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박사학위 논문

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Effects of Potassium on the Expression of Sodium Transporters in Salt-sensitive Hypertensive Rats induced by Uninephrectomy

by **Jung Ji Yong, M.D.**

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ABSTRACT

Effects of Potassium on the Expression of Sodium Transporters in Salt-sensitive Hypertensive Rats induced by Uninephrectomy

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Background

Dietary potassium is an important modulator of systemic blood pressure. The purpose of this study was to determine whether dietary potassium is associated with an altered abundance of major renal sodium transporters that may contribute to the modulation of systemic blood pressure. The experiments were performed by potassium repletion in salt-sensitive hypertensive rats induced by uninephrectomy (uNx-SSH rats).

Methods

Unilateral nephrectomy (uNx) was performed in male Sprague-Dawley rats, and they were fed a normal-salt diet (0.3% NaCl) for 4 weeks. Thereafter, the rats were fed a high-salt (HS) diet (3% NaCl) for the entire experimental period. The potassium repleted (HS+KCL) group was given a mixed solution of 1% KCl as a substitute for

drinking water. The expressions of major renal sodium transporters were determined sequentially at 1 and 3 wk by semi-quantitative immunoblotting. The level of expression of messenger RNA (mRNA) of With-No-Lysine (WNK) kinases was also determined sequentially at 1 and 3 wk by real-time PCR. Physiologic parameters and daily sodium and chloride balance were also measured on the day of sacrifice.

Results

In the HS+KCL group, the urine flow rate was increased (1.52 ± 0.16 vs. 1.22 ± 0.10 ml/hr/100g Bwt at 1 wk; 1.63 ± 0.23 vs. 1.18 ± 0.09 ml/hr/100gBwt at 3 wk); urine osmolality did not show any differences (603.9 ± 23.07 vs. 634.2 ± 19.55 mOsm/kg at 1 wk; 838.7 ± 57.87 vs. 810.5 ± 90.70 mOsm/kg at 3 wk) compared with the HS group and daily sodium and chloride balances were negative (-1.99 ± 0.48 and -2.61 ± 0.44 mmol/24hr/100g Bwt at 1 wk; -0.79 ± 0.65 and –2.56 ± 0.86 mmol/24hr/100g Bwt at 3 wk, respectively). The systolic BP of the HS+KCL group was decreased compared to the HS group (140.3 ± 2.97 vs. 150.9 ± 4.04 mmHg at 1 wk; 180.3 ± 1.76 vs. 207.7 ± 6.21 mmHg at 3 wk).

Protein abundances of NCC, ENaC-γ, and NHE3 in the HS+KCL group were significantly decreased (49 %, 33 %, and 21 % of the HS group at 1 wk, respectively; 16 %, 10 %, and 7 % of HS group at 3 wk). The expression of AQP2 was significantly reduced in response to the HS+KCL (1% of control) at 3 wk. In contrast, the expressions of NKCC2 and ROMK in the HS+KCL group did not show any significant alterations compared with those in the HS group. WNK4 mRNA expression was significantly increased in the HS+KCL group (1.4-fold of control at 1 wk and 1.9-fold

of control at 3 wk). The full-length WNK1 (L-WNK1) / kidney specific WNK1 (KS-WNK1) ratio was decreased in the HS+KCL group (0.6-fold of control at 1 wk and 0.6-fold of control at 3 wk).

Conclusions

We demonstrated a blood pressure lowering effect of dietary potassium supplementation in uNx-SSH rats associated with increased sodium and chloride excretion. Adaptive alterations of NCC, ENaC-γ, and NHE3 may partly play a role in the antihypertensive effect of dietary potassium. In the distal nephron, the WNK kinases pathway may be involved in the altered expression of NCC. The more relevant mechanism might be located in the proximal tubule. Further studies are needed to elucidate the precise mechanism.

Key words: Potassium, Salt-sensitive hypertension, Renal sodium transporters

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Contents

Abstract i

Contents iv

List of Tables v

List of Figures vi

Introduction 1

Materials and Methods 3

Results 9

Discussion 25

References 30

Korean Abstract 35

Acknowledgments 38

List of Tables

Table 1. Physiologic parameters ……. 13

List of Figures

Figure 1. Changes in systolic blood pressure ……13

Figure 2. Pressure-natriuresis curve during different potassium conditions…….. 14

Figure 3. Changes in fluid intake and urine volume. ……15

Figure 4. Changes in daily sodium and chloride balance...……...16

Figure 5. Protein abundance of NHE3 and Na^+-K^+-ATPase ……17

Figure 6. Protein abundance of NKCC2 and NCC …….....18

Figure 7. Protein abundance of ENaCs and ROMK …… 19

Figure 8. Protein abundance of AQP2 ……21

Figure 9. WNK kinase mRNA expression ……..22

Figure 10. Immunohistochemical analysis of NCC, NHE3, and AQP2…….... 24

Introduction

Hypertension is a major global health problem. The risk of developing cardiovascular, cerebrovascular, and kidney diseases are greatly increased in hypertensive individuals.

It is now well-accepted that the prevalence of hypertension is positively correlated with salt intake. Numerous clinical, epidemiologic, and animal studies have shown that an excess of dietary sodium is in some way related to the development of essential hypertension [1, 2]. The definitive connection between salt intake and hypertension was established by Dahl and colleagues [3]. These investigators clearly demonstrated the relationship between salt intake and hypertension, establishing the term 'salt-sensitive hypertension.'

In contrast, dietary potassium supplements have antihypertensive effect in excessive dietary sodium [4, 5]. The diuretic action of potassium has been described previously [6, 7]. In addition to its well-established diuretic effect, potassium has been reported to exert direct vasodilator effects and to attenuate vascular contractions induced by vasoactive substances [8, 9]. Although the precise mechanism underlying the antihypertensive action of potassium remains controversial, the natriuretic properties of potassium are thought to play an important role [6, 10].

The WNK (with no lysine) kinases are a novel family of serine/threonine kinases that play critical roles in the regulation of epithelial ion transport. Mutations of WNK kinases largely explain the pathogenesis of a genetic form of hypertension called familial hyperkalaemic hypertension (also known as pseudohypoaldosteronism type II or Gordon's syndrome) [11]. At present, there are four known mammalian WNK family

members (WNK1, WNK2, WNK3, and WNK4) [12]. WNK4 is widely expressed in epithelial tissues. The full-length WNK1 isoform is specifically expressed in the kidney, and is therefore known as kidney-specific WNK1 (KS-WNK1) [13]. The ubiquitous full-length WNK1 is also known as long WNK1 (L-WNK1). KS-WNK1 is an antagonist of L-WNK1 [14]. L-WNK1 has been shown to enhance the activity of epithelial sodium channel (ENaC) [15], whereas WNK4 inhibits sodium chloride cotransporter (NCC) [16]. Additionally, L-WNK1 increases NCC activity by antagonizing WNK4-mediated inhibition of this transporter [17]. Both L-WNK1 and WNK4 inhibit renal outer medullary potassium channel (ROMK) [18]. Thus, a positive ratio of L-WNK1-to-KS-WNK1 increases the rate of Na^+ re-absorption via ENaC and NCC, and decreases the rate of K^+ secretion via ROMK. These effects of L-WNK1 and KS-WNK1 thus allow Na^+ reabsorption to be uncoupled from K^+ secretion. The discovery of the renal WNK kinase pathway is offering new insight into sodium, potassium, and blood pressure regulation in the distal nephron [16]; however, it is insufficient to fully explain the mechanism underlying potassium-associated antihypertension.

Therefore, we studied the effect of potassium on the expression of renal ion transporters and renal WNK kinases to investigate whether long-term potassium supplementation can prevent the development of hypertension even when sodium intake is relatively high.

Materials and Methods

1. Experimental animals

After obtaining approval of the study protocol from the Institutional Animal Care and Use Committee, 24 specific pathogen-free male Sprague-Dawley rats (5-6 weeks, 160-190 g; Orient Bio Inc., 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 Purima Korea, Seongnam City, Korea) and deionized water for 1 week before undergoing surgical procedures.

2. High salt diet after uninephrectomy and potasssium repletion

All rats were anesthetized with isoflurane (Isoflu®, Abbott Laboratories, Chicago, IL, USA) and unilateral nephrectomy was performed by total extirpation of the left kidney. An normal-salt diet (0.3% NaCl) was provided for 4 weeks; thereafter, uninephrectomized rats were randomly allocated into two groups: (1) the HS group (n=12) was fed a high salt diet (3% NaCl); and (2) the HS+KCL group (n=12) was fed a high-salt diet and simultaneously given a mixed solution of 1% KCL as a substitute for drinking water. Rats in the cages were allowed to drink *ad libitum*.

3. Physiologic parameters

The BP and body weight were measured at the following three times: 1) baseline, 2)

prior to changing the diet, and 3) on the day of sacrifice. The BP was measured using the Non-invasive Blood Pressure System XBP1000 (Kent Scientific Corp., Torrington, CT, USA). During the last 2 days prior to sacrifice, the animals were placed in metabolic cages, and 24-hour urine samples were collected on the day before sacrifice for measurement of urine urea nitrogen, creatinine, protein, osmolality, sodium and potassium. Daily sodium, chloride, and potassium excretion were calculated from the urine volume and the urinary sodium and potassium concentration (mmol/L). Daily sodium, chloride, and potassium balance were 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 sacrifice 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 3000 rpm (Sorvall RT 6000 D; Sovall, Newtown, CT, USA) at 4 °C for 20 min to separate plasma. The plasma aldosterone level was measured by radioimmunoassay (RIA; Diagnostic Products Corporation, Los Angeles, CA, USA).

4. Quantitative real-time polymerase chain reaction (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, USA). The pieces were homogenized at 15,000 rpm with 3 strokes for 15 seconds with a tissue homogenizer (PowerGun 125; Fisher Scientific, Pittsburgh, PA, USA) and chloroform (Sigma), and mixed. After vortexing, 1 ml of the sample was incubated in room temperature for 3 minutes and centrifuged at 14,000 rpm for 15 minutes. The transparent portion was isolated and

isopropyl alcohol was added. After vortexing, the sample was incubated in room temperature for 10 minutes, followed by centrifugation at 14,000 rpm for 10 minutes. Isopropyl alcohol was removed for confirming RNA pellets at the bottom of each tube and 75% ethanol was added. Vortexing and centrifugation for 5 minutes were repeated. After discarding the ethanol, each pellet was dried and DEPC water (nuclease free water) was added. The RNA concentration was measured by a nano-drop spectrophotometer and purity was determined by measuring the A260/A280. After diluting the sample to a concentration of 5 μg/μL, complementary DNA (cDNA) was reverse-transcribed from RNA using PCR machine (PTC-200 Peltier Thermal Cycler; MJ RESEARCH, Watertown, MA, USA) and the Reverse Transcription System (Promega Corporation, Madison, WI, USA) consisting of 25 mM $MgCl_2$, 10x buffer, 10mM dNTPS, RNasin, AMV, oligo dT, and DEPC water. Real-time PCR was performed with 1 μL of cDNA and master mix containing 10 μL of TaqMan® 2X PCR Master Mix (Applied Biosystems, Branchburg, NJ, USA), 8 μL DEPC water, and 1 μL of probe and primer sets for WNK1, WNK4, and sodium-chloride cotransporter (NCC; all from Applied Biosystems, Branchburg, NJ, USA) using the Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, Foster city, CA, USA). Glutaraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene. Relative quantification was derived from comparing target genes and GAPDH.

5. Semi-quantitative immunoblotting

The right kidneys were dissected into small pieces and placed in a chilled isolation buffer containing 250 mM sucrose, 10 mM triethanolamine (Sigma), 1 μg/mL leupeptin

(Sigma), and 0.1 mg/mL phenylmethylsulfonylfluoride (Sigma) titrated to pH 7.6. Then, the pieces were homogenized at 15,000 rpm with 3 stokes for 15 seconds 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℃ for 15 minutes after adding 1 vol 5 X Laemmli sample buffer/4 vol sample.

Initially, loading gels were done on each sample set to allow fine adjustment of the loading amount to guarantee equal loading on subsequent immunoblots. Five micrograms of protein from each sample were loaded into each individual lane and electrophoresed on 12% polyacrylamide-SDS minigels by using a Mini-PROTEIN Ⅲ electrophoresis apparatus (Bio-Rad, Hercules, CA, USA) and then stained with Coomassie blue dye (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 semi-quantitatively determine density (Molecular Analyst version 1.5; Bio-Rad) and relative amounts of protein loaded in each lane. Finally, protein concentrations were corrected to reflect these measurements by the repetition of above process.

For immunoblotting, the proteins electrophoresed on gels were transferred from unstained gels to nitrocellulose membranes (Bio-Rad) with the electroelution method. After being blocked with 5% skim milk in PBS-T (80 mM Na_2HPO_4, 20 mM NaH_2PO_4, 100 mM NaCl, and 0.1% Tween-20 [pH 7.5]) for 30 minutes at room temperature, the membranes were probed overnight at 4℃ with the respective primary antibodies. For

probing blots, all primary antibodies were diluted into 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 an anti-rat Na^+/H^+ exchanger type 3 (NHE3; 1:200), anti-rat Na^+-K^+-$2Cl^-$ cotransporter (NKCC2; 1:2000), anti-rat NCC (1:1000), anti-rat α- (1:1000), β- (1:200), γ-ENaC (1:500), anti-rat Na^+-K^+-ATPase antibodies, and anti-rat aquaporin-2 (AQP2; 1:1000). The membranes were washed and incubated with secondary antibodies for 1 hour at room temperature. The secondary antibody was horseradish peroxidase-conjugated donkey anti-rabbit IgG (31458; Pierce, Rockford, IL, USA) diluted to 1:3000, except for Na^+-K^+-ATPase. The secondary antibody for Na^+-K^+-ATPase was horseradish peroxidase-conjugated rabbit anti-mouse IgG (31452; Pierce) diluted to 1:3000. Sites of antibody-antigen reaction were viewed with 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.

For semi-quantitative immunoblotting, we used previously characterized polyclonal antibodies. Affinity-purified polyclonal antibodies against NHE3 [19], NKCC2 [20], NCC [21], and α- and γ-ENaC [22] were used. Affinity-purified polyclonal antibodies against β-ENaC (sc-21013; Santa Cruz Biotechnology, Inc, CA, USA), ROMK (APC-001, Alomone Laboratories, Jerusalem, Israel), Na^+-K^+-ATPase (05-396; Millipore Corporation), and AQP2 (AQP-002, Alomone Laboratories) were also used.

6. Immunohistochemistry

The other one-half of the left kidney from some part of groups was immersed in 4% paraformaldehyde solution at 4 °C overnight. Each slice was dehydrated with a graded series of ethanol and embedded in paraffin. The embedded pieces of kidney were sectioned (3 nm thickness) on a microtome (RM 2145; Leica Instruments GmbH, Nussloch, Germany) and mounted on gelatin-coated glass slides. The sections were deparaffinized with xylene, dehydrated with a graded series of ethanol, and rehydrated. Antigen retrieval was done by repeated boiling and cooling in citric acid buffer. Endogenous peroxidase activity was blocked by incubation of the sections in 3% H_2O_2 for 10 minutes. Next, the immunostaining procedure was performed according to the protocol of the DakoCytomation kit (Envision® + Dual Link System-HRP, Carpinteria, CA, USA). The sections were incubated 1 hour with antibody against NCC and AQP2 at room temperature, and overnight with antibody against NHE3 at 4 °C.

7. Statistical analysis

All values are expressed as the mean ± standard error (SEM). The Mann-Whitney U-test (SPSS software version 15.0; SPSS Inc., Chicago, IL, USA) was used to compare groups. Band density values were standardized by dividing them with the average value of the control group. Thus, the mean for the control group was defined as 100%. A *P*-value < 0.05 was considered statistically significant.

Results

1. Physiologic parameters

As shown in Figure 1, after the uninephrectomy, a high salt diet produced a rise in systolic BP from 136.5 ± 1.22 mmHg to 150.9 ± 4.04 mmHg at 1 wk (P=0.03) and 207.7 ± 6.21 mmHg at 3 wk (P<0.01). After random allocation, the systolic BP was relatively decreased in the HS+KCL group (140.3 ± 2.97 mmHg at 1wk and 180.3 ± 1.76 mmHg at 3 wk, P=0.04) compared with the HS group (150.9 ± 4.01 mmHg at 1 wk and 207.7 ± 6.21 mmHg at 3 wk, P=0.01). The blood pressure-sodium excretion curve for the kidney of HS rats had shifted to the right, resulting in a blunted pressure natriuresis, and the HS+KCL rats had a relative shift to the left (Figure 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 the HS group (450.6 ± 6.98 g and 438.9 ± 16.52 g respectively; Table 1).

In uNx-SSH rats, the fluid intake and urine volume increased after a high salt diet. As shown in Figure 2, both the fluid intake and urine volume were further increased by potassium repletion. In addition, potassium repletion resulted in increased urinary excretion of sodium and potassium (Figure 3). Daily sodium, chloride and potassium balance were negative after potassium repletion (Figure 4). Despite an increased urine volume, urine osmolality was not different between the HS and HS+KCL groups (603.9 ± 23.07 vs. 634.2 ± 19.55 mOsm/Kg at 1 wk; and 838.7 ± 57.87 vs. 810.5 ± 90.70 mOsm/Kg at 3 wk, respectively). There were no significant differences in serum sodium and potassium by the end of the experiment (Table 1).

At 1wk, 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 enhanced 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 of all rats were depressed for the entire experimental period (Table 1).

2. Protein abundance using semi-quantitative immunoblotting

Semi-quantitative immunoblotting of homogenates of whole kidneys from HS+KCL rats showed a significant decrease in the protein abundance of NHE3 (21 % of the HS group at 1 wk; 7 % of the HS group at 3 wk) compared with the HS rats (Figure 5(A)). Immunohistochemical analysis also showed profoundly decreased NHE3 labeling in kidneys from HS+KCL rats (Figure 10). In contrast to the significant down-regulation of the NHE3, up-regulation (150% of the HS group) were observed in the expression levels of the Na^+-K^+-ATPase at 1 wk, but marked down-regulation (0.8 % of the HS group) was observed at 3 wk (Figure 5 (B)).

Expression of NKCC2 protein was not altered by potassium repletion at 1 and 3 wk of the experiment (Figure 6 (A)).

NCC abundance in the HS+KCL group significantly decreased (49 % of the HS group at 1 wk and 16 % of the HS group at 3 wk) compared with the HS group (Figure 6 (B)). This was confirmed by immunohistochemistry showing reduced labeling in the distal convoluted tubule of kidneys from the HS+KCL rats, whereas the HS rats demonstrated intense NCC labeling in the apical part of the distal convoluted tubules (Figure 10).

As shown in Figure 7 (A), semi-quantitative immunoblotting demonstrated that the abundance of ENaC-α was significantly increased (179% of the HS group at 1 wk and 185% of the HS group at 3 wk) in the HS+KCL rats. In contrast, the abundance of ENaC-β was decreased (72% of the HS group at 1 wk and 59% of the HS group at 3 wk) though not statistically significant (Figure 7 (B)). Also, ENaC-γ expression was significantly decreased (33% of the HS group at 1 wk and 10% of the HS group at 3 wk; Figure 7 (C)).

In contrast to the marked elevation of the plasma aldosterone level, the expression of ROMK was decreased (42% of the HS group) at 3 wk (Figure 7 (D)).

Semi-quantitative immunoblotting of kidneys from the HS+KCL group showed a significant decrease in the protein abundance of AQP2 (1% of the HS group) at 3 wk (Figure 8). Immunohistochemical analysis also showed a profoundly decreased AQP2 labeling in kidneys from the HS+KCL group. (Figure 10).

3. Results of WNK kinases and NCC mRNA expression

Expression of KS-WNK1 mRNA was significantly increased in the HS+KCL group (1.8- and 1.4-fold of the HS group at 1 and 3 wk) and the L-WNK1 / KS-WNK1 ratio of mRNA expression was significantly decreased in the HS+KCL group compared to the control group (Figure 9 (A), (B)). Also, expression of WNK4 mRNA was significantly increased in the HS+KCL group (1.4- and 1.9-fold of the HS group at 1 and 3 wk, respectively; Figure 9 (C), (D)). A significant decrease in NCC mRNA expression existed in the HS+KCL group (0.6- and 0.7-fold of HS group at 1 and 3 wk, respectively; Figure 9 (C), (D)).

	Basal (N=24)	*Post Op 4wk* (N=24)	*1wk after diet*		*3wk after diet*	
			HS (N=6)	HS+KCL (N=6)	HS (N=6)	HS+KCL (N=6)
Body weight (g)	275.50±2.23	442.40±6.41	450.60±6.98	411.50±7.03*	438.90±16.52	378.50±11.09*
Food intake (g/100gBwt/day)	7.14±0.27	6.16±0.18	21.53±3.37	16.26±1.82	17.61±0.40	14.47±1.08*
Ccr (ml/min/100gBwt)	0.60±0.06	0.38±0.02	0.54±0.05	0.46±0.03	0.85±0.13	0.64±0.07
Curea (ml/min/100gBwt)	0.39±0.03	0.32±0.02	0.40±0.04	0.36±0.03	0.70±0.11	0.46±0.05
CH20 (ml/min/100gBwt)	-0.01±0.00	-0.01±0.00	-0.02±0.00	-0.03±0.00	-0.03±0.00	-0.04±0.00
GFR (ml/min/100gBwt)	0.46±0.04	0.35±0.02	0.47±0.04	0.41±0.02	0.78±0.12	0.54±0.05
sBUN (mg/dL)	9.38±0.52	15.83±0.46	13.57±1.24	13.27±0.56	11.05±1.56	14.38±1.17
sCr (mg/dL)	0.40±0.02	0.59±0.02	0.50±0.04	0.57±0.02	0.38±0.05	0.47±0.04
sNa (mmol/L)	155.50±0.65	154.60±0.45	155.20±1.08	153.70±1.07	154.00±1.16	153.20±0.36
sK (mmol/L)	3.93±0.06	3.65±0.09	3.61±0.18	4.10±0.30	4.40±0.15	4.90±0.31
FENa (%)	0.45±0.04	0.55±0.02	8.34±0.51	10.80±1.75	5.17±0.68	6.03±0.78
FEK (%)	32.47±1.76	50.83±2.05	39.71±3.37	223.90±38.63*	25.54±4.43	137.40±26.59*
uOsm (mosm/kg)	1584.00±44.51	1354.00±37.75	603.90±23.07	634.20±19.55	838.70±57.87	810.50±90.70
PRA (ng/ml/hr)			1.02±0.68	1.25±0.43	0.14±0.04	0.20±0.07
PAC (pg/ml)			140.30±4.94	160.20±27.70	159.50±28.16	699.70±185.90*

Table 1. Physiologic parameters

Values are the means ± standard error; Ccr, Creatinine clearance; Curea, Urea clearance; CH2O, Free water clearance; sBUN, serum BUN; sCr, serum creatinine; 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.

*; $P<0.05$ when compared to the control rats.

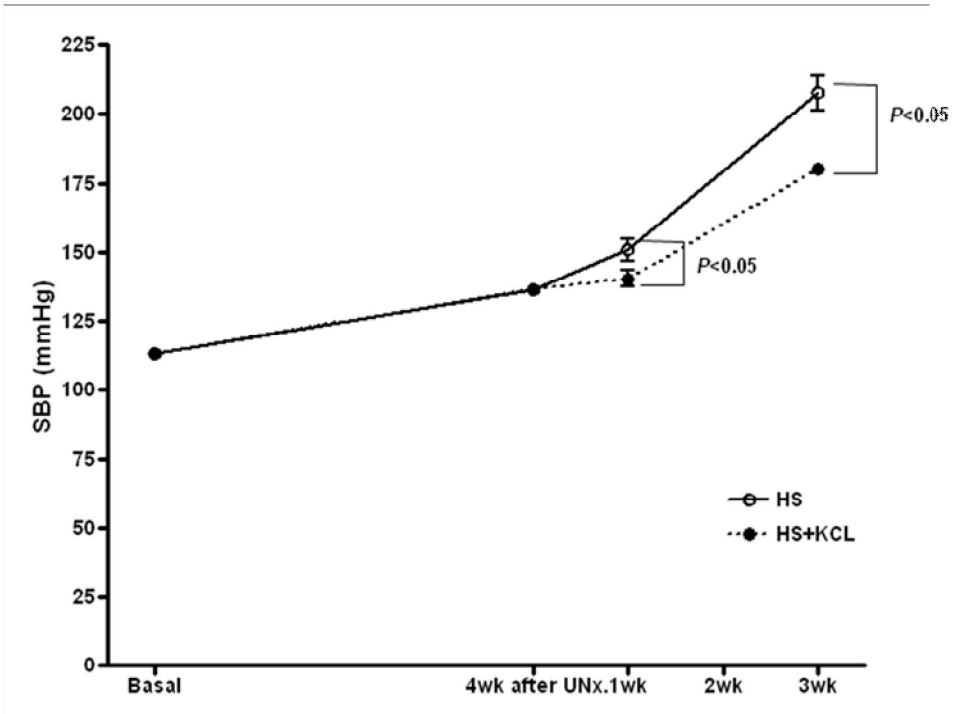


Figure 1. Changes in systolic blood pressure. After application of uninephrectomy, the BP in rats fed a high salt diet and tap water increased progressively. In rats given potassium, the elevation of BP was attenuated. All of the values are expressed as the mean ± standard error. HS, High-salt diet group after uninephrectomy; HS+KCL, High-salt diet with potassium repleted group after uninephrectomy.

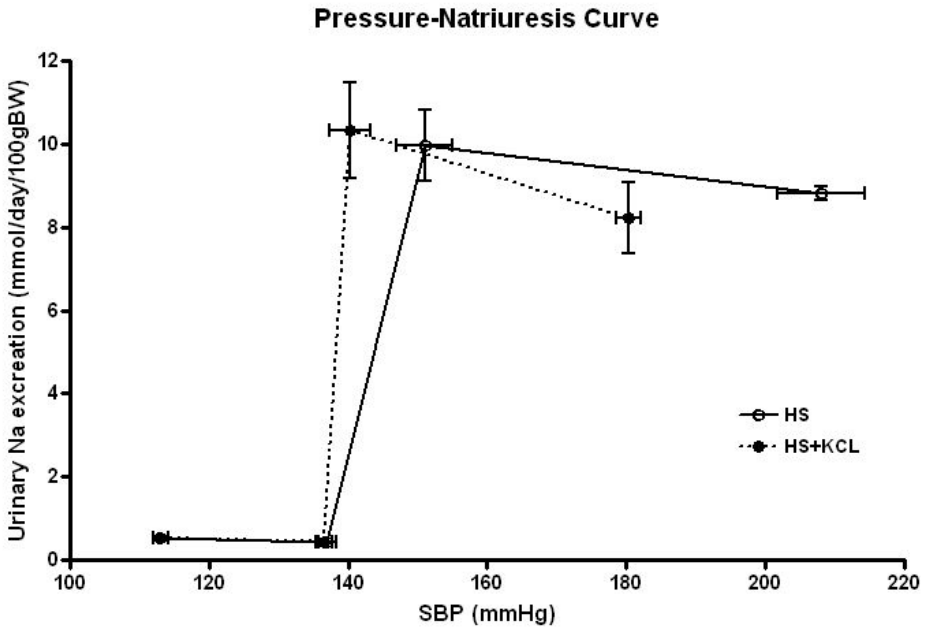


Figure 2. Pressure-natriuresis curve during different potassium condition. This curve illustrated the relationship between the SBP and 24-hour urinary sodium output. The pressure-sodium excretion curve for the kidney of HS rats had shifted to the right, and HS+KCL rats had a relative shift to the left. All of the values are expressed as the mean ± standard error. HS, High-salt diet group after uninephrectomy; HS+KCL, High-salt diet with potassium repleted group after uninephrectomy.

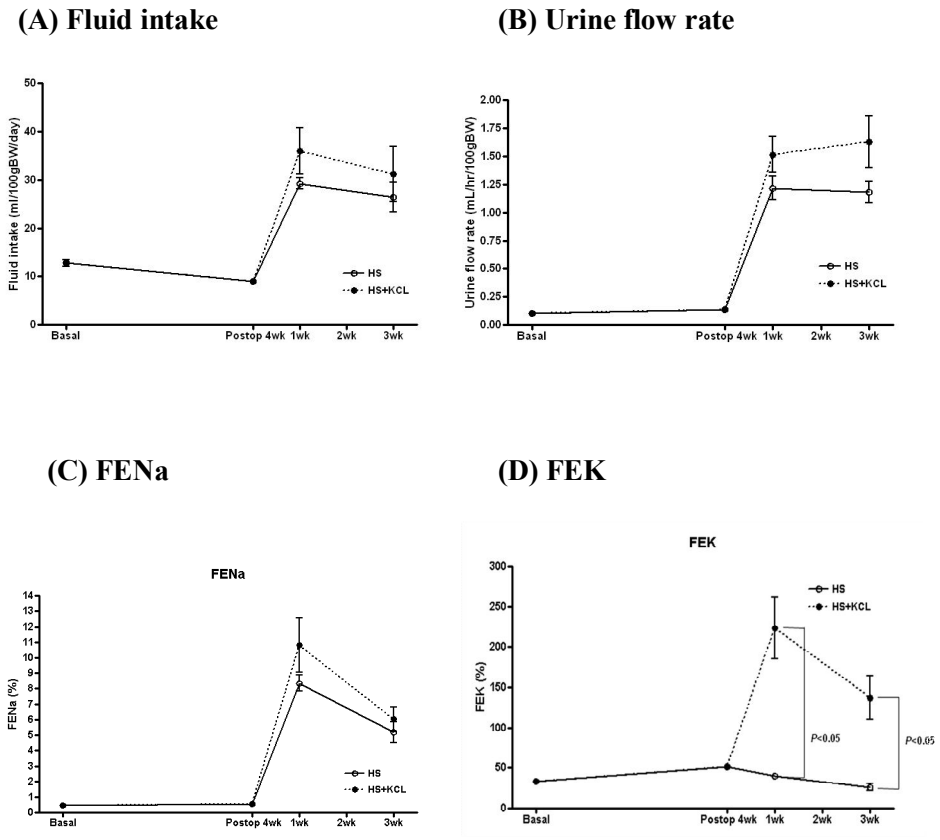
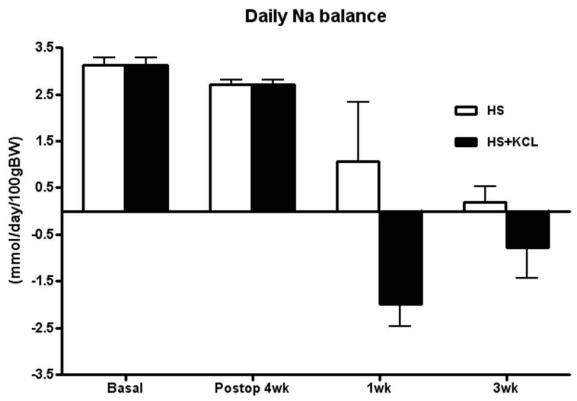


Figure 3. Changes in fluid intake and urine volume.

In the HS+KCL rats, both fluid intake and urine volume increased beginning in the first week of the experiment. FENa and FEK are also increased after potassium repletion. All of the values are expressed as the mean ± standard error. HS, High-salt diet group after uninephrectomy; HS+KCL, High-salt diet with potassium repleted group after uninephrectomy.

(A) Daily Sodium Balance



(B) Daily Chloride Balance

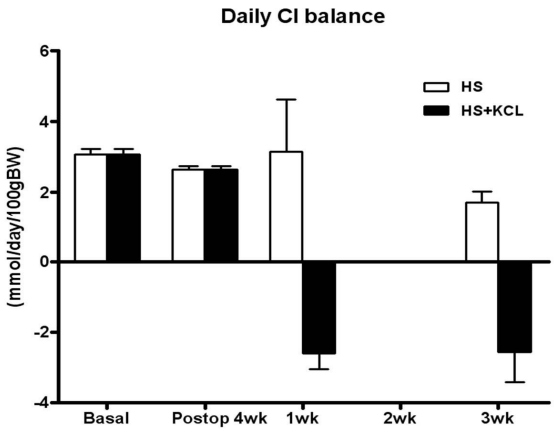


Figure 4. Changes in daily sodium and chloride balance.

In the HS+KCL rats, the daily sodium and chloride balance decreased beginning in the first week of the experiment. The daily sodium and chloride balance was also negative after potassium repletion. All of the values are expressed as the mean ± standard error. HS, High-salt diet group after uninephrectomy; HS+KCL, High-salt diet with potassium repleted group after uninephrectomy.

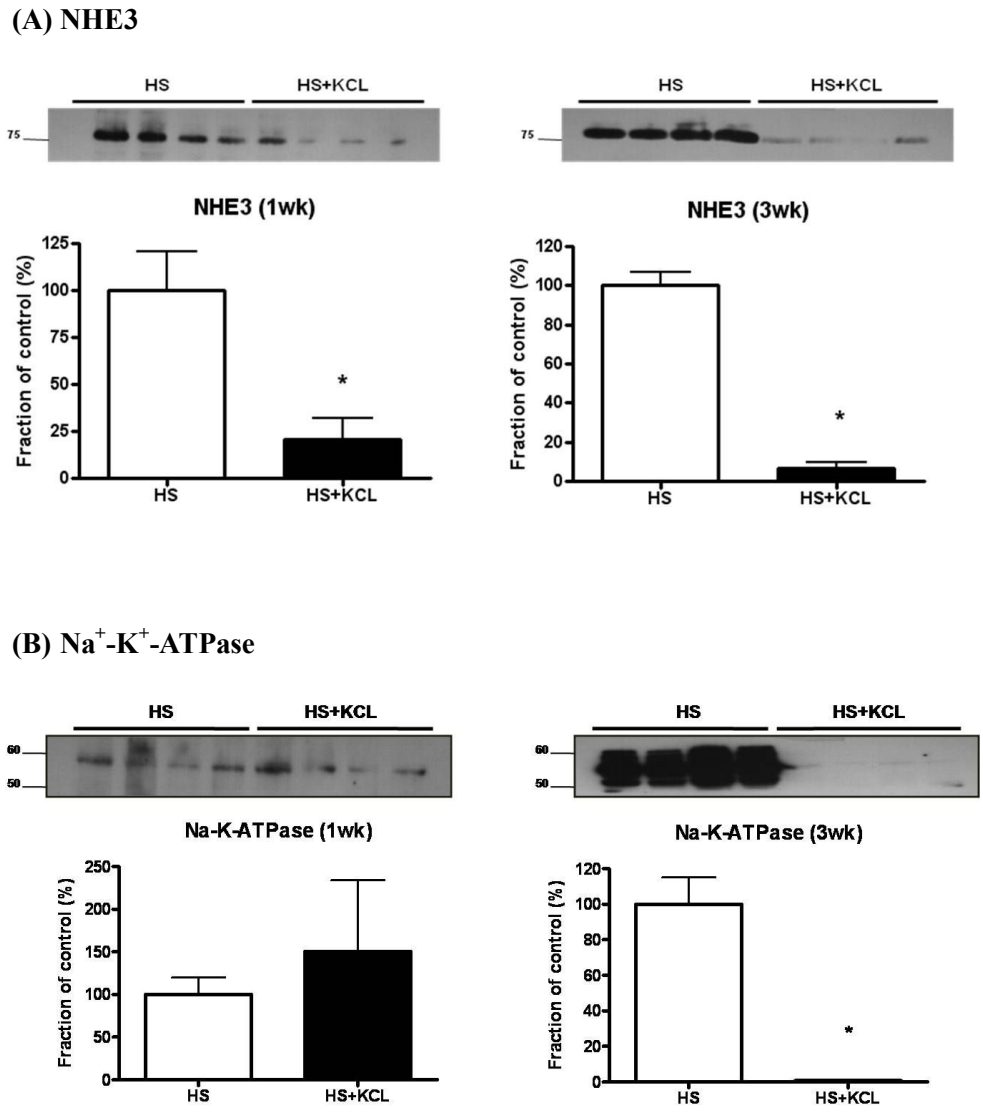


Figure 5. Protein abundance of NHE3 and Na^+-K^+-ATPase

(A) Protein abundance of NHE3 were significantly decreased compared with that of the HS group. (B) Na^+-K^+-ATPase were slightly increased at 1wk and significantly decreased at 3 wk compared with that of the HS group. Values are the means and bars indicate standard error. *; $p<0.05$ when compared with the HS group.

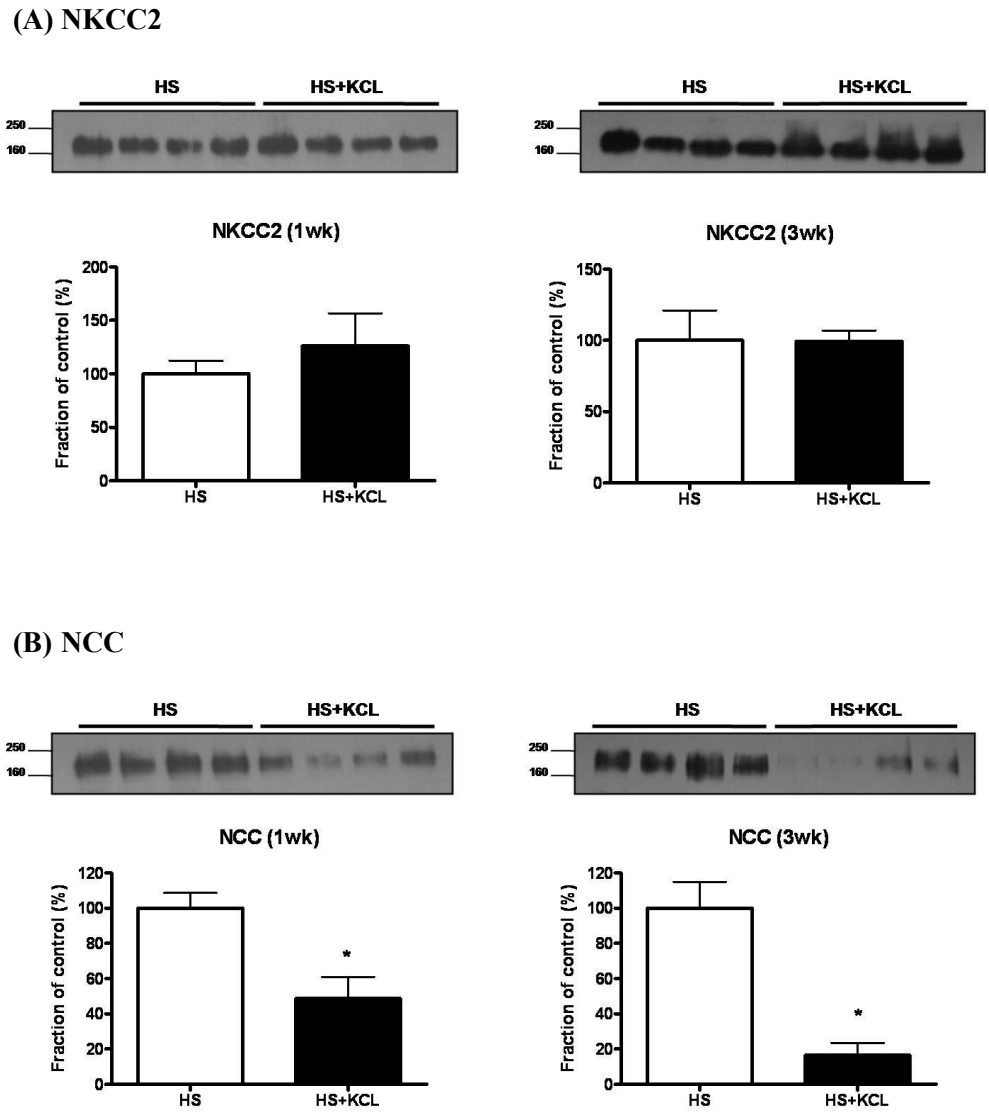
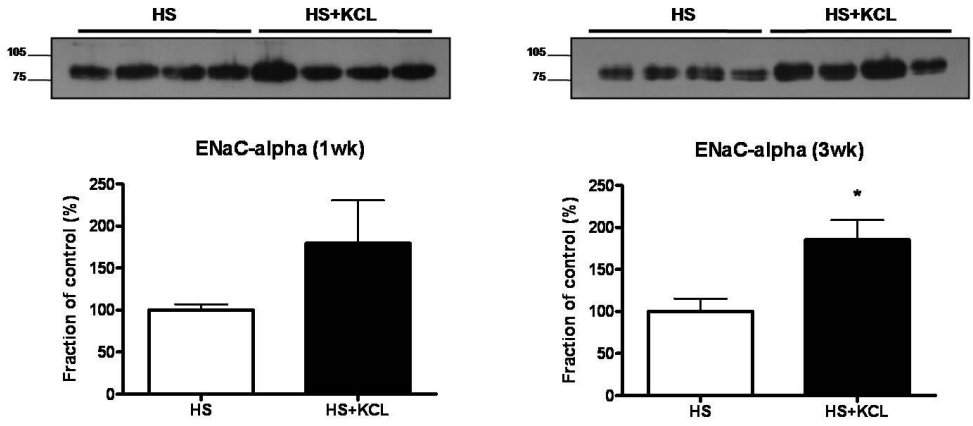


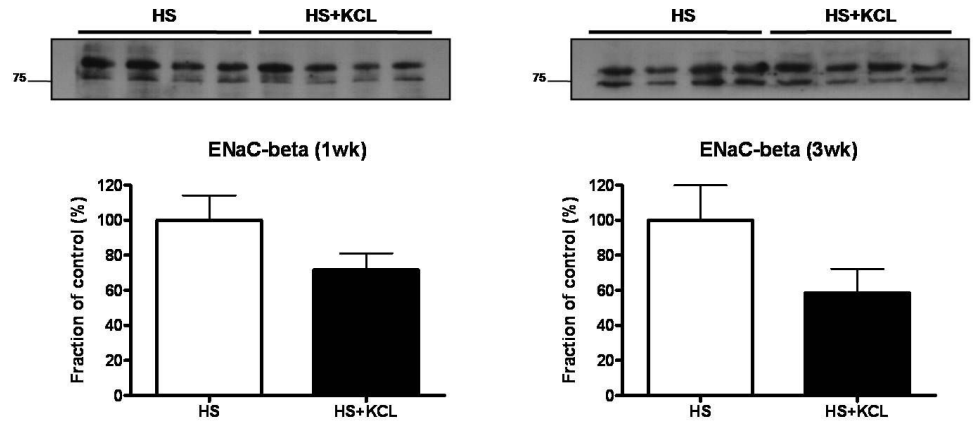
Figure 6. Protein abundance of NKCC2 and NCC

Protein abundance of NCC was significantly decreased compared with the HS group. In contrast, NKCC2 was not changed during potassium repletion. Values are the means and bars indicate standard error. *; $p<0.05$ when compared with the HS group.

(A) ENaC-α



(B) ENaC-β



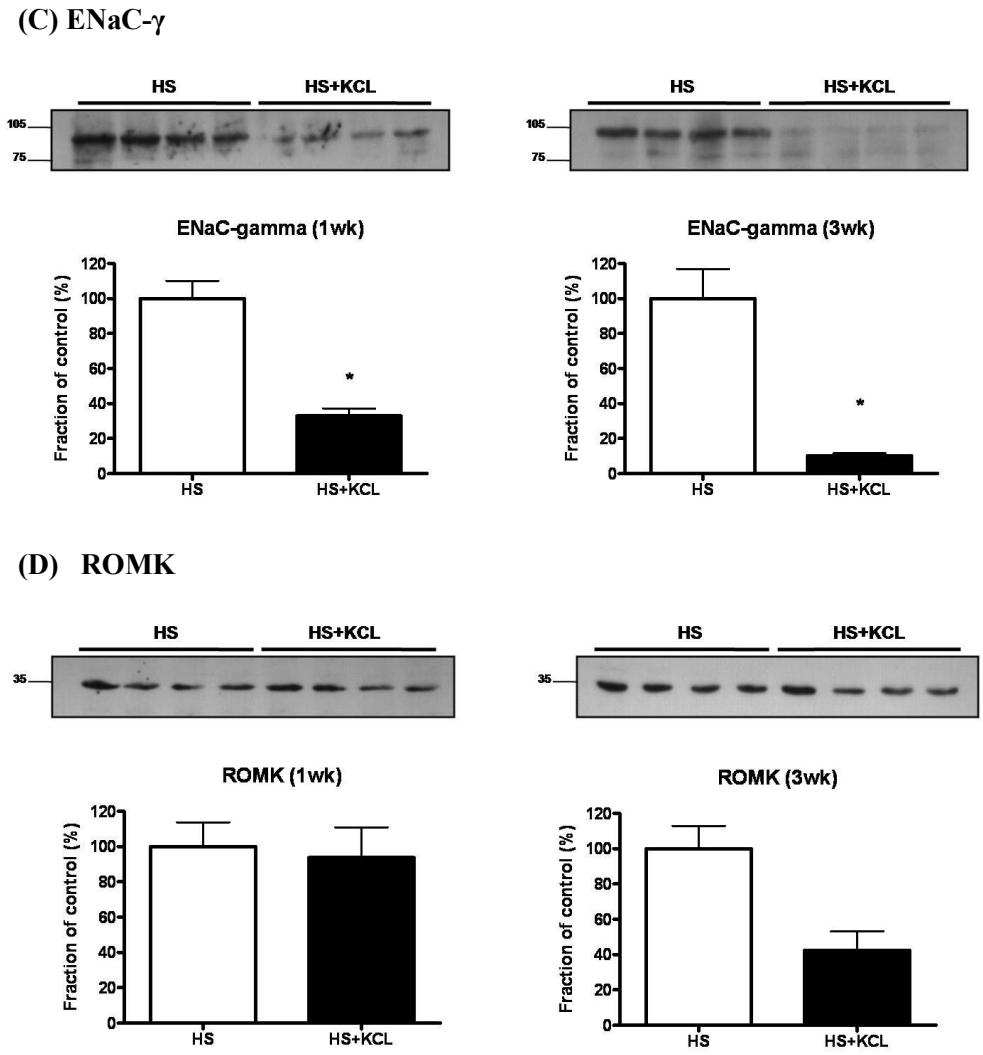


Figure 7. Protein abundance of ENaCs and ROMK.

Protein abundance of ENaC-γ in the HS+KCL group was significantly decreased. Also, the abundance of ENaC-β was decreased but, not statistically significant. In contrast, ENaC-α was significantly increased at 3 wk. In contrast to the marked elevation of the plasma aldosterone level, the expression of ROMK was decreased at 3 wk. Values are the means and bars indicate standard error. *; $p<0.05$ when compared with the HS group.

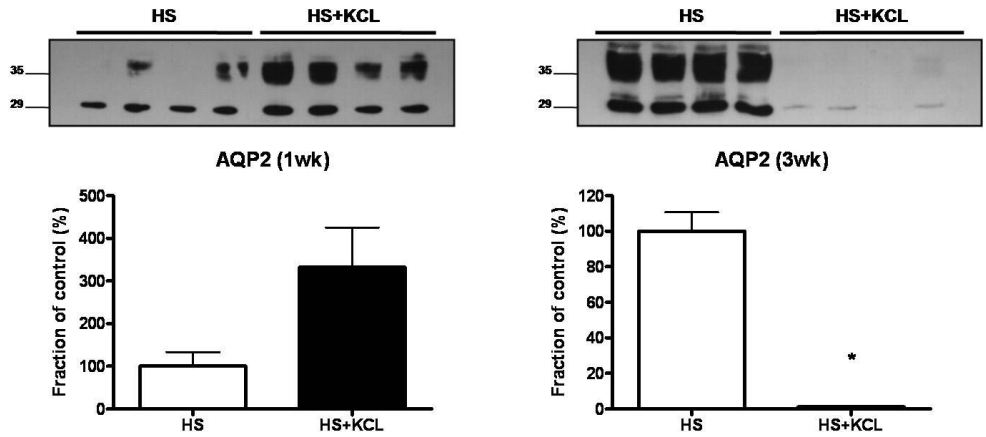
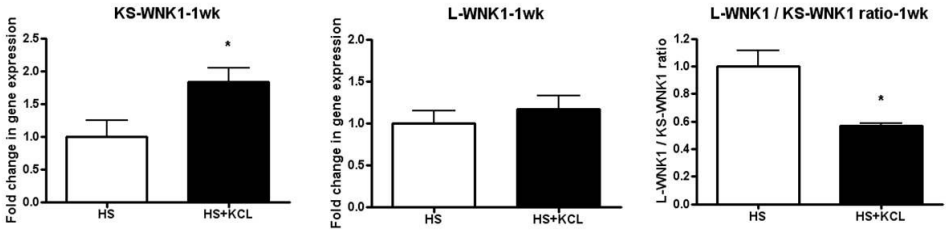


Figure 8. Protein abundance of AQP2.

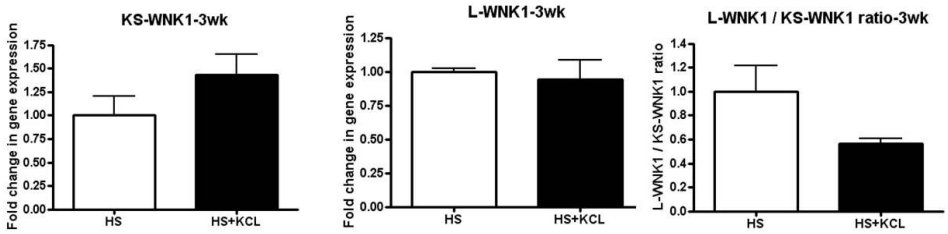
Protein abundance of AQP2 in the HS+KCL group significantly decreased at 3 wk.

*; $p<0.05$ when compared with the HS group.

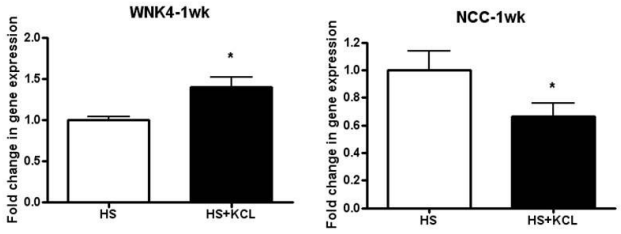
(A) WNK 1 mRNA expression at 1 wk



(B) WNK 1 mRNA expression at 3 wk



(C) WNK 4 and NCC mRNA expression at 1 wk



(D) WNK 4 and NCC mRNA expression at 3 wk

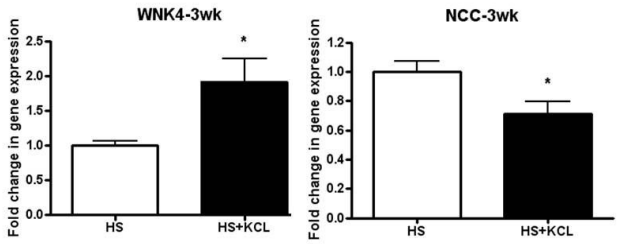


Figure 9. WNK kinase and NCC mRNA expression.

(A) At 1wk, the L-WNK1 / KS-WNK1 ratio of mRNA expression was significantly decreased in the potassium-repleted group compared to the control group, (B) At 3 wk, the L-WNK1 / KS-WNK1 ratio of mRNA expression was slightly decreased in the potassium-repleted group compared to the control group, (C) and (D) WNK 4 mRNA expression was significantly increased and NCC mRNA expression was decreased in potassium-repleted group compared to the control group at 1 and 3 wk.

The values are the means and the bars indicate the standard error. HS, High-salt diet group after uninephrectomy; HS+KCL, High-salt diet with potassium-repleted group after uninephrectomy. *; $p<0.05$ when compared with the HS group.

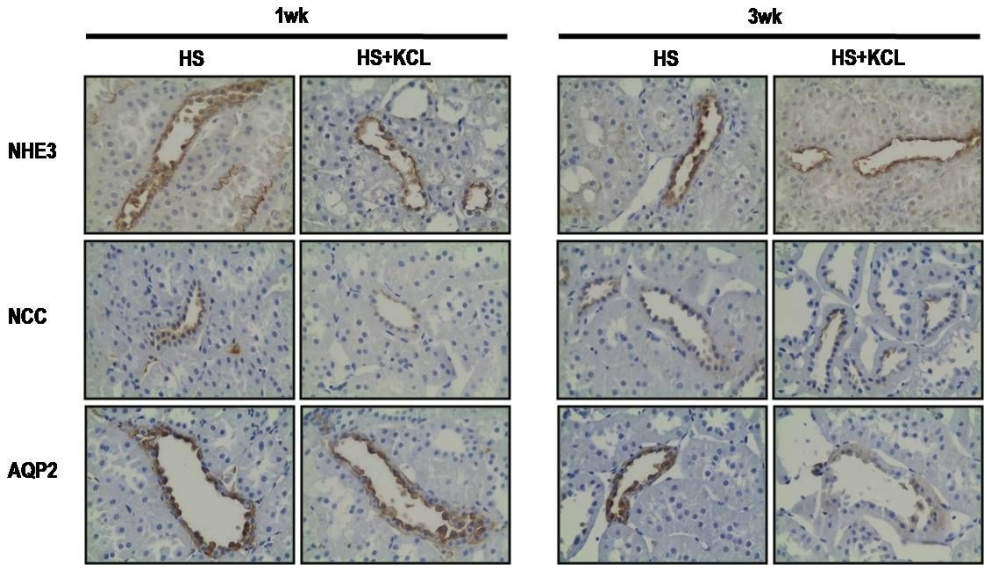


Figure 10. Immunohistochemical analysis of NHE3, NCC, and AQP2.

In the HS+KCL group, immunostaining of NHE3, NBC, NCC, and AQP2 significantly decreased at 1 and 3 wk compared with the HS group.

Discussion

The finding of these animal studies indicate that potassium supplements can promote daily sodium excretion, thus attenuate the rise in blood pressure and delay the onset of hypertension. The potassium loading was also effective in reducing blood pressure when given after uNx-SSH rats have been established. Therefore, potassium played an important role in the antihypertensive effect in the uNx-SSH model.

During the first week of potassium repletion, the HS+KCL group had a significantly negative sodium balance. As a result, blood pressure was not significantly increased in the HS+KCL group, but was significantly so in the HS group.

During the third week of potassium repletion, the daily sodium balance remained significantly negative in the HS+KCL group. Despite a significant rise in blood pressure, the HS rats retained more sodium than the HS+KCL group. This suggests that the blood pressure-sodium excretion curve for the kidneys of HS group had shifted to the right, resulting in a blunted pressure natriuresis, and the HS+KCL group could restore this blood pressure-urinary sodium excretion relationship more easily (Figure 2).

An increase in body weight was not observed in the HS and HS+KCL groups. One of the possible causes of this phenomenon is probably based on the reduction in food intake due to the massive rate of NaCl and/or KCl ingestion.

The mechanisms for the natriuresis induced by potassium loading were unknown until the WNK kinase pathway was discovered. Under normal conditions, WNK 4 inhibits the activity of NCC, but to date, no effects on ENaC activity have been described. L-WNK1 stimulates both ENaC- and NCC-mediated Na^+ 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 [23]. It has been reported that high and low K^+ intake increases and decreases expression of KS-WNK1, respectively [24, 25]. Low K^+ intake also leads to increased expression of L-WNK1 in the kidney [24]. Therefore, the ratio of L-WNK1-to-KS-WNK1 in the kidney is important in regulating Na^+ and K^+ homeostasis [26]. In the present study, KS-WNK1 was significantly up-regulated with potassium repletion, which led to a decreased ratio of L-WNK1-to-KS-WNK1 (Figure 9 (A), (B)). Thus, the previously reported *in vitro* evidence [16, 18] which suggests potassium intake up-regulates KS-WNK1 and WNK4 resulting in reduced sodium uptake via NCC was verified herein.

In our experiments, the natriuretic effect of KCl was not explained by changes in the plasma aldosterone concentration and was higher in the HS+KCL group. These finding could be explained by the concept of the 'aldosterone paradox,' the long-standing physiologic question of how aldosterone can be both a Na^+-retaining and K^+-secreting hormone. Specifically, if potassium loading (hyperkalemia) activates KS-WNK1 and WNK4, this will inhibit NCC and favor electrogenic sodium re-absorption by ENaC, thereby increasing the transepithelial voltage 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 re-absorption with a relative conservation of potassium [27]; thus, possible mechanism by which to explain why the HS+KCL group has increased urinary sodium excretion despite an elevated plasma aldosterone concentration.

Contrary to expectations, the expression of ROMK was not increased when there was

a marked elevation of plasma the aldosterone level (Figure 7 (D)). Although ROMK has been assigned a major role in K^+ secretion under physiologic conditions, ROMK activity is modulated by changes in K^+ intake, acid-base status, calcium, vasopressin [28], and maxi-K^+ channels with a large single-channel conductance and activated by apical membrane depolarization. Whereas basal K^+ secretion is mediated by the ROMK-type channel [29], the maxi-K^+ channel participates in distal K^+ secretion during high tubule fluid flow conditions [30]. Therefore, the maxi-K^+ channel may have played an important role in our study.

It is intriguing that we have found previously unexpected protein expression of NHE3. Significantly decreased expression of NHE3 after potassium repletion implicates another regulator that could influence proximal and thick ascending limb NaCl transport via regulation of NHE3. Renal ammonia production appears to be intimately related to potassium homeostasis, and the two may comprise the components of a closed loop regulatory system [31]. Potassium repletion is associated with decreased ammoniogenesis [32]. This effect may be related in part to a transcellular cation exchange. K^+ tends to enter into the cells with hyperkalemia in an attempt to decrease the extracelluar stores. Electroneutrality is maintained in this setting in part by H^+ moving out of the cells. The intracellular alkalosis could suppress renal ammoniogenesis and $HCO3^-$ re-absorption. Hyperkalemia, which accompanies intracellular alkalosis, have been implicated as factors inhibiting proximal $HCO3^-$ re-absorption and/or Na^+/H^+ exchange activity. Therefore, the effect of acidemia itself to increase proximal $HCO3^-$ re-absorption could be counterbalanced by these inhibiting effects on NHE3. The physiologic significance of the specific sites for the metabolic

effect of potassium on ammonia production is an interesting question, which may provide additional clues regarding the transport dynamics inter-relating these two variables.

Although the natriuretic properties of potassium are considered to play the most important role in the antihypertensive effects of potassium, several mechanisms have been demonstrated by which potassium may modulate hypertension [33, 34]. First, KCl loading is known to inhibit renin secrection [7, 35]. In the present study, however, plasma rennin activity was depressed in both groups. The depressed plasma rennin activity by potassium supplements may be due to increasing plasma aldosterone concentration induced by potassium. Second, potassium has been reported to have both direct and indirect effects on the vascular system [8]. Potassium may reduce the reactivity of the peripheral arterioles to vasoconstrictor agents such as norepinephrine and angiotensin II [36]. 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 [37]. Moreover, a report showed that the hyperactive central pressor response in Dahl salt-sensitive rats could be corrected largely with KCl supplementation [9]. However, potassium may influence the peripheral and central pressor responses indirectly by augmenting renal sodium excretion and thereby reducing body sodium content. It has been demonstrated that salt restriction induces a decreased pressor response to exogenous angiotensin II, which is explained solely by a decrease in the available angiotensin II receptor sites as a result of the increased concentrations of circulating angiotensin II [38]. Therefore, the present observation that potassium promoted natriuresis with the resultant decrease in the ECF volume in uNx.-SSH rats

suggests that it might change both the peripheral and the central pressor responses to angiotensin II.

In summary, a potassium supplement could prevent expansion of extracellular fluid in uNx-SSH rats and counteract the blood pressure raising effect. It is suggested, therefore, that potassium may attenuate the rise in blood pressure with a high salt diet in uninephrectomized rats, mainly as a result of the inhibition of sodium retention, by increasing urinary sodium excretion. The present study also demonstrated that potassium repletion in uNx-SSH rats is associated with substantial down-regulation of NHE3 and NCC in the rat kidney. Moreover, the protein abundance of AQP2 was markedly reduced in rats. Interestingly, reduced expression of NCC and ENaC-γ after potassium repletion is not consistent with the increased plasma aldosterone levels. In contrast, the abundance of the NKCC2 in the TALs was not changed. This suggests that NKCC2 is likely to play a role to maintain the urinary concentration associated with potassium loading.

In conclusion, although the hypotensive effect of potassium may be multifactorial, it may be associated with the natriuretic effect of potassium administration. NCC regulated by WNK4 was partially involved in our results. As shown by the alteration in NHE3 expression, the natriuretic action of potassium might be related to the proximal tubule. Further studies are needed to elucidate the functional correlation between potassium supplementation and attenuation of blood pressure.

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Korean Abstract

목적

포타시움은 혈압을 조절하는 중요한 요소로 알려져 왔다. 이 연구의 목적은 포타시움 식이가 혈압을 조절하는 기전에 있어 신 소디움 운반체의 발현의 변화와 연관이 있는지를 알아보고자 하는 것이다. 실험은 일측 신절제로 유도된 염분 민감성 랫드에서 포타시움 투여로 진행되었다.

방법

성숙 Sprague-Dawley 웅성 랫드에서 일측 신절제를 시행한 후 4주간 정상 식이 (0.3% NaCl)을 시행하였다. 이후 실험기간 내내 고염식이 (3% NaCl)를 진행하였다. 포타시움 투여군은 섭취 물을 1% KCl로 공급하였다. 주요 신 운반체의 발현은 Western 분석을 통해 각각 1주와 3주에 순차적으로 관찰하였다. WNK 키나아제의 메신저 리보핵산은 정량 중합효소 연쇄반응을 통해 각각 1주와 3주에 순차적으로 관찰하였다. 생리적 지표와 일일 소디움과 클로라이드 균형 역시 희생일에 측정되었다.

결과

고염식이 단독군에 비해 포타시움 투여군에서 소변량이 증가하였으나 (투여군 vs. 대조군; 1.52 ± 0.16 vs. 1.22 ± 0.10 ml/hr/100g Bwt 1주; 1.63 ± 0.23 vs. 1.18 ± 0.09 ml/hr/100g Bwt 3주). 소변 오스몰은 변화를 보이지 않았고 (투여군 vs. 대조군; 603.9 ± 23.07 vs. 634.2 ± 19.55 mOsm/Kg 1주; 838.7 ± 57.87 vs. 810.5 ±

90.70 mOsm/Kg 3주), 일일 소디움과 클로라이드 균형은 음의 값을 보였다 (투여군 vs. 대조군; -1.99 ± 0.48 vs. -2.61 ± 0.44 mmol/24hr/100g Bwt 1주; -0.79 ± 0.65 vs. –2.56 ± 0.86 mmol/24hr/100g Bwt 3주). 포타시움 투여군의 수축기 혈압은 고염식이 단독군에 비해 감소하였다(투여군 vs. 대조군; 140.3 ± 2.97 vs. 150.9 ± 4.04 mmHg 1주; 180.3 ± 1.76 vs. 207.7 ± 6.21 mmHg 3주).

포타시움 투여군에서 NCC, ENaC 그리고 NHE3의 단백 발현은 감소되었다(대조군의 각각 49 %, 33 %, 21 % 1주; 16 %, 10 %, 7 % 3주). AQP-2의 발현 또한 포타시움 투여군에서 3주째에 감소되었다(대조군의 1%). 이와는 반대로 NKCC2, ROMK는 포타시움 투여균에서 뚜렷한 변화를 보이지 않았다. WNK4 메신저 리보핵산의 발현은 포타시움 투여군에서 증가하였다 (대조군의 1.4배 1주; 1.9배 3주). KS-WNK1과 L-WNK1은 실험기간동안 뚜렷한 변화를 보이지 않았다.

결론

본 연구는 일측 신절제 유도 염분 민감성 랫드모델에서 소디움과 클로라이드의 배설과 관련하여 포타시움 식이의 혈압 강하효과를 설명하였다. NCC, ENaC-γ 그리고 NHE3의 적응성 변화가 포타시움 식이의 혈압 강하효과에 부분적인 역할을 할 것으로 생각된다. 원위 네프론에서는 WNK 키나아제 경로가 NCC 발현의 변화에 기여할 것으로 생각된다.

핵심어 : 포타시움, 염분미감성 고혈압, 신 소디움 운반체

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저작물 이용 허락서

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논문제목	한글 : 일측 신절제 유도 염분 민감성 고혈압 랫드에서 포타시움 투여로 인한 소디움 운반체의 발현 변화 영어 : Effects of Potassium on the Expression of Sodium Transporters in Salt-sensitive Hypertensive Rats induced by Uninephrectomy				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB 구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집 · 형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포 · 전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5 년으로 하고, 기간종료 3 개월 이내에 별도의 의사 표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1 개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음.
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송 · 출력을 허락함.

동의여부 : 동의(○) 반대( )

2009 년 8 월 25 일

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