Potassium intake modulates the thiazide-sensitive sodium-chloride cotransporter (NCC) activity via the Kir4.1 potassium channel

Ming-Xiao Wang[1,2,4], Catherina A. Cuevas[3,4], Xiao-Tong Su[2,4], Peng Wu[2], Zhong-Xiuzi Gao[2], Dao-Hong Lin[2], James A. McCormick[3], Chao-Ling Yang[2], Wen-Hui Wang[2,4] and David H. Ellison[3,4]

[1]*Department of Physiology, Zunyi Medical College, Zunyi, China;* [2]*Department of Pharmacology, New York Medical College, Valhalla, New York, USA; and* [3]*Department of Medicine, Oregon Health & Science University, Portland, Oregon, USA*

Kir4.1 in the distal convoluted tubule plays a key role in sensing plasma potassium and in modulating the thiazide-sensitive sodium-chloride cotransporter (NCC). Here we tested whether dietary potassium intake modulates Kir4.1 and whether this is essential for mediating the effect of potassium diet on NCC. High potassium intake inhibited the basolateral 40 pS potassium channel (a Kir4.1/5.1 heterotetramer) in the distal convoluted tubule, decreased basolateral potassium conductance, and depolarized the distal convoluted tubule membrane in *Kcnj10*flox/flox mice, herein referred to as control mice. In contrast, low potassium intake activated Kir4.1, increased potassium currents, and hyperpolarized the distal convoluted tubule membrane. These effects of dietary potassium intake on the basolateral potassium conductance and membrane potential in the distal convoluted tubule were completely absent in inducible kidney-specific Kir4.1 knockout mice. Furthermore, high potassium intake decreased, whereas low potassium intake increased the abundance of NCC expression only in the control but not in kidney-specific Kir4.1 knockout mice. Renal clearance studies demonstrated that low potassium augmented, while high potassium diminished, hydrochlorothiazide-induced natriuresis in control mice. Disruption of Kir4.1 significantly increased basal urinary sodium excretion but it abolished the natriuretic effect of hydrochlorothiazide. Finally, hypokalemia and metabolic alkalosis in kidney-specific Kir4.1 knockout mice were exacerbated by potassium restriction and only partially corrected by a high-potassium diet. Thus, Kir4.1 plays an essential role in mediating the effect of dietary potassium intake on NCC activity and potassium homeostasis.

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Correspondence: *Wen-Hui Wang, Department of Pharmacology, New York Medical College, Valhalla, New York 10595, USA. E-mail: wenhui_wang@nymc.edu. and David. H. Ellison, Oregon Health and Science Translational Research Institute, Oregon Health &Science University, Portland, Oregon 97239, USA. E-mail: ellisond@ohsu.edu*

[4]These authors contributed equally to this work.

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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 the 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] Because 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-rick kinase (SPAK) activity,[23] leading to the inhibition of NCC, because WNK and SPAK are required for 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.

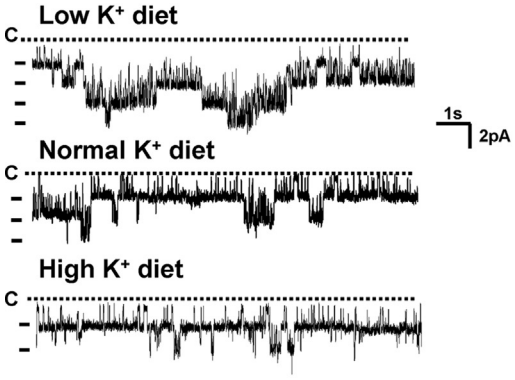
RESULTS

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 controls and referred to as "wild-type" (WT) mice. The mice were kept on a normal K^+ (1% K), LK (<0.001%), or HK (5%) diet for 4 days. We detected an inwardly-rectifying 40 pS K^+ channel in the basolateral membrane of the DCT. Figure 1a demonstrates that 40 pS K^+ channel activity defined by NP_o (a product of Channel Number and open probability) in the mice on an LK diet was higher than that of mice 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 diets (Figure 1a). Figure 1b summarizes the patch-clamp experimental results: the probability of finding 40 pS K^+ channels in the DCT was 65% (34 patches from 52 total experiments) in the mice on an NK diet, whereas it was 90% (18 patches from 20 total experiments) and 47% (8 patches from 17 total experiments) in animals on LK and HK diets, respectively. Moreover, the channel open probability (P_o) in the mice on an LK diet (0.45 ± 0.04) was slightly but significantly higher than that of mice on an NK diet (0.35 ± 0.03) or an HK diet (0.31 ± 0.02). Figure 1c depicts immunostaining showing the expression of Kir4.1 (no obvious difference) in the kidney of the mice on NK, LK, and HK diets, 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+}-

a Basolateral 40 pS K⁺ channel (Kir4.1/5.1) in DCT of control (WT) mice.



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.

c Effect of K⁺ diet on the Kir4.1 expression

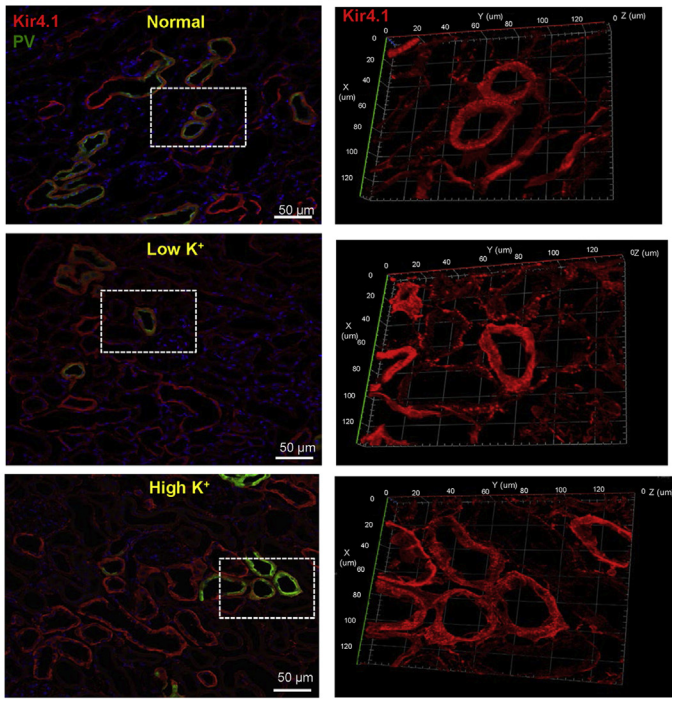


Figure 1 | Low K^+ (LK) intake stimulates and high K^+ (HK) inhibits basolateral K^+ channels in the distal convoluted tubule (DCT). (**a**) A single-channel recording shows the basolateral 40 pS K^+ channel activity in the DCT of control (wild-type [WT]) mice on an LK, normal K^+ (NK), or HK diet for 4 days, respectively. (**b**) Probability of finding 40 pS K^+ channel activity, mean NP_o, and P_o in the DCT of WT mice on NK, LK, and HK diets, respectively. Asterisk 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 $CaCl_2$, 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 (red) and parvalbumin (green) in DCT1 from kidneys of WT mice fed a normal (top), low (middle), or high K^+ diet (low panel) for 4 days. Three-dimensional reconstruction of z-stacks at 63x magnification shows Kir4.1 images at corresponding right panels. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.