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Potassium (K^+) intake modulates NCC activity via the K^+ channel, Kir4.1

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Abstract

Kir4.1 in the distal convoluted tubule (DCT) plays a key role in sensing plasma K^+ and in modulating the thiazide-sensitive Na-Cl cotransporter (NCC). The aim of current study was to test whether dietary K^+ intake modulates Kir4.1, and whether this is essential for mediating the effect of K^+ diet on NCC. High K^+ (HK) intake inhibited the basolateral 40 pS K^+ channel (a Kir4.1/5.1 heterotetramer) in the DCT, decreased basolateral K^+ conductance, and depolarized the DCT membrane in *Kcnj10*$^{flox/flox}$ (control or WT) mice. In contrast, low K^+ (LK) intake activated Kir4.1, increased K^+ currents, and hyperpolarized the DCT membrane. These effects of dietary K^+ intake on the basolateral K^+ conductance and membrane potential in the DCT were, however, completely absent in inducible kidney-specific Kir4.1 knockout (KS-Kir4.1 KO) mice. Furthermore, HK-intake decreased whereas LK-intake increases the abundance of NCC expression only in WT but not in KS-Kir4.1 KO mice. Renal clearance studies demonstrated that LK augmented, while HK diminished, hydrochlorothiazide (HCTZ)-induced natriuresis in WT mice. Disruption of Kir4.1 significantly increased basal urinary Na^+ excretion but it abolished the natriuretic effect of HCTZ. Finally, hypokalemia and metabolic alkalosis in KS-Kir4.1 KO mice were exacerbated by K^+ restriction and only partially corrected by HK diet. We conclude that Kir4.1 plays an essential role in mediating the effect of dietary K^+ intake on NCC activity and K^+ homeostasis.

Keywords

Kcnj10; *Kcnj16*; hyperkalemia; hypokalemia; EAST syndrome

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Disclosure

None

Introduction

During the past several years, it has become clear that the distal convoluted tubule (DCT), a short nephron segment that secretes little or no K^+ itself, plays a central and often dominant role in determining renal K^+ excretion [1]. This recognition has been driven largely by insights derived from rare monogenic human diseases and advances in transgenic and knockout technology [2–6]. These insights have converged to generate a working model postulating that sodium chloride reabsorption along the DCT, mediated by the thiazide-sensitive NaCl cotransporter (NCC), and working in concert with aldosterone, determines the amount of potassium secreted by more distal segments [1]. It is now clear that dietary K^+ intake is a dominant factor regulating NCC activity in animals and humans; low K^+ (LK) intake activates NCC, whereas high K^+ (HK) intake suppresses it [4;7–10]. LK intake-induced NCC stimulation should decrease K^+ secretion either through effects on Na^+ delivery to the aldosterone-sensitive distal nephron (ASDN) or by leading to its remodeling [11]. HK intake, conversely, should stimulate K^+ secretion by inhibiting NCC and increasing aldosterone secretion, thereby activating the epithelial Na^+ channel, ENaC.

Kir4.1 (encoded by *Kcnj10*) is expressed in the basolateral membrane of thick ascending limb, DCT, connecting tubule (CNT) and cortical collecting duct (CCD) [12–18]. Kir.4.1 interacts with Kir5.1 (encoded by *Kcnj16*) to form a 40 pS heterotetrameric K^+ channel in these segments [16;19]. Since Kir4.1/5.1 heterotetramer is the only type of K^+ channel expressed in the basolateral membrane of the DCT, this 40 pS K^+ channel plays a dominant role in determining the basolateral K^+ conductance and the membrane potential [13;20;21]. We recently demonstrated that Kir4.1 activity in the DCT plays a key role in regulating NCC activity, to maintain potassium homeostasis, by showing that *kcnj10* knockout strikingly reduced the expression of total NCC (tNCC) and phosphorylated NCC (pNCC) [13;22]. The mechanism by which Kir4.1 activity regulates NCC activity along the DCT likely involves a Cl^--sensitive with-no-lysine kinase (WNK) in the DCT [4;23;24]. Because Kir4.1 activity in the DCT determines the inside-negativity of the membrane potential, which provides a driving force for Cl^- exit across the basolateral membrane through ClC-Kb [25;26], alteration of Kir4.1 activity would be expected to affect Cl^- exit. Our previous experiments demonstrated that the deletion of Kir4.1 in the DCT depolarized the basolateral membrane and inhibited Cl^- channels [13;22]. The inhibition of Cl^- channels would be expected to raise intracellular Cl^- (Cl_i^-) concentration thereby inhibiting WNK and ste20 proline-alanine-rich kinase (SPAK) activity [23], leading to the inhibition of NCC, since WNK and SPAK are required its activation [27–33]. Our previous work showed the central importance of Kir4.1 and linked its activity to the ability of DCT basolateral membranes to ‘sense’ K^+[22], but we speculated that the effects of dietary K^+ intake on NCC might involve additional changes. Here, we tested the hypothesis that dietary K^+ alterations modulate the activity of Kir4.1, and that this channel is essential for the DCT to sense and respond to dietary perturbation.

Results

LK intake stimulates and HK inhibits basolateral K^+ channels in the DCT

We first used single channel recording to examine the basolateral K^+ channel activity in the DCT of *Kcnj10*$^{flox/flox}$ mice, which were used as control and referred as "wild type" (WT) mice, and the mice were kept on a normal K^+ (1% K), a LK (<0.001%) or a HK (5%) diet for 4 days. We detected an inwardly-rectifying 40 pS K^+ channel in the basolateral membrane of the DCT. From the inspection of Fig.1A, it is apparent that 40 pS K^+ channel activity defined by NP_o in the mice on a LK diet was higher than that on a normal K^+ (NK) diet. In contrast, the 40 pS K^+ channel activity in the DCT of WT mice on a HK diet for 4 days was lower than those on NK and LK diet (Fig. 1A). Fig. 1B summarizes the patch-clamp experimental results: the probability of finding 40 pS K^+ channels in the DCT was 65% (34 patches from total 52 experiments) in the mice on a NK whereas it was 90% (18 patches from total 20 experiments) and 47% (8 patches from total 17 experiments) in animals on a LK and HK diet, respectively. Moreover, the channel open probability (P_o) in the mice on a LK diet (0.45±0.04) was slightly but significantly higher than that on a NK diet (0.35±0.03) and on a HK diet (0.31±0.02). Fig.1C is immunostaining showing the expression of Kir4.1 in the kidney of the mice on NK, LK and HK, respectively.

The notion that LK intake stimulates while HK intake inhibits the basolateral 40 pS K^+ channel activity was further examined with the whole-cell recording to measure Ba^{2+}-sensitive K^+ currents in the early part of DCT (DCT1). As reported previously [13], the whole-cell K^+ current in the DCT1 is equal to the basolateral Kir4.1/5.1 channel activity because there is no detectable ROMK channel activity in the apical membrane of DCT1. Fig. 2A is a set of representative current traces showing Ba^{2+}-sensitive whole-cell K^+ currents measured from −100 to 60 mV with a 20 mV step in the DCT of the mice on a LK (N=18), NK (N=16) and HK diet (N=12), respectively. It is apparent that LK intake for 4 days increases, while HK intake for 4 days decreases, whole cell K^+ currents in comparison to that of the mice on a NK diet. Fig. 2B is a trace of Ba^{2+}-sensitive K^+ currents measured with RAMP protocol from −100 to 100 mV, showing that LK intake increases while HK intake decreases the K^+ currents. A bar graph summarizing results measured at −60 mV shows that K^+ currents are 2680±145 pA (LK, N=20), 1420±50 pA (NK, N=18) and 560±35 pA (HK, N=13), respectively (Fig.2B). We then repeated the above experiments in the DCT1 of kidney specific (KS)-Kir4.1 knockout (KO) mice on different K^+ diets. (The method for creating KS-Kir4.1 KO mice were described previously)[22] Fig. 2C is a set of traces showing Ba^{2+}-sensitive K^+ currents measured with step protocol clamped from −100 to 60 mV (20 mV step) and Fig. 2D is a trace showing K^+ currents measured with RAMP protocol from −100 to 100 mV. It is apparent neither LK intake increases nor HK intake decreases K^+ currents in the DCT1 of KS-Kir4.1 KO mice (NK, 110±15 pA; LK, 105±12 pA; HK, 110±20 pA) (Fig.2D, N=9–11). Thus, dietary K^+ intake-induced regulation of the basolateral K^+ conductance is the results of either stimulation of Kir4.1 during LK or the inhibition of Kir4.1 during HK.

LK intake hyperpolarizes while HK intake depolarizes the membrane in the DCT

Since basolateral K^+ channels participate in generating the membrane potential in the DCT, we expected that LK intake-induced activation of Kir4.1 should increase, while HK intake-induced inhibition of the K^+ channel should decrease, the negativity of DCT membrane potentials. Thus, we measured the K^+ reversal potential, which serves as an index of the membrane potential, with 140 mM KCl or 125 mM K^+ -gluconate +15 mM KCl (low Cl^-) in the pipette (intracellular solution) and 140 mM Na^+/5 mM K^+ in the bath. Since we did not find a significant difference between two solutions, data were pooled. Fig. 3A is a trace showing that the K^+ reversal potential of the DCT is more negative (red line) in mice on LK diet for 4 days than on a NK diet (black line). In contrast, a HK intake decreases the negativity of the DCT membrane potential (green line). Results are summarized in Fig. 3B showing that LK intake significantly hyperpolarizes the membrane of DCT from −61±2 mV to −75±3 mV (N=8) while HK intake depolarizes the membrane to −48±2 mV (N=8). We have also measured K^+ reversal potential in KS-Kir4.1 KO mice on a NK, LK and HK diets for 4 days. Fig. 3C is a set of traces demonstrating that K^+ reversal potential of the DCT of KS-Kir4.1 KO mice on a NK diet (black line, on a LK diet (red line) and a HK diet (green line). We confirmed that the deletion of Kir4.1 in the DCT significantly depolarized the membrane (−41±2 mV, N=6) [22]. Moreover, neither LK nor HK intake has any significant effect on the membrane potential in the DCT of KS-Kir4.1 KO mice. Fig.3B summarizes the results showing that K^+ reversal potentials in the DCT of KS-Kir4.1 KO mice on a LK or a HK diet are −42±2 mV (N=7) and −39 ±2mV (N=7), respectively.

The negative cell membrane potential is the driving force for Cl^- exit across the basolateral membrane through a 10 pS Cl^- channel, encoded by ClC-Kb, which is the only type of Cl^- channel in the DCT [26;34]. Thus, an alteration of Kir4.1 activity would be expected to affect Cl^- movement across the basolateral membrane, as hyperpolarization should stimulate, and depolarization would diminish, Cl^- exit. Our previous experiments also demonstrated that the deletion of Kir4.1 channel inhibited Cl^- conductance in the DCT [22]. Thus, we next examined the NPPB-sensitive Cl^- conductance in the DCT of WT and KS-Kir4.1 KO mice with whole-cell recording. Fig. 3D summarizes the results showing that LK intake increased NPPB-sensitive Cl^- currents from 1040±65 pA (N=13) to 1445±120 pA (N=6) while HK intake decreased Cl^- currents to 810±50 pA (N=6). However, the deletion of Kir4.1 in the DCT not only decreased Cl^- currents under control conditions but also abolished the effect of dietary K^+ intake on Cl^- channel activity (Control, 170±15 pA; LK, 175±12 pA; HK, 150±10 pA) (Fig.3D, N=6). Thus, deletion of Kir4.1 also inhibited the basolateral Cl^- channel activity and abolished the effect of dietary K^+ intake on Cl^- conductance in the DCT.

Kir4.1 activity is essential for the effect of dietary K^+ intake on NCC

It is well established that dietary K^+ intake plays a key role in the regulation of NCC such that LK intake stimulates while HK intake inhibits NCC activity in the DCT [4;8–10]. We hypothesized that deletion of Kir.4.1 should diminish or abolish the effect of dietary K^+ intake on NCC expression, if the regulation of Kir.4.1 activity is essential for initiating dietary K^+ intake-induced modulation of NCC. Thus, we first examined the role of Kir4.1 in mediating the effect of LK intake on NCC activity. Fig. 4A is a Western blot showing the

effect of LK intake on the expression of tNCC and pNCC (at Thr^{53}) in WT and KS-Kir4.1 KO mice. We confirm the previous report that a LK intake increased the expression of tNCC (160±5% of the control) and pNCC (165±5% of the control) in comparison to NK [4]. However, the deletion of Kir4.1 not only decreased the expression of tNCC (48±4% of WT control) and pNCC (35±5% of WT control) under control conditions but also abolished the effect of LK intake on tNCC and pNCC (Fig. 4C and 4D, N=5). We next examined the effect of HK intake on tNCC and pNCC in WT and KS-Kir4.1 KO mice (Fig.4B). We confirm that a HK intake decreased the expression of tNCC (60±3% of the control) and pNCC (60±3% of the control) in comparison to NK [9]. The parallel change in tNCC and pNCC may be due to the possibility that un-phosphorylated NCC may not be stable and could be degraded [35]. However, the deletion of Kir4.1 also abolished the effect of HK intake on tNCC and pNCC (Fig.4C and 4D, N=5).

We next examined the effect of HCTZ (25 mg/1 kg body weight) on urinary Na^+ excretion using renal clearance method in WT and KS-Kir4.1 KO mice on a NK, HK or LK diet for 4 days. During the experiments the mice were intravenously perfused with isotonic saline for 4 hr (0.2–0.3 ml/1 hr) to maintain hemodynamics. Urine collections started one hr after infusion of 0.3 ml saline and total 6 collections were performed (every 30 minutes). Fig. 5A summarizes results from each individual experiment and Fig. 5B demonstrates the mean value and statistical information. From the inspection of Fig. 5, it is apparent that LK intake significantly decreased basal level of urinary Na^+ excretion (E_{Na}) in WT mice from 0.68±0.04 (NK, n=13) to 0.15±0.01 μEq/min/100g BW (LK, n=8) whereas HK significantly increased E_{Na} to 1.36±0.09 μEq/min/100g BW (HK, n=7). Moreover, the natriuretic effect of HCTZ was significantly larger in the mice on a LK diet for 4 days (from 0.15±0.01 to 2.0 ±0.26 μEq/min/100g BW) than those on a NK diet (from 0.68±0.04 to 1.73±0.15 μEq/min/100g BW) whereas it was smaller in the mice on a HK diet for 4 days (from 1.36±0.09 to 1.97 ±0.13 μEq/min/100g BW). This suggests that LK intake enhances and HK intake decreases NCC function in WT mice. The deletion of Kir4.1 increased the urinary Na^+ excretion under control conditions in comparison to those of corresponding WT (1.02±0.08 μEq/min/100g BW) (n=10). Moreover, the HCTZ-induced natriuretic effect was completely absent in KS-Kir4.1 KO mice (1.17±0.07 μEq/min/100g BW), suggesting that NCC activity was completely inhibited. Furthermore, unlike in WT mice, LK intake not only failed to significantly decrease E_{Na} in KS-Kir4.1 KO mice (0.8±0.07 μEq/min/100g BW) but also abolished the effect of HCTZ on urinary Na^+ excretion (1.15±0.12 μEq/min/100g BW) (n=5). Finally, Fig.5 also demonstrates that HK intake fails to increase E_{Na} (0.93±0.09 μEq/min/100g BW) and that HCTZ had no effect on Na^+ excretion (1.05±0.14 μEq/min/100g BW) in KS-Kir4.1 KO mice (n=6). Thus, Kir4.1 activity is essential for mediating the dietary K^+ intake-induced modulation of NCC function.

Deletion of Kir4.1 impairs the renal ability to preserve K^+

We next examined the effect of HCTZ (25 mg/1 kg body weight) on urinary K^+ excretion (E_K) using renal clearance method in WT and KS-Kir4.1 KO mice on a NK (N=12), HK (N=9) or LK diet (N=7) for 4 days. Fig. 6A shows the results from each individual experiment and Fig. 6B demonstrates the mean value and statistical information. The application of HCTZ modestly but significantly increased renal K^+ excretion in WT mice on

a NK diet (control, 0.64±0.06; HCTZ, 1.19±0.08 μEq/min/100g-BW). This finding is different from the report that acute application of HCTZ did not affect urinary K^+ excretion in c57/Bl6 mice [36]. However, the discrepancy may be caused by different K^+ content in the control diet (0.67% vs 1.0% used in the present study). LK intake significantly suppressed basal level of E_K to 0.05±0.01 μEq/min/100g–BW while HK intake increased basal level of E_K to 1.53±0.14 μEq/min/100g-BW. HCTZ did not significantly change E_K in the mice on a LK (0.11±0.02 μEq/min/100g–BW) or a HK diet (1.66±0.10 μEq/min/100g–BW). The deletion of Kir4.1 caused K^+ wasting under control conditions (NK, E_K =1.02±0.05 μEq/min/100g –BW, N=9) and during K^+ restriction (LK, E_K =0.31±0.04 μEq/min/100g –BW, N=6) (Fig. 6A and 6B). Only increasing dietary K^+ intake prevented K^+ wasting in KS-Kir4.1 KO mice (HK, E_K =1.54±0.18 μEq/min/100g –BW). Furthermore, application of HCTZ had no significant effect on E_K in KS-Kir4.1 KO mice (NK, E_K =1.16±0.07; LK, E_K =0.45±0.06; HK, E_K =1.79±0.16 μEq/min/100g –BW).

The notion that the deletion of Kir4.1 caused K^+ wasting in animals on a NK and LK diets was also supported by metabolic cage study. Fig. 7A, summarizing the results from 5–7 WT and KS-Kir4.1 KO mice, shows that urinary K^+ excretion rate per day in KS-Kir4.1 KO mice was significantly larger (NK, 31±1.6 μmol/g BW; LK, 3.4±0.25 μmol/g BW) than it was in WT mice (NK, 21.7 ±3.3 μmol/g BW; LK, 0.5±0.15 μmol/g BW). The KS-Kir4.1 mice also developed hypochloremic metabolic alkalosis and hypokalemic under control conditions (Plasma K^+ of KS-Kir4.1 KO=2.57±0.10 mM, N=6; WT=3.62 ±0.12 mM, N=6) (Fig.7B). Moreover, the KO mice developed even more severe hypokalemia after 4 days K^+ restriction (plasma K^+ =1.21±0.12 mM, N=7) than did control mice (2.60±0.10 mM, N=6). Although urinary K^+ excretion of KS-Kir4.1 KO mice on a HK diet was not significantly different from that of WT (Fig.7A), KS-Kir4.1 KO mice were still mildly hypokalemic (Plasma K^+=3.28±0.12 mM, N=6) in comparison to WT mice (Plasma K^+=4.02±0.10 mM, N=6) (Fig.7B). This suggests that Kir4.1 activity plays an important role in maintaining K^+ homeostasis.

Discussion

The classic view regarding renal K^+ secretion is focused on connecting tubule and cortical collecting duct in which aldosterone stimulates ENaC-dependent Na^+ absorption thereby enhancing K^+ excretion [37;38]. Recently, a large body of studies has indicated a key role of NCC in mediating the effect of dietary K^+ intake on K^+ excretion by the kidney, such that HK intake inhibits whereas LK intake stimulates NCC activity [4;8–10;39]. The regulation of NCC activity by dietary K^+ intake has important physiological effects on renal K^+ excretion: low NCC activity resulting from HK intake, stimulates K^+ secretion, either by increasing Na^+ delivery or causing tubule remodeling [11], whereas high NCC activity, induced by LK-intake, suppresses K^+ secretion, either by limiting Na^+ delivery, or by inducing tubule remodeling [11], thereby preventing K^+ wasting. The role of NCC in regulating renal K^+ secretion and K^+ homeostasis is also convincingly established by human genetic and clinical studies demonstrating that an abnormal NCC activity is responsible hyperkalemia or hypokalemia. For instance, familial hyperkalemic hypertension (FHHt, also called pseudohypoaldosteronism type II), is caused by high activity of NCC [40–42] whereas hypokalemia in patients with Gitelman syndrome is caused by loss-function

mutations of NCC [3]. Thus, proper regulation of NCC activity and ENaC/ROMK activity in ASDN are essential for maintaining body K^+ homeostasis.

We demonstrated previously that the basolateral Kir4.1 activity is closely related to the NCC activity in the DCT because deletion of Kir4.1 reduced NCC expression and function [22]. In contrast, the basolateral K^+ channel activity in the DCT in NCC null mice (Wang's unpublished observation) or in *Wnk4*^{PHAII} transgenic mice with high NCC was similar to WT mice[43]. Here, we show that Kir4.1 activity plays a key role in mediating the effect of dietary K^+ intake on NCC activity, because the effect of dietary K^+ intake on NCC activity is completely absent in KS-Kir4.1 KO mice. Although dietary K^+ intake also affects Cl^- conductance, Kir4.1 should be a more suitable candidate than ClC-Kb for serving as a K^+ sensor because dietary K^+ intake alters the plasma K^+ rather than Cl^- concentration. However, the Cl^- channel is an important component for K^+-sensor mechanism in the DCT. For instance, HK intake decreases K^+ conductance more than Cl^- conductance thereby changing membrane permeability ration towards to Cl^- equilibrium potential. Conversely, LK diet increases K^+ conductance more than Cl^- conductance thereby shifting membrane potential close to K^+ equilibrium potential.

Two lines of evidence suggest that the LK-induced upregulation of NCC depends on Kir4.1 activation in the DCT. First, LK-intake significantly augments the basolateral K^+ conductance and increases the inside-negativity of DCT membrane potential (hyperpolarization). Since LK intake did not increase the basolateral K^+ conductance in the DCT of KS-Kir4.1 KO mice, this suggests that the activation of Kir4.1 is responsible for LK-intake induced increase in the basolateral K^+ conductance. Second, LK-intake fails to increase the expression and function of NCC in KS-Kir4.1 KO mice. This finding indicates that the activation of basolateral Kir4.1 channel activity is an essential step for LK intake-induced stimulation of NCC function. The activation of the basolateral K^+ channels in the DCT by LK intake may be induced by the stimulation of existing Kir4.1 channels and by the augmentation of Kir4.1 insertion. This notion is supported by single channel patch-clamp experiments showing that LK-intake increases NP_o and the probability of finding the 40 pS K^+ channel, a Kir4.1/Kir5.1 heterotetramer.

While activation of Kir4.1 is required for the effect of LK intake on NCC, HK-induced inhibition of NCC is related to the suppression of the basolateral Kir4.1 in the DCT. This notion is supported by two pieces of evidence: First, HK-intake significantly reduces the basolateral K^+ conductance and decreases the inside-negativity of DCT membrane potential (depolarization). Second, HK intake fails to further decrease NCC activity in KS-Kir4.1 KO mice, suggesting that inhibition of Kir4.1 in the DCT is necessary for the down-regulation of NCC induced by HK intake. Moreover, the finding that HK intake decreases NP_o and the probability of finding channel the basolateral 40 pS K^+ channel in the DCT strongly suggests that HK-intake induced suppression of the basolateral K^+ channels results from inhibition of Kir4.1 activity and insertion.

Thus, we have established a positive correlation between NCC activity and basolateral Kir4.1 activity in the DCT. However, the mechanism by which Kir4.1 activity regulates NCC function is still not completely understood. We speculate that Cl^--sensitive WNK-

SPAK pathway may be a key linker between Kir4.1 and NCC activity. It is possible that LK-induced membrane hyperpolarization in the DCT should increase Cl^- exit across the basolateral membrane thereby decreasing the intracellular Cl^- level while HK intake has an opposite effect. Our finding that LK-intake increases while HK-intake decreases NPPB-sensitive Cl^- conductance in the DCT also suggests that Cl^- exit in the DCT should be enhanced during LK intake but decreased during HK intake. Taking together, the present study strongly indicates that the cross-talk between basolateral Kir4.1 and apical NCC plays a role in maintaining body K^+ homeostasis since KS-Kir4.1 KO mice are unable to maintain plasma K^+ concentrations in a normal range even during increasing dietary K^+ intake. However, Cl^- measurement would be required to finally prove the hypothesis.

Fig. 8 is a scheme illustrating the role of Kir4.1 in mediating the effect of dietary K^+ intake on NCC function. LK intake activates the basolateral K^+ channel activity and increases the K^+ permeability in the DCT. Since LK intake also decreases plasma K^+ concentration, an increased K^+ permeability and a decreased plasma K^+ concentration hyperpolarize the membrane of the DCT. Because membrane potential provides the driving force for Cl^- exit across the basolateral membrane, a LK intake induced hyperpolarization is expected to favor Cl^- exit across the basolateral membrane and to decrease intracellular Cl^- concentration thereby activating Cl^--sensitive WNK-SPAK signaling which stimulates NCC. In contrast, a HK intake should have opposite effect on DCT membrane potential and WNK-SPAK pathway thereby inhibiting NCC. We conclude that the presence of Kir4.1 is essential for mediating the dietary K^+ intake on NCC such that an increase in the basolateral K^+ conductance is required for LK-induced activation of NCC whereas a decrease is essential for HK -induced inhibition of NCC.

Materials and Methods

Animals

All animal studies were approved by the Institutional Animal Care and Use Committees of Oregon Health & Science University and New York Medical College. Mice expressing Pax8-rtTA and tet-on LC-1 transgene were crossed with *Kcnj10*$^{flox/flox}$ mice to generate inducible kidney-specific *Kcnj10*$^{-/-}$ (KS-*Kcnj10*$^{-/-}$ or KS-Kir4.1 KO) (detailed information is provided in supplement). *Kcnj10* deletion was carried out in 8–10 week-old male and/or female mice homozygous for floxed *Kcnj10* gene and hemizygous for Pax8-rtTA/LC-1 transgene by providing doxycycline (5mg/ml, 5% sucrose) in the drinking water for 2 weeks. This was followed by at least 2 additional weeks without doxycycline treatment, before performing experiments and mice were kept under a 12-h light and dark cycle, and were fed with normal rodent diet and plain drinking water. Littermate mice of the same age and genetic background drinking 5% sucrose were used as controls (*Kcnj10*$^{flox/flox}$ or WT). After two weeks on the control diet (NK), the mice are then fed with NK, LK (<0.001%) or HK (5%) for additional 4 days before experiments. The method of mouse genotyping and DCT preparation has been described previously and is included in supplement [22].

Patch-clamp experiment

A Narishige electrode puller (Narishige, Japan) was used to manufacture the patch-clamp pipettes from Borosilicate glass (1.7-mm OD). The resistance of pipette was 5 MΩ (for single channel recording) or 2 MΩ (for whole cell-recording) when it was filled with solution contained (in mM) 140 KCl, 1.8 $MgCl_2$ and 10 HEPES (titrated with KOH to pH 7.4). For the measurement of K^+ reversal potential we used either high Cl^- pipette solution (140 mM KCl) or low Cl^- pipette solution containing (in mM) 125 K^+-gluconate, 15 KCl, 2 MgATP, 1 EGTA and 10 HEPES (titrated with KOH to PH=7.4). For single channel recording, the bath solution contained (in mM) 140 NaCl, 5 KCl, 2 $MgCl_2$, 1.8 $CaCl_2$, and 10 HEPES (pH=7.4) while the pipette was filled with 140 mM KCl solution. The detail for the single channel and whole-cell recording has been described previously and also in supplement [22].

Immunoblotting

The method for Western blot has been described previously and also in supplement[22]. Anti-NCC and anti-pNCC at threonine-53 antibodies (both 1:6000, developed in laboratory of DHE) were used for Western blot (see supplement). An Odyssey infrared imaging system (LI-COR) was used to capture the images at a wave-length of 680 or 800 nM.

Procedures for renal clearance and urine and blood analysis

The detailed method for renal clearance is described in supplemental material. After surgery mice were perfused with saline intravenously for 4 hr (0.2–0.3 ml/1 hr and total 0.8–1.2 ml) to maintain hemodynamics. Urine collections started one hr after infusion of 0.3 ml saline and total 6 collections (every 30 minutes) were performed (2 for controls and 4 for experiments). All mice were placed in individual metabolic cages and fed with normal K^+ diet for 2 days, and then mice were divided in three groups: NK, LK or HK diet. Mice were studied after 4 days on the assigned diet. Urine was collected for 24-h under saturated mineral oil and the urine potassium concentrations were measured using a dual-channel flame photometer with internal lithium standard (Cole-Parmer Instrument, Vernon Hills, IL). Blood from the same mice was obtained via cardiac puncture and analyzed using an i-STAT® analyzer (Abbott Pointe, Princeton, NJ). Plasma K^+ concentration was measured using the flame photometer as mentioned above.

Statistical analysis.

Data were analyzed using student *t* test for comparisons between two groups or one-way ANOVA for comparisons between more than 2 groups. *P*-values <0.05 were considered statistically significant. Data are presented as the mean ± SEM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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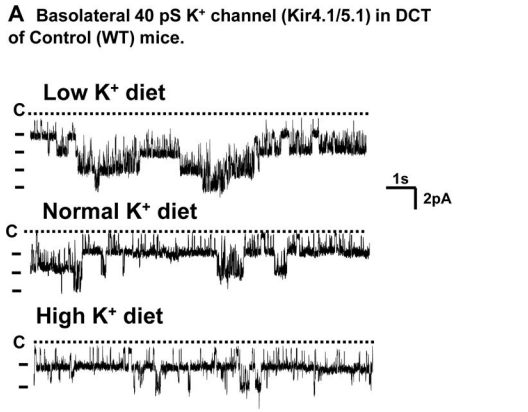

B Effect of K^+ diet on the 40 pS K^+ channel(Kir4.1/5.1) activity

K^+ diet	Numbers of total patches	Numbers of patches with Kir4.1	Mean NP_o /patch	Mean P_o
Normal K^+	52	34(65%)	1.34±0.15	0.35±0.03
Low K^+	20	18(90%)*	1.69±0.09*	0.45±0.04*
High K^+	17	8 (47%)*	0.98±0.06*	0.31±0.02

"*" indicates that the difference is significant in comparison to the control.

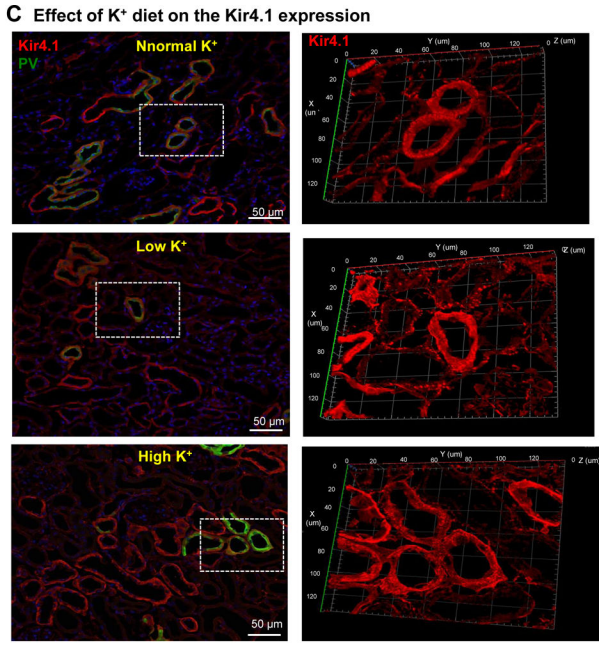

Fig. 1. LK intake stimulates and HK inhibits basolateral K^+ channels in the DCT.

(A) A single channel recording shows the basolateral 40 pS K^+ channel activity in the DCT of control mice (considered as WT) on a low K^+ (LK), normal K^+ (NK) or a high K^+ (HK) diet for 4 days, respectively. (B) A table showing the probability of finding the 40 pS K^+ channel activity, mean NP_o and P_o in the DCT of WT mice on a NK, LK and HK, respectively. "*" indicates significant difference. For single channel recording, the DCT was bathed in a solution containing (mM) 140 NaCl, 5 KCl 2 $MgCl_2$, 1.8 mM $CaCl_2$ and 10 HEPES (pH=7.4) and the pipette solution contains 140 KCl, 2 $MgCl_2$, 1 EGTA, and 5 HEPES. (C) Immunostaining showing the expression of Kir4.1 in the kidney of mice on NK, LK and HK diets, respectively.

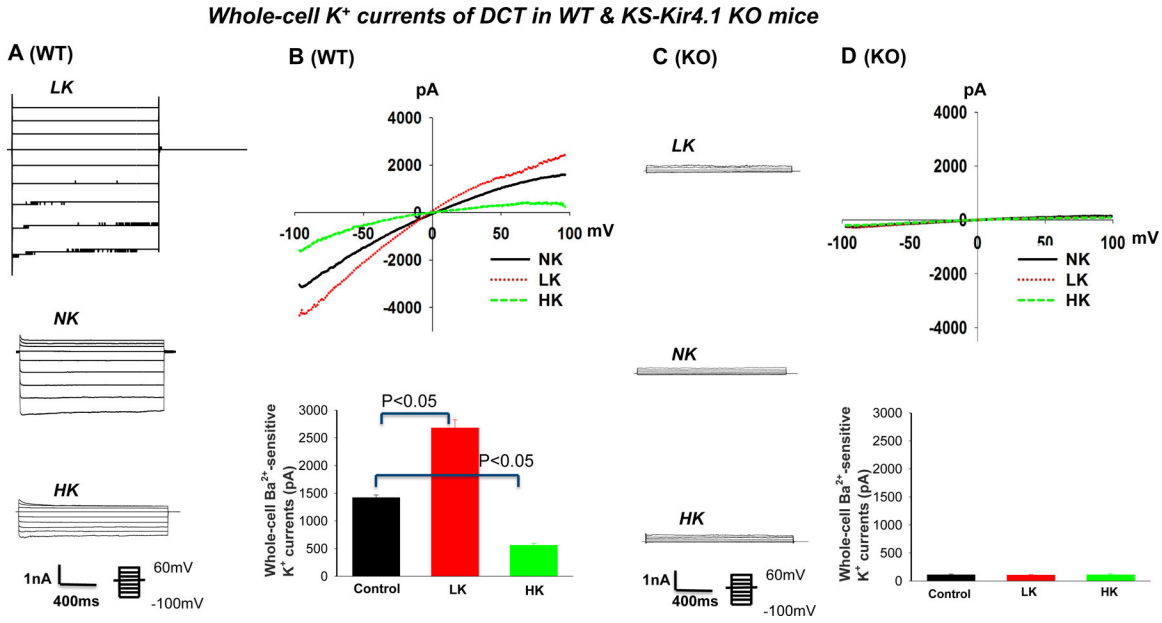

Fig. 2. LK increases and HK decreases Ba^{2+}-sensitive whole-cell K^+ currents.

(A) A set of traces shows Ba^{2+} -sensitive K^+ currents measured with step protocol from −100 to 60 mV in the DCT of WT mice on a LK, NK and HK, respectively. (B) A set of recording shows Ba^{2+} -sensitive K^+ currents measured with RAMP protocol from −100 to 100 mV in the DCT of WT mice on a NK (black), LK (red) or HK diet (green). A bar graph summarizes the values measured at −60 mV with whole-cell recording. For the whole-cell recording, a symmetric 140 mM KCl solution as described in Fig. 1 was used for the bath and the pipette. (C) A set of traces shows Ba^{2+} -sensitive K^+ currents measured with step protocol from −100 to 60 mV in the DCT of KS-Kir4.1 KO mice on a LK, NK and HK, respectively. (D) Whole-cell recording shows Ba^{2+} -sensitive K^+ currents measured with RAMP protocol from −100 to 100 mV in the DCT of KS-Kir4.1 KO mice on a NK (black), LK (red) or HK diet (green). A bar graph summarizes the values measured at −60 mV (bottom panel).

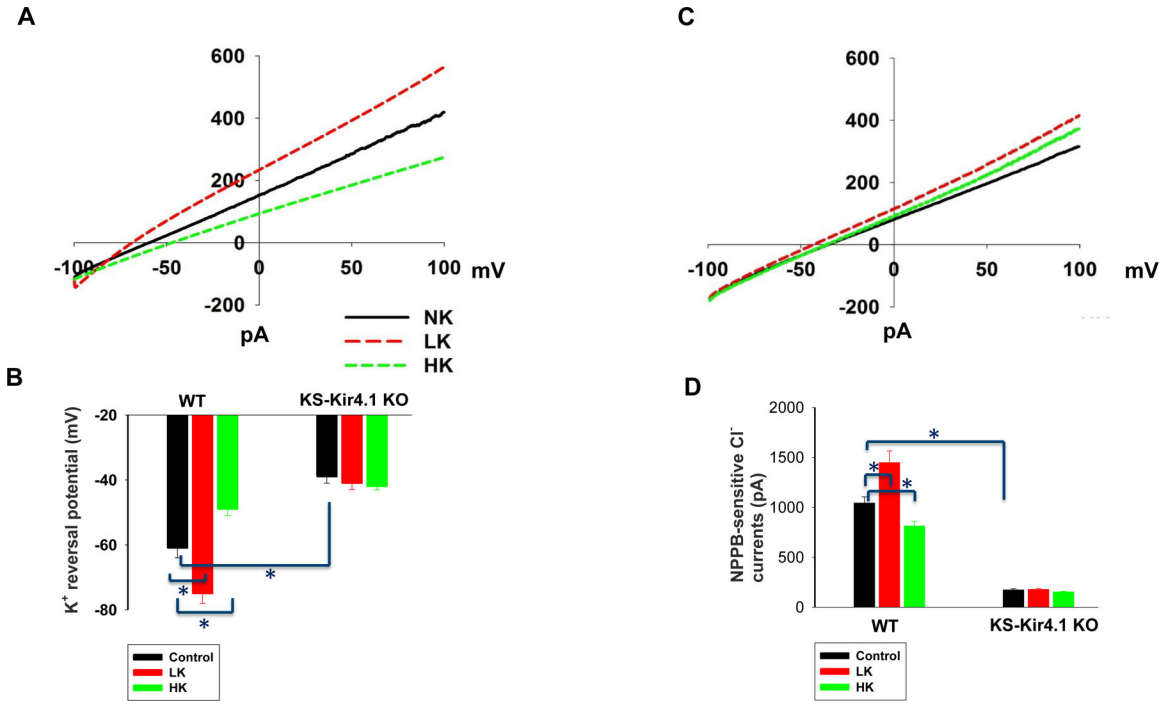

Fig. 3. LK intake hyperpolarizes and HK intake depolarizes the membrane of the DCT in WT but not in KS-Kir4.1 KO mice.

(A) A perforated whole-cell recording showing K^+ reversal potential in the DCT cells of WT mice on a NK (black), a LK (Red) or a HK diet (green) for 4 days. (B) A bar graph summarizes the results of six similar experiments performed in WT and KS-Kir4.1 KO mice, respectively. (C) A recording showing K^+ reversal potential in the DCT cells of KS-Kir4.1 KO mice on a NK (black), a LK (Red) or a HK diet (green) for 4 days. For the measurement of K^+ reversal potential, the bath solution contains 140 mM NaCl and 5 mM KCl while the pipette solution has 140 mM KCl. (D) Bar graph summarizes the results of experiments in which NPPB (10 μM)–sensitive Cl^- currents in the DCT are measured at −60 mV with the whole-cell recording in the WT mice or in KS-Kir4.1 KO mice on a NK (black), a LK (Red) or a HK diet (green) for 4 days. The measurements were carried out with whole-cell recording with symmetrical 140 mM KCl in the bath and pipette.

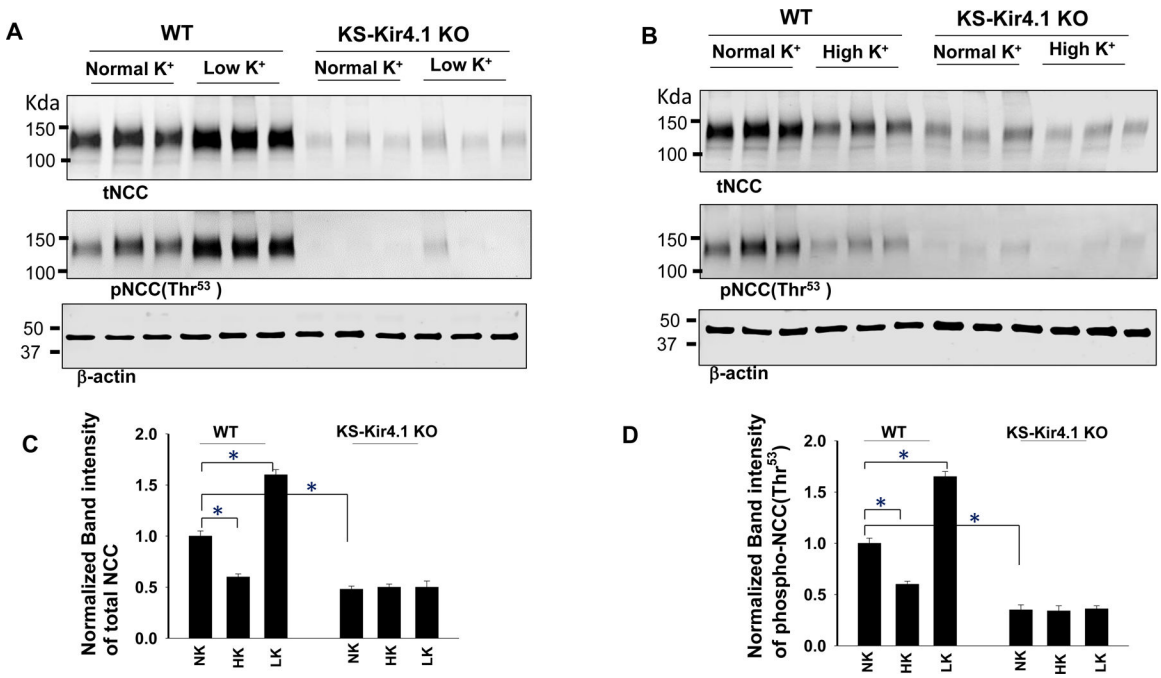

Fig. 4. Dietary K^+ intake regulates the expression of tNCC and pNCC in WT but not in KS-Kir4.1 KO mice.

Immunoblot showing the expression of total NCC (tNCC) and phosphor-NCC (pNCC) in WT and KS-KS-Kir4.1 KO mice on a NK or a LK diet for 4 days (A) and on a NK or a HK diet for 4 days (B). A bar graph summarizes the normalized band intensity of above experiments for tNCC (C) and pNCC (D), respectively.

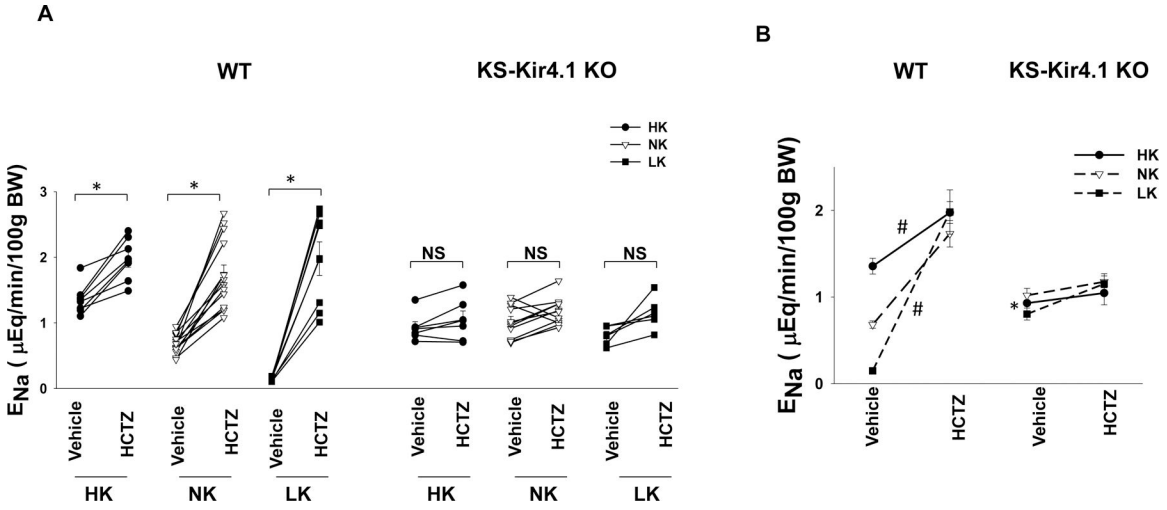

Fig. 5. Deletion of Kir4.1 induces Na^+ wasting and abolishes the effect of dietary K^+ intake on NCC function.

(A) A line graph shows the results of each experiment in which the effect of single dose HCTZ (25 mg/1 kg body weight) on urinary Na^+ excretion (E_{Na}) within 120 min was measured with renal clearance method in WT or KS-Kir4.1 KO mice on a NK (triangle), a HK (circle) and a LK (square) diet, respectively. "*" indicates the significant difference ($P<0.05$) between vehicle and HCTZ treatment groups. (B) A line graph shows the mean value and statistical information for all above experiments. "#" indicates the significant difference of HCTZ natriuretic effect between HK or LK groups and NK group ($P<0.05$). "*" indicate that the basal level E_{Na} between WT and KS-Kir4.1 KO mice on NK is significantly different.

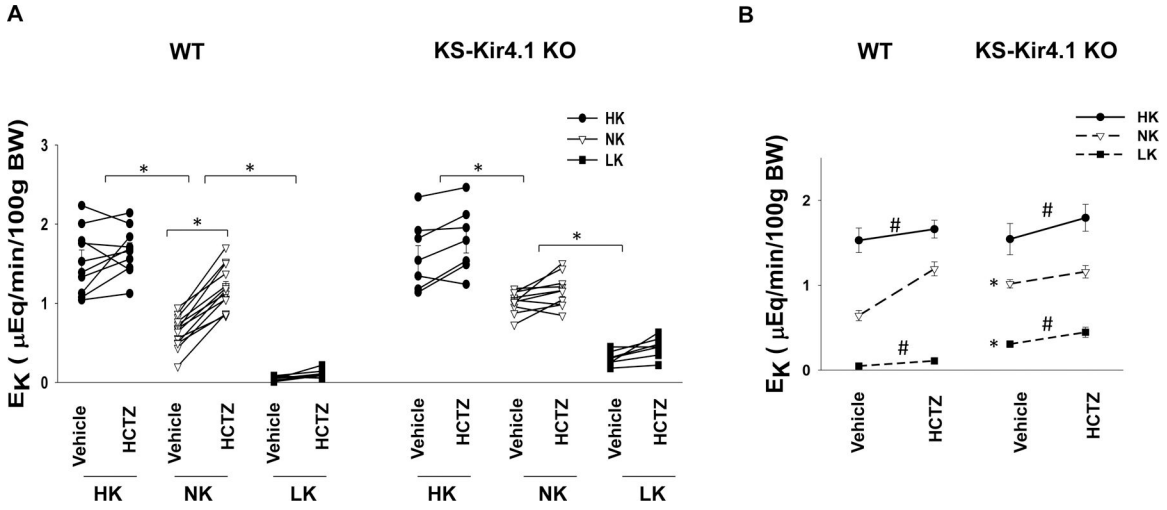

Fig. 6. Ks-Kir4.1 mice are K^+ wasting under control conditions and during K^+ restriction.

(A) A line graph shows the results of each experiment in which the effect of single dose HCTZ on urinary K^+ excretion (E_K) within 120 min was measured with renal clearance method in WT or KS-Kir4.1 KO mice on a NK (triangle), a HK (circle) and a LK (square) diet, respectively. "*" indicates the significant difference ($P<0.05$) between vehicle and HCTZ or between NK group and HK/LK groups. (B) A line graph shows the mean value and statistical information for all above experiments. "#" indicates a significant difference between HK or LK group and NK group. "*" indicates that basal level E_K of KS-Kir4.1 KO mice is significantly higher than the corresponding value of WT mice.

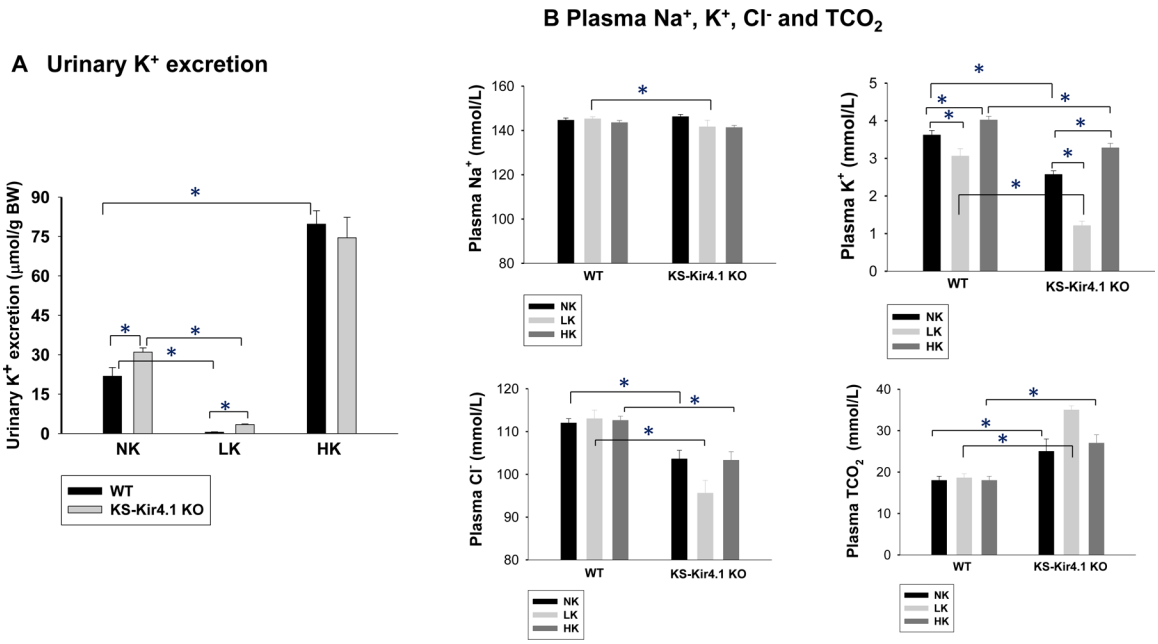

Fig. 7. KS-Kir4.1 mice are hypokalemic and metabolic alkalosis.

(A) 24 hr urinary K^+ excretion measured with metabolic cage in WT and KS-Kir4.1 KO mice on different K^+ diets. (B) Plasma Na^+, K^+, Cl^- and total CO_2 in WT and KS-Kir4.1 KO mice on different K^+ diets. "*" indicates that the difference between groups is significant ($P<0.05$).

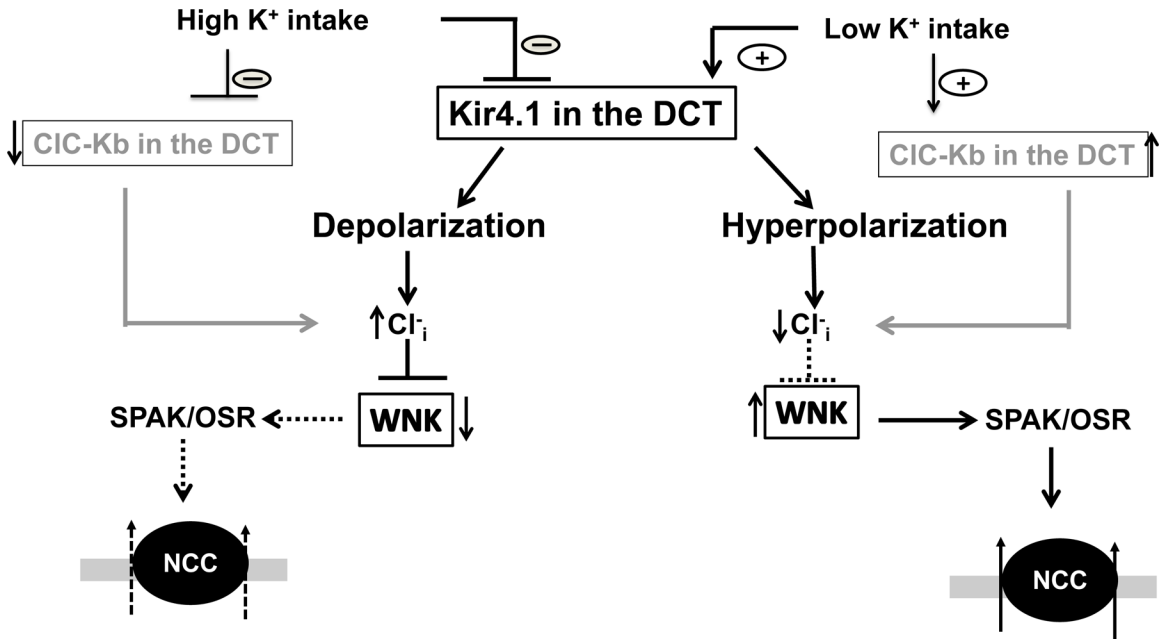

Fig. 8.

A cell scheme illustrating the role of Kir4.1 in mediating the effect of dietary K^+ intake on NCC activity. Solid and dotted lines represent an enhanced or a diminished pathway. Cl^-_i means the intracellular Cl^- level.