REVIEW

Kir4.1/Kir5.1 in the DCT plays a role in the regulation of renal K⁺ excretion

Xiao-Tong Su, David H. Ellison, and Wen-Hui Wang

INTRODUCTION

Potassium (K⁺) is critical for maintaining cellular function. Normal K⁺ balance is maintained over the short term through the uptake by cells, comprising a cellular storage reservoir (including muscle, liver, and red blood cells). Longer term balance, however, is dependent on excretion via the urine, during both potassium loading and depletion (13). The classic view of K⁺ excretion is focused on the endothelial sodium (Na⁺) channel (ENaC) and secretory potassium channels, including renal outer medullary K⁺ channel (ROMK) and calcium-dependent big-conductance potassium channels, in the aldosterone-sensitive distal nephron (ASDN), where Na⁺ entry stimulates the basolateral potassium channel (a Kir4.1/Kir5.1 heterotrimer) in the DCT. The alteration of basolateral potassium conductance is essential for the effect of dietary K⁺ intake on NCC because deletion of Kir4.1 in the DCT abolished the effect of dietary K⁺ intake on NCC. Since potassium intake-mediated regulation of NCC plays a key role in regulating renal K⁺ excretion and potassium homeostasis, the deletion of Kir4.1 caused severe hypokalemia and metabolic alkalosis under control conditions and even during increased dietary K⁺ intake. Finally, recent studies have suggested that the angiotensin II type 2 receptor (AT2R) and bradykinin-B2 receptor (BK2R) are involved in mediating the effect of high dietary K⁺ intake on Kir4.1/Kir5.1 in the DCT.

distal convoluted tubule; K⁺ excretion; NCC; NKCC2

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Kir4.1 also interacts with Kir5.1 (encoded by Kcnj16) to form a functional 40-pS heterotetrameric potassium channel under physiological conditions (12, 17). While the role of Kir4.1 in the heterotetramer is to provide potassium permeability (44), Kir5.1 plays a role in regulating Kir4.1 function. It has been shown that the pH sensitivity of Kir4.1/Kir5.1 heterotetramers is greater than that of Kir4.1 homotetramers (31). Recently, Wang et al. (36) have demonstrated that Kir5.1 is associated with E3 ubiquitin ligase, Nedd4-2, bound to its C terminus thereby regulating the ubiquitination of Kir4.1. The notion that Kir5.1 may regulate the ubiquitination of Kir4.1 is supported by the finding that the deletion of Kir5.1 or Nedd4-2 significantly increased Kir4.1 expression. These results suggest that Kir5.1 is a regulatory subunit for Kir4.1/Kir5.1 heterotetramers.

Kir4.1/Kir5.1 REGULATES NCC

Loss-of-function mutations of Kir4.1 in the kidney caused tubulopathy characterized by mild salt wasting, hypokalemic metabolic alkalosis, hypomagnesemia, hypocalciuria, and elevated levels of renin and aldosterone (2). Thus the phenotype of Kir4.1 loss-of-function mutations resembles Gitelman syndrome, an inherited tubule disease resulting from mutations in the SLC12A3 gene encoding NCC (24). The notion that Kir4.1/Kir5.1 regulates the membrane transport in the DCT was recapitulated in both global Kcnj10−/− and kidney-specific (KS)-Kcnj10−/− mice. It was observed that the deletion of Kir4.1 reduced NCC expression in both global kcnj10−/− and KS-Kcnj10−/− mice (4, 44). Renal clearance studies performed in KS-kcnj10−/− mice demonstrated that the deletion of Kir4.1 abolished the thiazide-induced natriuretic effect. Moreover, metabolic cage studies showed that the mice had severe K+ wasting and hypokalemic metabolic alkalosis. Thus the mouse model recapitulates the renal phenotype of EAST/SeSAME syndrome. Because Kir4.1 deletion strikingly depolarizes the DCT membrane, the inhibition of NCC in KS-kcnj10−/− mice may be related to raising the intracellular chloride concentration ([Cl−]). Indeed, deletion of Kir4.1 also inhibited basolateral chloride channels in the DCT (4). It is well established that high [Cl−] inhibits the activity of the with-no-lysine kinase (WNK) by regulating autoinhibition and autophosphorylation (1, 18, 28, 38). Since WNK is required to activate ste20-proline-alanine-rich kinase (SPAK) and oxidative-sensitive response kinase 1 (OSR1), which stimulate NCC activity (7, 8, 21, 40), high [Cl−] is also expected to inhibit SPAK/OSR1, thereby decreasing NCC phosphorylation (1). A decrease in NCC phosphorylation enhances the internalization and ubiquitination of NCC (22, 39). Thus Kir4.1/Kir5.1 activity in the DCT plays a key role in regulating NCC activity. Moreover, Kir4.1/Kir5.1 may also play a role in the regulation of Na-K-2Cl cotransporter (NKCC2) in the TAL since the deletion of Kir4.1 also decreased NKCC2 activity (4). However, because potassium channels other than Kir4.1/Kir5.1 are also expressed in the basolateral membrane of the cTAL (16, 43), NKCC2 in KS-kcnj10−/− mice was reduced only modestly.

Kir4.1/Kir5.1 IS ESSENTIAL FOR THE EFFECT OF DIETARY K+ INTAKE ON NCC

Regulation of NCC plays an essential role in modulating K+ excretion (5). For instance, high K−induced inhibition of NCC should augment Na+ delivery to the CNT and CCD, thereby stimulating K+ secretion. It has been reported that NCC dephosphorylation (an indication of inhibition) occurs within minutes in response to an acute rise in plasma K+ concentrations after a meal (25). Since high K+ intake should also stimulate aldosterone secretion and activate apical ENaCs, increased Na+ delivery and activated ENaCs should enhance renal K+ excretion. Conversely, low dietary K+ intake should have an opposite effect on renal K+ excretion by increasing NCC activity in the DCT (33, 35). Therefore, dietary K+ intake-induced modulation of NCC activity plays a key role in controlling K+ excretion in the ASDN and in maintaining potassium homeostasis. Terker et al. (28) have elegantly demonstrated the relationship between phosphorylated NCC (an indicator of NCC activity) and plasma K+ concentrations such that decreasing plasma K+ increased, while increased plasma K+ decreased, NCC activity. Furthermore, they have suggested that the cell membrane potential of the DCT may be responsible for the effect of dietary K+ intake on NCC (29). Indeed, we have observed that high K+ intake depolarized, while low K+ intake hyperpolarized, DCT membranes in the mouse kidney (35).

The negative membrane potential in the DCT is mainly determined by basolateral potassium conductance (permeability) and plasma K+ concentration. Although high K+ intake tends to increase the plasma K+, the increase in plasma K+ concentration is modest under physiological conditions. Thus it is unlikely that change in plasma K+ concentrations alone would be mainly responsible for the depolarization of the DCT basolateral membrane observed in vivo. Therefore, it is conceivable that the change in basolateral K+ permeability is mainly responsible for dietary K+ intake-induced alteration of DCT membrane potential. This notion is confirmed by the finding that high K+ intake significantly inhibited, while low K+ intake increased, basolateral potassium permeability (35). The changes in basolateral K+ conductance and DCT membrane potential induced by dietary K+ intake completely depend on Kir4.1/Kir5.1 since the deletion of Kir4.1 not only abolished basolateral potassium conductance but also the effect of dietary K+ intake on membrane potential. In addition, dietary K+ intake failed to regulate NCC expression and activity in KS-Kcnj10−/− mice, suggesting that Kir4.1/Kir5.1 is essential for the effect of dietary K+ intake on NCC. It is possible that high K+ intake-induced inhibition of Kir4.1/Kir5.1 leads to inhibiting the chloride-sensitive WNK pathway, thereby inhibiting NCC.

REGULATION OF Kir4.1/Kir5.1

Dietary K+ intake plays an important role in the regulation of Kir4.1/Kir5.1 not only in the DCT but also in the CCD. However, unlike in the DCT, high K+ intake has been shown to stimulate Kir4.1/Kir5.1 in the CCD (30). One possible reason for this difference may be that aldosterone is more active in the CCD, where it plays a dominant role in regulating ENaC; thus the rise in aldosterone that occurs during dietary potassium loading may activate Kir4.1/Kir5.1 in the CCD but not in the DCT. The different responses of Kir4.1/Kir5.1 to high K+ intake between DCT and CCD are physiologically significant since high K+−induced inhibition of Kir4.1/Kir5.1 in the DCT is essential for the inhibition of NCC, whereas high
K⁺-induced stimulation of Kir4.1/Kir5.1 in the CCD should increase the driving force for Na⁺ absorption, thereby stimulating K⁺ excretion. In addition, it has been reported Kir4.1/Kir5.1 in the CCD is stimulated by insulin/IGF-1 and inhibited by dopamine (42).

Although the effect of dietary K⁺ intake on Kir4.1/Kir5.1 in the DCT is well established (35), the mechanism by which dietary K⁺ intake alters Kir4.1/Kir5.1 activity is not fully understood. Our recent studies have suggested the role of AT2R and BK2R in mediating the effect of high K⁺ intake on basolateral Kir4.1/Kir5.1 in the DCT. We observed that AT2R inhibition significantly increased basolateral potassium conductance in the DCT, hyperpolarized the DCT membrane, and augmented NCC activity (37). Because the stimulatory effect of AT2R inhibition on NCC was absent in KS-kcnj10⁻/⁻ mice, it suggests the possibility that AT2R inhibition-induced activation of Kir4.1/Kir5.1 was required for the upregulation of NCC. Since high K⁺ intake has been reported to increase the expression of AT2R, it raises the possibility that AT2R may be involved in mediating the effect of high K⁺ intake on Kir4.1/Kir5.1 in the DCT. This notion was also supported by the finding that application of an AT2R agonist inhibited Kir4.1/Kir5.1 in the DCT. However, inhibition of AT2R may indirectly enhance AT1R activity, which has been reported to directly activate NCC (3, 6, 23, 32, 46). Further investigation is required to explore the role of angiotensin II in regulating Kir4.1/Kir5.1 and NCC.

Three lines of evidence have suggested that BK2R is possibly involved in mediating the effect of high K⁺ intake on Kir4.1/Kir5.1 and NCC (45). First, bradykinin inhibited Kir4.1/Kir5.1 in the DCT and depolarized the DCT membrane, an effect blocked by the specific BK2R antagonist. Second, immunohistochemistry has shown that BK2R is highly expressed in the lateral and apical membrane of the DCT. Also, a previous study demonstrated that kininogen was detected in the early distal tubule and neighboring CCD (19). Third, bradykinin infusion inhibited NCC activity and increased Na⁺ excretion, an effect which was absent in KS-kcnj10⁻/⁻ mice. High K⁺ intake has been reported to increase renal BK2R expression and renal tissue kallikrein expression levels (11, 34). Thus we speculate that BK2R may be involved in mediating the effect of high K⁺ intake on basolateral Kir4.1/Kir5.1 in the DCT. Accordingly, the stimulation of BK2R in the DCT is expected to inhibit NCC, thereby increasing Na⁺ delivery to the collecting duct and facilitating K⁺ excretion. In this regard, stimulation of BK2R has been shown to inhibit ENaC (41), a mechanism which should work in concert with BK2R-induced inhibition of NCC to stimulate K⁺ excretion and to prevent excessive Na⁺ absorption in the CCD. Thus activation of both AT2R and BK2R by high dietary K⁺ intake should play a key role in the regulation of basolateral potassium conductance in the DCT and NCC.

Fig. 1. Schematic model illustrating the role of Kir4.1/Kir5.1 in mediating the effect of dietary K⁺ intake on NaCl cotransporter (NCC) activity and how ion transport is altered in the response to dietary K⁺ intake to mediate sodium and potassium homeostasis. High K⁺ intake (green arrow or symbol) decreases Kir4.1/Kir5.1 channel activity, which subsequently inhibits the WNK-SPAK pathway and NCC activity in the early distal convoluted tubule (DCT1) cells. The sodium reabsorption and potassium excretion rates increase along the downstream segments. AT2R, angiotensin II type 2 receptor; BK2R, bradykinin type 2 receptor; MR, mineralocorticoid receptor. The dotted line indicates diminished effects.
PHYSIOLOGICAL PERSPECTIVE

High K⁺ intake is known to stimulate aldosterone synthesis and increases its plasma level. However, high K⁺ intake causes natriuresis and increases renal K⁺ excretion simultaneously despite the high aldosterone level. The finding that high K⁺ intake inhibits NCC by suppressing Kir4.1/Kir5.1 in the DCT explains the puzzle of the “aldosterone paradox.” Figure 1 is a cell scheme illustrating the role of Kir4.1/Kir5.1 in the regulation of renal K⁺ excretion in ASDN during increased dietary K⁺ intake. High K⁺ intake inhibits basolateral Kir4.1/Kir5.1 in the DCT by stimulating AT2R and BK2R, thereby inhibiting NCC and increasing Na⁺ and volume delivery to the latter part of the ASDN. Because aldosterone stimulates ENaC in the ASDN, NCC inhibition promotes ENaC-dependent and flow-stimulated K⁺ secretion in the ASDN without increasing net Na⁺ absorption. Thus high K⁺ intake-induced inhibition of Kir4.1/Kir5.1 serves as a switch to turn off Na⁺ absorption in the DCT, and this mechanism is essential for facilitating renal K⁺ excretion without causing volume expansion.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

X.-T.S. and W.-H.W. conceived and designed research; X.-T.S. performed experiments; X.-T.S., D.H.E., and W.-H.W. analyzed data; X.-T.S., D.H.E., and W.-H.W. interpreted results of experiments; X.-T.S. prepared figures; X.-T.S. drafted manuscript; X.-T.S., D.H.E., and W.-H.W. edited and revised manuscript; X.-T.S., D.H.E., and W.-H.W. approved final version of manuscript.

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