Effects of potassium on expression of renal sodium transporters in salt-sensitive hypertensive rats induced by uninephrectomy

Ji Yong Jung,1 Sejoong Kim,1 Jay Wook Lee,2 Eun Sook Jung,4 Nam Ju Heo,6 Min-Jeong Son,3 Yun Kyu Oh,4 Ki Young Na,5 Jin Suk Han,6,7 and Kwon Wook Joo6,7

1Department of Internal Medicine, Gachon University of Medicine and Science, Incheon; 2Department of Internal Medicine, Chung-Ang University Yong-San Hospital, Seoul; 3Department of Internal Medicine, The Armed Forces Capital Hospital, Sungnam; 4Department of Internal Medicine, Seoul National University Boramae Medical Center, Seoul; 5Department of Internal Medicine, Seoul National University Bundang Hospital, Sungnam; and 7Clinical Research Institute, Seoul National University Hospital, Seoul, Korea

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HYPERTENSION IS A MAJOR GLOBAL health problem. The risks of cardiovascular and kidney diseases are greatly increased in hypertensive individuals.

The prevalence of hypertension is positively correlated with salt intake (8). Numerous studies have shown that an excess of dietary sodium is related to the development of hypertension in some way (10, 35). It has been reported recently that both a reduction in nephron number by uninephrectomy (uNx) and chronic salt loading in young rats caused salt-sensitive hypertension (SSH) (5). By contrast, potassium has usually been viewed as a minor factor in the pathogenesis of hypertension. However, abundant evidence indicates that potassium depletion has a critical role in hypertension (7, 26, 27). Many studies examined the effect of potassium on blood pressure, and most of them identified a beneficial effect (16, 28, 41). As an antihypertensive effect, the diuretic action of potassium has been described previously (22, 42). Although potassium has been regarded as having various antihypertensive effects, its precise mechanism remains uncertain.

The With-No-Lysine (WNK) kinases are a novel family of serine/threonine kinases that play critical roles in the regulation of epithelial ion transport. At present, there are four known mammalian WNK family members: WNK1, WNK2, WNK3, and WNK4 (43). WNK4 is widely expressed in epithelial tissues. The full-length WNK1 (L-WNK1) has intact kinase activity and is expressed ubiquitously. In contrast, kidney-specific WNK1 (KS-WNK1) is a kinase-deficient isoform with a truncated N terminus. This isoform is expressed in the distal nephron at high levels (34). The discovery of the renal WNK kinase pathway has offered new insight into sodium, potassium, and blood pressure regulation in the distal nephron (48); however, it is insufficient to fully explain the mechanism underlying potassium-associated antihypertensive effects.

In this study, we evaluated the effects of potassium supplementation on the hypertension induced by both uNx and a high salt intake and on the expression of renal ion transporters to investigate the underlying mechanisms of the antihypertensive effects of potassium replacement.

MATERIALS AND METHODS

Experimental animals. After approval for the study protocol was obtained from the Institutional Animal Care and Use Committee, 24 specific pathogen-free male Sprague-Dawley rats (5–6 wk, 160–190 g; Orient Bio, Seongnam City, Korea) were placed in cages. Investigations were conducted in accordance with the Guide for Care and Use of Laboratory Animals (National Academy of Science, 1996). The animals were kept in a light- and temperature-controlled room with free access to standard rat chow (Agribrand Purina Korea, Seongnam City, Korea) and deionized water for 1 wk before undergoing surgical procedures.

High-salt diet after uNx and potassium repletion. All rats were anesthetized with isoflurane (Isoflur, Abbott Laboratories, Chicago, IL), and uNx was performed by total extirpation of the left kidney. A normal-salt diet (0.3% NaCl) was provided for 4 wk; thereafter, uNx rats were randomly allocated into two groups: 1) the HS group (n = 12), which was fed a high-salt diet (3% NaCl); and 2) the HS + KCl group (n = 12), which was fed a high-salt diet and simultaneously given a mixed solution of 1% KCl as a substitute for drinking water.

Address for reprint requests and other correspondence: K. W. Joo, Dept. of Internal Medicine, Seoul National Univ. Hospital, 28 Yongon-Dong, Chongno-Gu, Seoul, 110-744, Korea (e-mail: junephro@snu.ac.kr).
Rats in both cages were allowed to drink ad libitum. The potassium contents were the same as 0.82% (basic content of standard rat chow from Agibrand Purina Korea, Seoul, Korea) between the normal salt and high salt diets. The HS+KCl group was provided with additional mixed solution of 1% KCl as a substitute for drinking water.

**Physiological parameters.** Physiological parameters of all animals (n = 24) were assessed at the basal and 4-wk postoperation levels. To reconcile them with the abundances of protein and mRNA, the parameters from animals (n = 6) to be euthanized were assessed at 1 and 3 wk.

Blood pressure (BP) and body weight were measured at the following three time points: 1) baseline, 2) before a change in diet, and 3) on the day of death. BP was measured using the Noninvasive Blood Pressure System XBP1000 (Kent Scientific, Torrington, CT). During the 2 days before death, the animals were placed in metabolic cages, and 24-h urine samples were collected on the day before death for the measurement of urine urea nitrogen, creatinine, protein, osmolality, sodium, and potassium. Daily sodium, chloride, and potassium excretions were calculated from the urine volume and the urinary sodium and potassium concentration (mmol/l). Daily sodium, chloride, and potassium balance was calculated by total sodium, chloride, and potassium intake minus urinary sodium, chloride, and potassium excretion, respectively. Blood samples were collected from the abdominal aorta at the time of death for measurement of blood urea nitrogen, creatinine, osmolality, sodium, and potassium. Creatinine and urea clearances were calculated using the standard formula. Whole blood was centrifuged at 3,000 rpm (Sorvall RT 6000 D; Sorvall, Newtown, CT) at 4°C for 20 min to separate plasma. The plasma aldosterone level was measured by RIA (Diagnostic Products, Los Angeles, CA).

**Quantitative real-time PCR.** One-half of the right kidney from the rats was dissected into small pieces and placed in chilled TRIZol solution (Sigma, St. Louis, MO). After homogenization, RNA was extracted from the kidney using RNAzol (Invitrogen) according to the manufacturer’s instructions. The RNA concentration was measured by a nanodrop spectrophotometer, and purity was determined by A260/A280. After dilution of the sample to a concentration of 5 μg/μl, cDNA was reverse-transcribed from RNA using a PCR machine (PTC-200 Peltier Thermal Cycler; MJ Research, Watertown, MA) and the Reverse Transcription System (Promega, Madison, WI), consisting of 25 mM MgCl₂, 10× buffer, 10 mM dNTPs, RNasin, AMV, oligo dT, and DEPC water.

Real-time PCR was performed with 1 μl of cDNA and a master mix containing 10 μl of TaqMan 2× PCR Master Mix (Applied Biosystems, Branchburg, NJ), 8 μl of DEPC water, 1 μl of probe, and primer sets for WNK1, WNK4, and the Na⁺K⁺2Cl⁻ cotransporter (NCC; all from Applied Biosystems) using the Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). Forward and reverse primers and the TaqMan probe from the 5′ sequence of exon 1 (WNK1 forward, 5′-GGG ACT CCT GGC TTC CTT TC-3′; reverse, 5′-ATC GGA GCT TGA GCC ATT CCT-3′; TaqMan probe, 5′-CCTCCGGCTCCAGTCTC-3′) were used to amplify full-length WNK1 (29). Primers from exon 4a (RP forward, 5′-GCT CCT GGT CTC AAA AGG ATT GTA T-3′), exon 5 (RP reverse, 5′-CAG GAA TGT CTA CTT TGT CAA AAC TG-3′) and the TaqMan probe (5′-TGA GGG AGT GAA GCC A-3′) were used to amplify kidney-specific WNK1 (29). GAPDH was used as a control housekeeping gene. Relative quantification was derived by comparing target genes and GAPDH.

**Semiquantitative immunoblotting.** The right kidneys were dissected into small pieces and placed in chilled isolation buffer containing 250 mM sucrose, 10 mM triethanolamine (Sigma), 1 μg/ml leupeptin (Sigma), and 0.1 mg/ml PMSF (Sigma) titrated to pH 7.6. The pieces were then homogenized at 15,000 rpm with three strokes for 15 s with a tissue homogenizer (PowerGun 125). After homogenization, the total protein concentration of the homogenate was measured by the bicinchoninic acid protein assay method (BCA Reagent Kit; Sigma) and diluted to 2.05 μg/μl using the isolation buffer solution. The samples were then stabilized by heating to 60°C for 15 min after adding 1 vol 5× Laemmli sample buffer/4 vol sample.

Initially, loading gels were performed on each sample set to allow the fine adjustment of the loading amount to guarantee equal loading on subsequent immunoblots. Five micrograms of protein from each sample was loaded into each individual lane and electrophoresed on 12% polyacrylamide-SDS minigels using a Mini-PROTEIN electrophoresis apparatus (Bio-Rad, Hercules, CA) and then stained with Coomassie blue dye (a 0.025% solution made in 4.5% methanol and 1% acetic acid, G-25; Bio-Rad). Selected bands from these gels were scanned with densitometry (GS-700 Imaging Densitometry; Bio-Rad) to semiquantitatively determine the density (Molecular Analyst version 1.5; Bio-Rad), and relative amounts of protein were loaded in each lane. Finally, protein concentrations were corrected to reflect these measurements by repeating the above process.

For immunoblotting, the proteins electrophoresed on gels were transferred from unstained gels to nitrocellulose membranes (Bio-Rad) by the electroelution method. After being blocked with 5% skim milk in PBS-T [80 mM Na₂HPO₄, 20 mM NaH₂PO₄, 100 mM NaCl, and 0.1% Tween 20 (pH 7.5)] for 30 min at room temperature, the membranes were probed overnight at 4°C with the respective primary antibodies. For the probing blots, all primary antibodies were diluted using a solution containing 150 mM NaCl, 50 mM sodium phosphate, 10 mg/dl sodium azide, 50 mg/dl Tween 20, and 0.1 g/dl bovine serum albumin (pH 7.5). Immunoblotting was performed using anti-rat Na⁺/H⁺ exchanger type 3 (NHE3; 1:200), anti-rat Na⁺-K⁺-ATPase, anti-rat Na⁺/HCO₃⁻ cotransporter type 1 (NBC1; 1:1,000), anti-rat Na⁺-K⁺-2Cl⁻ cotransporter (NKCC2; 1:2,000), anti-rat NCC (1:1,000), and anti-rat α (1:1,000), β (1:200), and γ-epithelial Na channel (ENaC; 1:500). The membranes were washed and incubated with secondary antibodies for 1 h at room temperature. The secondary antibody was horseradish peroxidase-conjugated donkey anti-rabbit IgG (31458; Pierce, Rockford, IL) diluted to 1:3,000. The sites of antibody-antigen reaction were visualized using an enhanced chemiluminescence system (ECL RPN 2106; Amersham Pharmacia Biotech, Buckinghamshire, UK) before exposure to X-ray film (Hyperfilm, Amersham Pharmacia Biotech). Band density was measured by densitometry (GS-700 Imaging Densitometry; Bio-Rad) and calculated as a value relative to the average value of the control group.

![Fig. 1. Changes in systolic blood pressure (SBP).](http://ajprenal.physiology.org/DownloadedFrom)
Table 1. Physiological parameters

<table>
<thead>
<tr>
<th></th>
<th>Basal (n = 24)</th>
<th>4 wk Postoperation (n = 24)</th>
<th>1 wk After Diet</th>
<th>3 wk After Diet</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>275.50 ± 2.23</td>
<td>442.40 ± 6.41</td>
<td>450.60 ± 6.98</td>
<td>438.90 ± 16.52</td>
</tr>
<tr>
<td>Food intake, g/100 g body wt⁻¹·day⁻¹</td>
<td>7.14 ± 0.27</td>
<td>6.16 ± 0.18</td>
<td>21.53 ± 3.37</td>
<td>17.61 ± 0.40</td>
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<tr>
<td>Fluid intake, ml/100 g body wt⁻¹·day⁻¹</td>
<td>12.78 ± 0.73</td>
<td>8.92 ± 0.35</td>
<td>29.22 ± 1.17</td>
<td>26.45 ± 3.07</td>
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<tr>
<td>Urine volume, ml·h⁻¹·100 g body wt⁻¹</td>
<td>0.10 ± 0.01</td>
<td>0.13 ± 0.01</td>
<td>1.22 ± 0.10</td>
<td>1.18 ± 0.10</td>
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<tr>
<td>sNa, mmol/l</td>
<td>155.50 ± 0.65</td>
<td>154.60 ± 0.45</td>
<td>155.20 ± 1.08</td>
<td>154.00 ± 1.16</td>
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<tr>
<td>sK, mmol/l</td>
<td>3.93 ± 0.06</td>
<td>3.65 ± 0.09</td>
<td>3.61 ± 0.18</td>
<td>4.40 ± 0.15</td>
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<td>FENa, %</td>
<td>0.45 ± 0.04</td>
<td>0.55 ± 0.02</td>
<td>8.34 ± 0.51</td>
<td>5.17 ± 0.68</td>
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<tr>
<td>FEK, %</td>
<td>32.47 ± 1.76</td>
<td>50.83 ± 2.05</td>
<td>39.71 ± 3.37</td>
<td>25.54 ± 4.43</td>
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<td>uOsm, mosmol/kgH₂O</td>
<td>1584.00 ± 44.51</td>
<td>1354.00 ± 37.75</td>
<td>603.90 ± 23.07</td>
<td>838.70 ± 57.87</td>
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<tr>
<td>PRA, ng·ml⁻¹·h⁻¹</td>
<td>1.02 ± 0.68</td>
<td>1.25 ± 0.43</td>
<td>1.04 ± 0.04</td>
<td>0.20 ± 0.07</td>
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<tr>
<td>PAC, pg/ml</td>
<td>140.30 ± 4.94</td>
<td>160.20 ± 27.70</td>
<td>159.50 ± 28.16</td>
<td>699.70 ± 185.90</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = no. of animals. sNa, serum Na; sK, serum K; FENa, fractional excretion of Na; FEK, fractional excretion of K; uOsm, urine osmolality; PRA, plasma renin activity; PAC, plasma aldosterone concentration; HS, high-salt diet group after uninephrectomy; HS+KCl, high-salt diet with potassium-repleted group after uninephrectomy. *P < 0.05 compared with HS rats.
RESULTS

Physiological parameters. As shown in Fig. 1, after uNx there was a high-salt diet-induced rise in systolic BP from 136.5 ± 1.22 to 150.9 ± 4.04 mmHg at 1 wk (P = 0.03) and 207.7 ± 6.21 mmHg at 3 wk (P < 0.01). The systolic BP was less increased in the HS+KCl group (140.3 ± 2.97 at 1 wk and 180.3 ± 1.76 mmHg at 3 wk, P = 0.04) compared with that in the HS group (150.9 ± 4.01 at 1 wk and 207.7 ± 6.21 mmHg at 3 wk, P = 0.01). The BP-sodium excretion curve for the kidneys of the HS group rats shifted to the right, resulting in a blunted pressure natriuresis, and the HS+KCl rats had a relative shift to the left (Fig. 2). The body weights at 1 and 3 wk in the HS+KCl group (411.5 ± 7.03 g, P < 0.01; 378.5 ± 11.09 g, P = 0.03, respectively) were lower than those of the HS group (450.6 ± 6.98 and 438.9 ± 16.52 g, respectively; Table 1). In the uNx-SSH rats, the fluid intake and urine volume increased after a high-salt diet. As shown in Table 1, both the fluid intake and the urine volume were further increased by potassium repletion. In addition, potassium repletion resulted in the increased urinary excretion of sodium and potassium, although this increase was not statistically significant. The daily sodium and chloride balances were negative after potassium repletion at 3 wk, although this increase was not statistically significant. The daily sodium and chloride balances were negative after potassium repletion (Fig. 3). Despite increased urine volume, urine osmolality was not different between the HS and HS+KCl groups (603.9 ± 23.07 vs. 634.2 ± 19.55 mosmol/kgH2O at 1 wk; and 838.7 ± 57.87 vs. 810.5 ± 90.70 mosmol/kgH2O at 3 wk, respectively; Table 1). There were no significant differences in serum sodium and potassium by the end of the experiment (Table 1).

At 1 wk, the plasma aldosterone concentration was 140.30 ± 4.94 pg/ml in the HS group and 160.20 ± 27.70 pg/ml in the HS+KCl group. Potassium repletion significantly increased the plasma aldosterone concentration at 3 wk (HS vs. HS+KCl: 159.50 ± 28.16 vs. 699.70 ± 185.90 pg/ml, respectively; P < 0.05; Table 1). The plasma renin activity was depressed for the entire experimental period in both groups (Table 1).

Semiquantitative immunoblotting. As shown in Fig. 4, the semiquantitative immunoblotting of the whole kidney homogenates from the HS+KCl rats showed a significant decrease in the protein abundance of NHE3 and NBC1 (53 and 54% of those in the HS group at 1 wk; 19 and 59% of those in the HS group at 3 wk, respectively) compared with the HS rats. Immunohistochemical analysis also showed profoundly decreased NHE3 and NBC1 labeling in kidneys from HS+KCl rats (see Fig. 7). The expression of NKCC2 protein was increased by potassium repletion at 3 wk.

As can be seen in Fig. 5, NCC abundance in the HS+KCl group significantly decreased (45% of the HS group at 1 wk and 8% of the HS group at 3 wk) compared with that in the HS group. This result was confirmed by immunohistochemistry showing reduced labeling in the distal convoluted tubule of kidneys from the HS+KCl rats, while the HS rats demonstrated intense NCC labeling in the apical part of the distal convoluted tubules (see Fig. 7). The abundance of ENaC-α was significantly increased (126% of the HS group at 1 wk and 144% of the HS group at 3 wk) in the HS+KCl rats. By contrast, the expression of ENaC-β (97% of the HS group at 1 wk and 74% of the HS group at 3 wk) and ENaC-γ (63% of the HS group at 1 wk and 45% of the HS group at 3 wk) were decreased, although the decrease was not statistically significant. Accompanying the marked elevation of the plasma aldosterone concentration at 3 wk, both the plasma sodium and potassium concentrations were blunted for the entire experimental period in both groups (Table 1).
sterone level (Table 1), the expression of ROMK was significantly increased (302% of the HS group at 1 wk and 882% of the HS group at 3 wk).

Results of WNK kinases and NCC mRNA expression. The expression of KS-WNK1 mRNA was significantly increased in the HS/H11001 KCl group (1.8- and 1.4-fold of the HS group at 1 and 3 wk, respectively), and the L-WNK1/KS-WNK1 ratio of mRNA expression was significantly decreased in the HS/H11001 KCl group compared with the control group (Fig. 6, A and B). Also, the expression of WNK4 mRNA was significantly increased in the HS/H11001 KCl group (1.4- and 1.9-fold of the HS group at 1 and 3 wk, respectively; Fig. 6, C and D). A significant decrease in NCC mRNA expression was seen in the HS/H11001 KCl group (0.6- and 0.7-fold of HS group at 1 and 3 wk, respectively; Fig. 6, C and D).

DISCUSSION

We observed that potassium repletion attenuated the rise in BP in this animal model. We used a recently developed a rat model (4, 5) where the animals are unilaterally nephrectomized at a young age and then subjected to a high-salt diet. A reduced nephron number, one of acquired subtle renal injuries, may predispose an animals to development of pregglomerular renal microvascular disease, which is a common mechanism for SSH (19, 20). From a clinical point of view, this animal model could be linked to a human disease. A reduction in salt intake should be suggested particularly in young patients that already have subtle renal injuries. Furthermore, our result might be one explanation of how a potassium-rich diet ameliorates the development of hypertension. This BP-lowering effect of potassium was associated with the inhibition of sodium retention, which was mainly explained by increasing urinary sodium excretion. During the experimental period, the daily sodium balance remained negative in the HS/H11001 KCl group. Despite a significant rise in BP, the HS rats retained more sodium than the HS/H11001 KCl group. This corresponds with our result that the BP-sodium excretion curve of the HS group had been shifted relatively to the right, resulting in blunted pressure natriuresis. On the other hand, the HS/H11001 KCl group showed a BP-sodium excretion curve shifted relatively to the left compared with the HS group (Fig. 2).
Fig. 6. With-No-Lysine (WNK) kinase and NCC mRNA expression. 

A: at 1 wk, the full-length (L-WNK1)/kidney-specific WNK1 (KS-WNK1) ratio of mRNA expression was significantly decreased in the potassium-repleted group compared with that of the HS group. 

B: at 3 wk, the L-WNK1/KS-WNK1 ratio of mRNA expression was slightly decreased in the potassium-repleted group compared with that of the HS group. 

C and D: WNK 4 mRNA expression was significantly increased and NCC mRNA expression was decreased in the potassium-repleted group compared with that of the HS group at 1 and 3 wk. The values are the means, and the bars indicate SD. *P < 0.05 compared with the HS group.
An increase in body weight was not observed in the HS and HS/KCl groups. One of the possible causes of this phenomenon is the reduction in food intake due to the massive rate of NaCl and/or KCl ingestion.

NHE3 is an apically expressed transporter protein in the proximal tubule cells that mediates transcellular sodium and HCO$_3^-$ reabsorption, in conjunction with Na$^+/K^+$-ATPase and NBC1 in the basolateral plasma membrane (Fig. 7). The present study revealed that NHE3 abundance was significantly decreased. The decreased apical NHE3 expression may mediate the apical component of the decreased transcellular sodium transport in the proximal tubule. This result provides evidence of an important role for NHE3 in sodium reabsorption in the proximal nephron (2). Thus it is likely that the downregulation of NHE3 by potassium repletion represents a compensatory response preventing sodium retention due to the consequences of increased aldosterone levels. Hyperkalemia also suppresses H$^+$ secretion and HCO$_3^-$ reabsorption. NHE3 is considered to play an important role in sodium reabsorption in the proximal tubule (1). This role suggests that the downregulation of NHE3 may be associated not only with decreased sodium reabsorption but also with decreased HCO$_3^-$ reabsorption. In addition, potassium repletion is associated with decreased ammoniagenesis (40). This effect is related to a transcellular cation exchange. Potassium tends to enter into the cells with hyperkalemia, and electroneutrality is maintained in part by H$^+$ moving out of the cells. The intracellular alkalosis could suppress renal ammoniagenesis and HCO$_3^-$ reabsorption. Thus it is possible that the decreased expression of NHE3 in potassium repletion could also be associated with indirect effects of intracellular alkalization related to hyperkalemia.

The abundance of NKCC2 was not decreased in potassium-repleted rats. This result suggests that NKCC2 is likely to play a role in maintaining the urinary concentration, but not in renal sodium regulation, in response to potassium loading.

Mutations of WNK kinases largely explain the pathogenesis of a genetic form of hypertension called familial hyperkalemic hypertension (also known as pseudohypoaldosteronism type II or Gordon’s syndrome) (46). The ubiquitous full-length WNK1 is also known as long WNK1 (L-WNK1). KS-WNK1 is an antagonist of L-WNK1 (44). L-WNK1 has been shown to enhance the activity of ENaC (47), whereas WNK4 inhibits NCC (48). Additionally, L-WNK1 increases NCC activity by antagonizing WNK4-mediated inhibition of this transporter (49). Both L-WNK1 and WNK4 inhibit ROMK (21). Thus a positive L-WNK1-to-KS-WNK1 ratio increases the rate of sodium reabsorption via ENaC and NCC and decreases the rate of potassium secretion via ROMK. Under normal conditions, ENaC stimulates both ENaC- and NCC-mediated sodium transport. Consequently, KS-WNK1 suppresses NCC transport via its dominant-negative effect on L-WNK1 and enhances ENaC-mediated Na$^+$ transport through a different process (17). It has been reported that high and low potassium intake increase and decrease expression of KS-WNK1, respectively (29, 33). Low potassium intake also leads to increased expression of L-WNK1 in the kidney (29). Therefore, the L-WNK1-to-KS-WNK1 ratio in the kidney is important in regulating sodium and potassium homeostasis (18). A recent study re-

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**Fig. 7. Immunohistochemical analysis of NHE3, NBC1, and NCC.** In the HS+KCl group, immunostaining of NHE3, NBC1, and NCC significantly decreased at 1 and 3 wk compared with that in the HS group.
ported that the KS-WNK1 knockout mice exhibit increased abundance of NCC and ROMK (13). These findings are partially compatible with our observations in this study. The KS-WNK1 knockout model, which has an infinitely high L-WNK1/KS-WNK1 ratio, led to increased expression of NCC. On the other hand, the increased expression of ROMK in KS-WNK1 knockout mice was intriguing, which was counter to other reports that suggested that KS-WNK1 stimulates ROMK expression by inhibiting L-WNK1 (6, 29, 30, 44). In the present study, KS-WNK1 was significantly upregulated with potassium repletion, which led to a decreased L-WNK1-to-KS-WNK1 ratio (Fig. 6, A and B), leading to decreased NCC and increased ROMK abundances. Thus the previously reported in vitro evidence (21, 48) suggesting that potassium intake upregulated KS-WNK1 and WNK4, resulting in reduced sodium uptake via NCC, was well in accord with our results.

In our experiments, the natriuretic effect of KCl was not explained by changes in the plasma aldosterone concentration, which was higher in the HS + KCl group. This finding could be explained by the concept of the “aldosterone paradox” (15). Aldosterone must at times become a NaCl-retaining hormone, while at other times it must function primarily or exclusively as a kaliuretic hormone. The secretion of NaCl-retaining vs. kaliuretic actions of aldosterone depends on events other than control of ENaC. Specifically, if potassium loading (hyperkalemia) activates KS-WNK1 and WNK4, this will inhibit NCC and favor electrogenic sodium reabsorption by ENaC, thereby increasing the transepithelial voltage difference and stimulating potassium secretion. The opposite occurs when a low-sodium diet (hypovolemia) does not affect or even decrease KS-WNK1 and WNK4 because this will activate the NCC and favor electroneutral sodium reabsorption with a relative conservation of potassium (38). Aldosterone stimulates sodium reabsorption in part through its action of increasing the abundance of NCC in the distal convoluted tubules (25) and ENaC-α in the collecting duct principal cells (31). Moreover, it has been demonstrated that the effect of aldosterone in stimulating renal sodium retention can be overridden by the phenomenon of “aldosterone escape,” resulting in a marked decrease in NCC abundance in the distal convoluted tubules, but not ENaC-α in the collecting duct (45). Thus this phenomenon may be a possible mechanism explaining why the HS + KCl group has increased urinary sodium excretion despite an elevated plasma aldosterone concentration.

Although the natriuretic properties of potassium are considered to play an important role in the antihypertensive effects of potassium, several mechanisms have been demonstrated by which potassium may modulate hypertension (11, 39). First, KCl loading inhibits renin secretion (9, 42). In the present study, however, plasma renin activity was depressed in both groups. The depressed plasma renin activity by potassium supplements may be due to the increased plasma aldosterone concentration induced by potassium. Second, potassium has been reported to have both direct and indirect effects on the vascular system (3). Potassium may reduce the reactivity of the peripheral arterioles to vasoconstrictor agents such as norepinephrine and angiotensin II (14). The increased pressor response to intravenous angiotensin II in rats maintained on a high-sodium diet does not occur among rats receiving KCl in addition to NaCl (36). Moreover, a report showed that the hyperactive central pressor response in Dahl salt-sensitive rats could be corrected largely with KCl supplementation (12).

In summary, potassium may attenuate the rise in BP with a high-salt diet in uNx rats, mainly as a result of the inhibition of sodium retention, by increased urinary sodium excretion. The present study also demonstrated that potassium repletion in uNx-SSH rats is associated with substantial downregulation of NHE3 and NCC in the rat kidney.

In conclusion, potassium supplementation attenuated hypertension in uNx-SSH rats fed a high-salt diet. Although this BP-lowering effect of potassium may be multifactorial, it may be associated with its natriuretic effect, which is associated with the decreased expression of NCC regulated by WNK4 and NHE3. Further studies will be needed to elucidate the functional correlation between potassium supplementation and the attenuation of blood pressure.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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