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Basolateral Kir4.1 activity in the distal convoluted tubule regulates K secretion by determining NCC activity

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Abstract

Purpose of Review—Renal potassium (K) secretion plays a key role in maintaining K homeostasis. The classic mechanism of renal K secretion is focused on the connecting tubule (CNT) and cortical collecting duct (CCD) in which K is uptaken by basolateral Na-K-ATPase and it is secreted into lumen by apical ROMK (Kir1.1) and Ca^{2+} –activated big conductance K channel (BK). Recently, genetic studies and animal models have indicated that inwardly rectifying K channel 4.1 (Kir4.1 or *Kcnj10*) in distal convoluted tubule (DCT) may play a role in the regulation of K secretion in the aldosterone-sensitive distal nephron (ASDN) by targeting NaCl cotransporter (NCC). This review summarizes recent progresses regarding the role of Kir4.1 in the regulation of NCC and K secretion.

Recent Findings—Kir4.1 is expressed in the basolateral membrane of the DCT and plays a predominant role in contributing to the basolateral K conductance and in participating in the generation of negative membrane potential. Kir4.1 is also the substrate of src-family tyrosine kinase (SFK) and the stimulation of SFK activates Kir4.1 activity in the DCT. The genetic deletion or functional inhibition of Kir4.1 depolarizes the membrane of the DCT, inhibits ste20-proline-alanine rich kinase (SPAK) and suppresses NCC activity. Moreover, the down-regulation of Kir4.1 increases ENaC expression in the collecting duct and urinary K excretion. Finally, the mice with low Kir4.1 activity in the DCT are hypomagnesemia and hypokalemia.

Summary—Recent progress in exploring the regulation and the function of Kir4.1 in the DCT strongly indicates that Kir4.1plays an important role in initiating the regulation of renal K secretion by targeting NCC and it may serves as a K sensor in the kidney.

Keywords

Kir.5.1; ROMK; ENaC; NCC; With-No-lysine kinase; Ste20-proline-alanine rich kinase

Introduction

Hyperkalemia, a potentially fatal disorder, occurs commonly in the setting of chronic kidney disease and heart failure. Its incidence appears to have increased because the most effective

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Conflicts of Interest

The author has no conflicts of interest

cardio- and reno-protective agents that block the renin/angiotensin/aldosterone system (RAAS), all impair renal K disposition. To prevent hyperkalemia induced by using RAAS inhibitors has led to the development of new agents to treat hyperkalemia [1]. Although these agents appear to be effective, they do not address the fundamental cause of the hyperkalemia, which is inappropriate kidney K retention. The aldosterone-sensitive distal nephron (ASDN) including the late distal convoluted tubule (DCT2), connecting tubule (CNT) and cortical collecting duct (CCD) is responsible for K secretion [2;3]. The classic mechanism for the K secretion is that K ions are secreted to the lumen of the CNT and CCD via ROMK (Kir1.1) and Ca^{2+}-activated BK channels along a favorable electrochemical gradient created by Na absorption via ENaC [3–8]. Recent paradigm-shifting discoveries concerning the control of systemic K balance have identified a previously unrecognized system that maintains K homeostasis normally. Surprisingly, the key player in this system is the thiazide-sensitive NCC of the DCT [9–12]. 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 for causing hyperkalemia or hypokalemia. For instance, pseudohypoaldosteronism type II (PHAII) or familial hyperkalemic hypertension (FHHt), is caused by high activity of NCC [13–15] whereas hypokalemia in patients with Gitelman syndrome is due to the loss-function mutations of NCC [16]. Although numerous factors have been shown to regulate NCC activity [17–19], we previously demonstrated that the depletion of Kir.4.1 activity in the DCT inhibited the expression of NCC[20**]. Loss-of-function mutations of *Kcnj10* cause EAST/SeSAME syndrome in humans (seizures, sensorineural deafness, ataxia, mental retardation and electrolyte imbalance) [21–24]. The renal phenotype of the disease is reminiscent to Gitelman syndrome including hypomagnesemia, hypokalemia and metabolic alkalosis, suggesting that the disruption of Kir4.1 mainly impairs transport in the DCT [25;26]. Thus, the aim of the current review is an attempt to provide an overview regarding the role of Kir4.1 in regulating renal Na and K transport.

Expression of Kir4.1 along the nephron segments

Fig. 1A is a nephron scheme illustrating the expression of Kir4.1 along the nephron in the mouse kidney and the corresponding native tubule is shown in Fig. 1B. Kir4.1 is expressed in the basolateral membrane of the cortical TAL (cTAL), DCT, CNT and CCD. Fig. 1C is a scheme showing the expression of NKCC2, NCC, ENaC and ROMK in the apical membrane of the cTAL, DCT and CNT/CCD, respectively. However, experiments performed in post-neonatal 9 day (p9) mice have showed that the expression of Kir4.1 in the cTAL is mainly limited in the late part of cTAL rather than in the whole length of the cTAL of the mouse kidney [27*]. Kir4.1 expression was also detected in human TAL, however, loss-of-function mutations of Kir4.1 in humans did not have phenotype of defective membrane transport in the TAL, suggesting that Kir4.1 function in the TAL may not be indispensable [21–23]. The possibility that Kir4.1 function in the cTAL could be compensated is also supported by patch-clamp experiments performed in p9 neonatal *Kcnj10*$^{-/-}$ mice demonstrating that the disruption of Kir4.1 stimulates the activity of Na^+ and Cl^--activated 80- to 150-pS K^+ channel (K_{ca}4.1 or *slo*2.2) in the cTAL [27;28]. The upregulation of Na-activated 80–150 pS K channel in the TAL may be induced by high vasopressin level in p9

neonatal Kir4.1 knockout mice as evidenced by the finding that AQP2 expression was upregulated [29].

Kir.4.1 is also expressed in the basolateral membrane of the DCT along the whole length [22]. The DCT reabsorbs 5–9% of filtered Na load and it is functionally divided into the early part (DCT1) and the late portion (DCT2) [30–32]. While thiazide-sensitive NCC is expressed in the apical membrane of both DCT1 and DCT2, ROMK and ENaC activity are only detected in the apical membrane of DCT2 [33;34]. In the DCT1, Na and Cl enter the cells across the apical membrane through the NCC and Na is then pumped out of the cell through the basolateral Na-K-ATPase while Cl exits the cell along its electrochemical gradient by basolateral Cl channels (ClC-kb) or KCl cotransporter [35;35–37]. In the DCT2, Na enters the cell across the apical membrane not only through NCC but also by ENaC [38;39].

Kir4.1 interacts with Kir5.1 to form an inwardly-rectifying 40 pS K channel in the basolateral membrane of DCT1 and DCT2. Moreover, the 40 pS K channel is the only K channel type detected in the basolateral membrane of the DCT. Our previous experiments performed in p9 neonatal WT and *Kcnj10*$^{-/-}$ mice have demonstrated that Kir4.1 plays a dominant role in determining the basolateral K conductance in the DCT1 because the disruption of Kir4.1 almost completely eliminates the basolateral K conductance [20**]. The notion that Kir4.1 is a major type of K channel and it determines the membrane potential in the DCT is also supported by our recent unpublished observations made from kidney-specific Kir4.1 knockout mice (Wang's observation). We have observed that Kir4.1/Kir5.1 heterotetramer is also the only K channel type detected in the basolateral membrane of DCT1 and DCT2 of adult mice.

In addition to cTAL and DCT, immunostaining and the patch-clamp experiments have detected Kir4.1 expression and activity in the basolateral membrane of the CNT and CCD [40*]. Like in the DCT, Kir4.1 interacts with Kir5.1 to form a 40–45 pS inwardly-rectifying K channels in the CNT and CCD [41;42]. However, experiments performed in p9 c57/bl6 mice have showed that the positive staining of Kir4.1 was limited in the top portion of the renal cortex but was almost absent in the low portion of the renal cortex. Moreover, K channels other than Kir4.1/5.1 heterotetramer were also detected in the basolateral membrane of the CNT and CCD of both adult and p9 neonatal mice [40*]. Therefore, unlike in the DCT, Kir4.1 participates only partially in generating the membrane potential in the CNT and CCD [40]. This notion is suggested by experiments performed in p9 neonatal Kir4.1 knockout mice in which the K reversal potential (an index of the cell membrane potential) in the CNT/CCD was only modestly depolarized in the knockout mice in comparison to those of WT littermates.

Regulation of Kir4.1

Kir4.1 is inhibited by protein kinase C (PKC) and stimulated by src-family protein tyrosine kinase (SFK) which phosphorylates Kir4.1 at tyrosine residues 8 and 9 of the N-terminus [43;44]. PKC has been shown to mediate the inhibitory effect of dopamine on the basolateral Kir4.1–Kir5.1 heterotetramer in the CCD [42]. On the other hand, the SFK-induced tyrosine phosphorylation of Kir4.1 stimulates the basolateral K conductance in the DCT [44].

Moreover, caveolin-1 plays a role in mediating the stimulatory effect of SFK on Kir4.1 [45**]. Caveolin-1 is highly expressed in the basolateral membrane of the DCT, CNT and CCD and the disruption of caveolin-1 inhibits the Kir4.1/5.1 activity in the mouse DCT. The basolateral Kir4.1–Kir5.1 channel activity was inhibited by acidic pH [41] and Kir5.1 may play a role in the regulation of pH sensitivity of Kir4.1 [46;47]. This notion is supported by the finding that the pH sensitivity of Kir4.1 is blunted in *Kcnj16*$^{-/-}$ mice [48]. Kir4.1 was immunoprecipitated from rat renal tissue extracts with Ca^{2+}-sensing receptor (CaSR) and the expression of CaSR decreased the surface expression of Kir4.1 in HEK293 cells, suggesting the possibility that CaSR may regulate the activity of the basolateral 40 pS K channel [49;50].

Kir4.1 regulates NCC expression

Our recent experiments have demonstrated that Kir4.1 activity in the DCT determines the expression of NCC [20**;45**]. The role of NCC in the regulation of K excretion and K homeostasis has been well recognized by several independent studies [9–11;51**]. It has been demonstrated that an increase in dietary K intake inhibits the expression and activity of NCC and increases ENaC [9]. This should inhibit Na transport in the DCT thereby increasing Na delivery to the distal nephron segments such as DCT2 and CNT. Since high K intake stimulates ENaC activity [9], this should enhance K secretion in the CNT and CCD without the stimulation of overall Na absorption. On the other hand, a decrease in dietary K intake has been shown to increase the expression and activity of NCC thereby decreasing Na delivery to the distal nephron segments, leading to suppressing K secretion [9]. Thus, NCC activity plays a key role in controlling Na delivery to the DCT2, CNT and CCD and in the regulation of K secretion in the ASDN. It is well documented the NCC activity is regulated by with-no-lysine kinase (WNK), ste20-proline-alanine rich kinase (SPAK) and oxidative-sensitive responsive kinase (OSR) [18;52–55]. WNK phosphorylates and activates SPAK or OSR which in turn stimulates NCC activity by phosphorylation [54;56]. Gain-of-function mutations of WNK1 and WNK4 are responsible for increasing NCC activity in the DCT thereby causing familial hyperkalemic hypertension [57;58]. Thus, WNK-SPAK-mediated NCC phosphorylation plays a key role in the regulation of K excretion and K homeostasis. Recently, an elegant experiment by Terker et al has convincingly demonstrated that SPAK-induced NCC phosphorylation is closely controlled by plasma K level such as hyperkalemia inhibits while hypokalemia stimulates NCC phosphorylation [51**].

Although the role of plasma K level in the regulation of NCC activity is well established, it is not clear how NCC could sense the change in dietary K intake. Because Kir4.1 is the only type K channel in the basolateral membrane of the DCT, we hypothesize that Kir4.1 is actually a mediator between NCC and plasma K. This possibility is strongly suggested by the finding that the deletion or the inhibition of Kir4.1 decreases NCC expression [20**; 45**]. Fig. 2 illustrates the mechanism by which the changes in Kir4.1 activity regulate NCC activity. The inhibition of the basolateral K conductance depolarizes the membrane and decreases Cl exit across the basolateral membrane of the DCT thereby increasing the intracellular Cl concentrations (Fig. 2A). Because WNK is a Cl-sensitive kinase such that a high intracellular Cl (Cl_i) concentration inhibits WNK activity [59*;60*], the inhibition of WNK leads to suppression of SPAK activity thereby inhibiting the NCC expression. On the

other hand, the activation of Kir4.1 should decrease Cl_i and it activates WNK-SPAK/OSR pathway which in turn stimulates NCC activity by phosphorylation (Fig. 2B). It has been shown that SPAK-induced phosphorylation not only activates NCC but also increases NCC expression by inhibiting degradation [18;52;54;61]. The activity of Kir4.1 plays a role in the regulation of NCC activity has also been demonstrated by Terker *et al* and they have demonstrated that the change in the intracellular Cl concentration induced by membrane voltage is responsible for the inhibition of NCC activity in DCT cells expressing loss-function of Kcnj10 mutants [62**]. Thus, it is conceivable that the activity of the basolateral Kir4.1 activity in the DCT controls the NCC activity through a Cl-sensitive WNK pathway. Because the activity of NCC determines the Na transport in the DCT and controls the Na delivery to the distal nephron including DCT2, CNT and CCD, a change in Kir4.1 activity is expected to affect K secretion in the distal nephron segments. This also explains why hypokalemia is an essential feature of the tubulopathy in SeSAME syndrome [22].

Inhibition of Kir4.1 stimulates ENaC activity

Experiments performed in global Kir4.1 knockout mice have shown that the depletion of Kir4.1 stimulates the expression of ENaC-β, γ subunits and cleaved ENaC-α in the collecting duct [40*]. The upregulation of ENaC expression may explain the fact why patients with EAST/SeSAME syndrome have a modest phenotype of salt wasting despite of the inhibition of Na transport in the DCT [23]. We suspect that increased expression of ENaC-β, γ subunits and cleaved ENaC-α should be a compensation action as consequence of the down-regulation of NCC. It is possible that the inhibition of NCC is expected to cause a volume depletion which should increase the aldosterone and vasopressin level thereby stimulating ENaC expression. Moreover, we reasoned that a high vasopressin level may be mainly responsible for the stimulation of ENaC-β and γ expression in p9 neonatal *Kcnj10*$^{-/-}$ mice because vasopressin has been shown to stimulate the expression of ENaC-β subunit [63]. Furthermore, our previous studies showed that the disruption of Kir4.1 increased AQP2 expression, suggesting Kcnj10$^{-/-}$ mice have a high vasopressin level [29].

Inhibition of Kir4.1 impairs magnesium absorption

Hypomagnesemia is one phenotype of tubulopathy in SeSAME syndrome, suggesting the role of Kir4.1 in the regulation of magnesium absorption in the DCT [22]. Although the mechanism by which the inhibition of Kir4.1 causes hypomagnesemia is not completely understood, a decrease in the membrane potential in the DCT should partially contribute to inhibiting magnesium absorption in the DCT because transcellular magnesium transport process is electrogenic. Thus, a decrease in the basolateral K conductance in the DCT should diminish the driving force for the entry of magnesium ions across the apical membrane thereby inhibiting their absorption in the DCT. This notion is supported by the experiments performed in caveolin-1 knockout mice which have a low basolateral Kir4.1 activity and less negative membrane potential in the DCT in comparison to WT mice [45**]. Consequently, the caveolin-1 knockout mice are associated with increased magnesium excretion and have hypomagnesemia. Because intracellular magnesium ions have a strong inhibitory effect on ROMK outward current, a decrease in intracellular magnesium ion concentrations should increase ROMK outward currents and cause K wasting [64]. Thus, in addition to the

regulation of NCC, Kir4.1 activity may affect renal K secretion through the modulation of magnesium absorption in the DCT.

Conclusion

The emerged evidence suggests that NCC plays a central role in the regulation of renal K secretion in response to hypokalemia or hyperkalemia and that Kir4.1 in the DCT mediates the effect of dietary K intake on NCC activity. Since Kir4.1 determines the basolateral K conductance and the membrane potential in the DCT, an alteration of Kir4.1 activity should affect WNK-SPAK activity through an intracellular Cl-sensitive mechanism thereby modulating NCC activity. Thus, Kir4.1 in the DCT may be a plasma K sensor and the regulation of Kir4.1 initiates the regulation of K secretion in the ASDN.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

*of special interest

**of outstanding interest

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Key points

- Kir4.1 is a main contributor to the basolateral K conductance in the DCT.
- The inhibition of Kir4.1 suppresses the expression of SPAK and NCC.
- The down-regulation of Kir4.1 causes hypokalemia and hypomagnesimia.

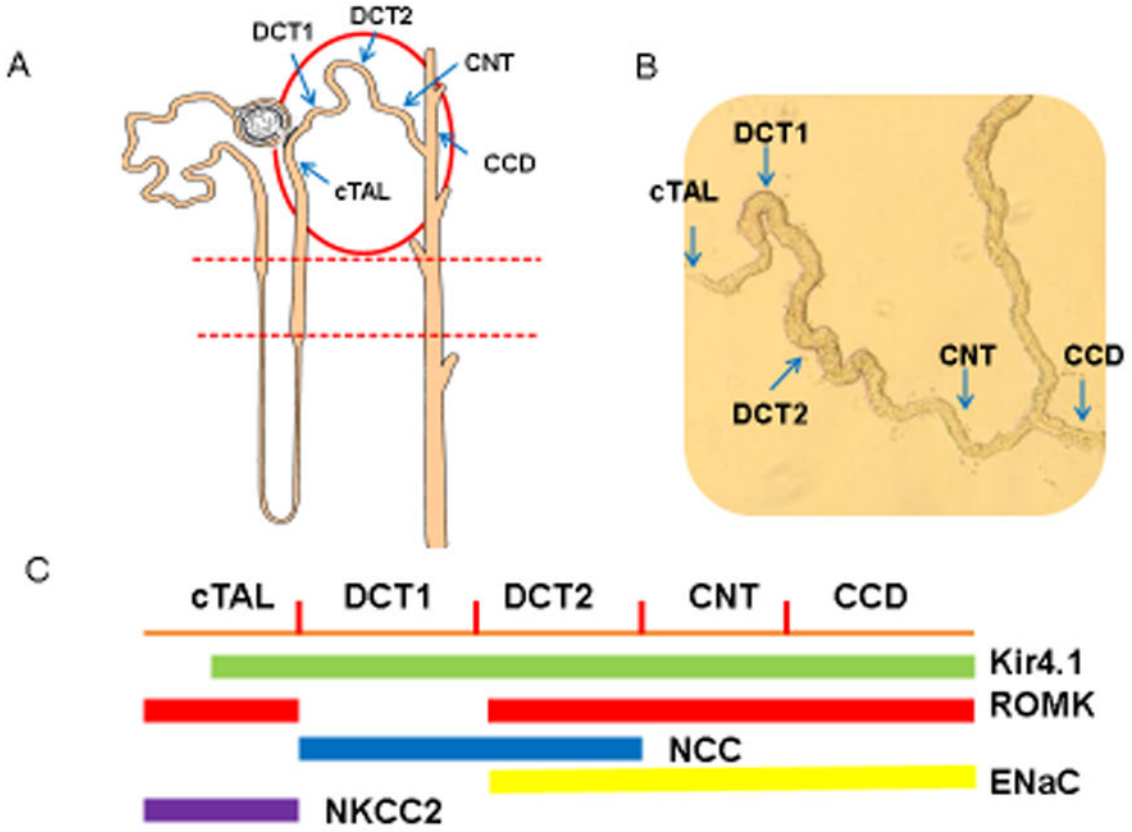

Fig. 1.

(A) A nephron scheme illustrating the expression of Kir4.1 in nephron segments circled by a red oval. (B) An image of isolated nephron segments which corresponds to the portion indicated by a red oval in Fig. 1A. The kir4.1 is expressed in the basolateral membrane from cortical thick ascending limb (cTAL) to the cortical collecting duct (CCD). (C) A scheme illustrating that NKCC2, NCC, ENaC and ROMK are expressed in the apical membrane of tubules from cTAL to CCD where Kir4.1 is expressed.

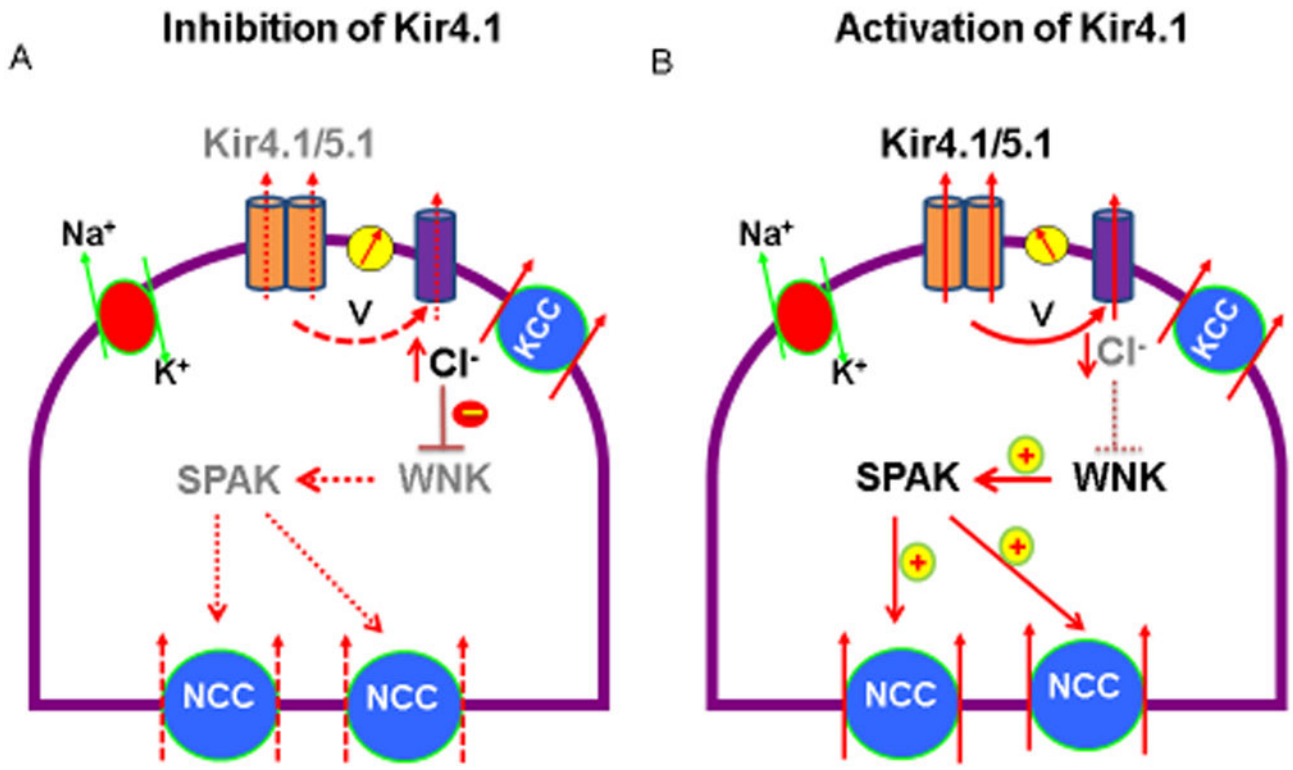

Fig. 2.

A scheme illustrating the mechanism by which the inhibition of Kir4.1 decreases (A) or the stimulation of Kir4.1 increases activity of NCC in the DCT (B). Dotted and solid lines represent a diminished and an enhanced function, respectively. Gray font means an inhibition or a decrease. Abbreviation: V, cell voltage; WNK, with-no-lysine kinase, SPAK, ste20-proline-alanine rich kinase.