle cells (20) and may induce vascular smooth muscle relaxation and thus account for the blood pressure-lowering effect in hypertensive rats after high potassium intake.

The long-term effects of dietary potassium on the renal end-organ damage were investigated in WKY rats and SHR. Albumin excretion rate (AER) was higher in SHR than in WKY rats. AER rose further with high sodium intake and was ameliorated by an addition of equimolar potassium in SHR. The graded histopathologic injury correlated well with measured AER. Major improvement in hypertensive renal lesions occurred in SHR with potassium supplement and salt loading. Potassium supplement has been shown to attenuate renal injury in SHR without affecting the blood pressure (12). These results show that potassium protected against renal lesions induced by salt loading independent of blood pressure effect in SHR. Also, a previous study suggested that low renal kallikrein levels may contribute to hypertension and renal disease (37). Reduced urinary or renal kallikrein levels have also been observed in a number of genetically hypertensive rats (1, 22). Long-term infusion of purified tissue kallikrein attenuated glomerular sclerotic lesions and tubular injury in hypertensive Dahl salt-sensitive rats without causing an apparent blood pressure reduction (44). Our recent study showed that adenoviral-mediated kallikrein gene delivery into Dahl salt-sensitive rats attenuated hypertension and renal injury induced by a high-salt diet (6). Taken together, these results demonstrated a direct linkage between tissue kallikrein expression and renal protection in hypertensive rats. The present studies show that high potassium intake upregulated tissue kallikrein and bradykinin B2 receptor gene expression in hypertensive rats, and elevated renal kallikrein-kinin system components may attribute, in part, to the protective effects of potassium against renal injury and hypertension.

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