The expression, regulation, and function of Kir4.1 (Kcnj10) in the mammalian kidney

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Su X-T, Wang W-H. The expression, regulation, and function of Kir4.1 (Kcnj10) in the mammalian kidney. Am J Physiol Renal Physiol 311: F12–F15, 2016. First published April 27, 2016; doi:10.1152/ajprenal.00112.2016.—Kir4.1 is an inwardly rectifying potassium (K⁺) channel in the mammalian kidney. The expression, regulation, and function of Kir4.1 (Kcnj10) in the mammalian kidney has been shown to be expressed in the brain, inner ear, eye, gastric parietal cells, and kidney (7, 14, 21, 28, 30). Loss-of-function mutations of Kir4.1 cause epilepsy, ataxia, sensorineural deafness, and renal tubulopathy/seizures, sensorineural deafness, ataxia, intellectual deficit, and electrolyte imbalance syndrome (1). In the brain, Kir4.1 is expressed in glial cells, particularly in astrocytes, and it is involved in the “K⁺ spatial buffering” process, which is essential for maintaining resting membrane potential of neurons (21). In the inner ear, Kir4.1 is required for generating the endocochlear potential of intermediate cells and for maintaining high K⁺ content of the endolymph (30). In the eye, Kir4.1 is expressed in retina Muller glia cells and in corneal epithelial cells, and it plays a role in the regulation of the extracellular K⁺ level and in regulating the healing process of cornea epithelial cells, respectively (14, 16). In the kidney, Kir4.1 interacts with Kir5.1 to form a 40-pS inwardly rectifying K⁺ channel in the basolateral membrane of the late thick ascending limb (TAL), distal convoluted tubule (DCT), connecting tubule (CNT)/cortical collecting duct (CCD). It plays a role in K⁺ recycling across the basolateral membrane in corresponding nephron segments and in generating negative membrane potential. The renal phenotypes of the loss-function mutations of Kir4.1 include mild salt wasting, hypomagnesemia, hypokalemia, and metabolic alkalosis, suggesting that the disruption of Kir4.1 mainly impairs the transport in the DCT. Patch-clamp experiments and immunostaining demonstrate that Kir4.1 plays a predominant role in determining the basolateral K⁺ conductance in the DCT. However, the function of Kir4.1 in the TAL and CNT/CCD is not essential, because K⁺ channels other than Kir4.1 are also expressed. The downregulation of Kir4.1 in the DCT reduced basolateral chloride (Cl⁻) conductance, suppressed the expression of ste20 proline-alanine-rich kinase (SPAK), and decreased Na–Cl cotransporter (NCC) expression and activity. This suggests that Kir4.1 regulates NCC expression by the modulation of the Cl⁻-sensitive with-no-lysine kinase–SPAK pathway.

Kcnj10; kidney

AN INWARDLY RECTIFYING POTASSIUM (K⁺) CHANNEL, Kir4.1 has been shown to be expressed in the brain, inner ear, eye, gastric parietal cells, and kidney (7, 14, 21, 28, 30). Loss-of-function mutations of Kir4.1 cause epilepsy, ataxia, sensorineural deafness, and renal tubulopathy/seizures, sensorineural deafness, ataxia, intellectual deficit, and electrolyte imbalance syndrome (1). In the brain, Kir4.1 is expressed in glial cells, particularly in astrocytes, and it is involved in the “K⁺ spatial buffering” process, which is essential for maintaining resting membrane potential of neurons (21). In the inner ear, Kir4.1 is required for generating the endocochlear potential of intermediate cells and for maintaining high K⁺ content of the endolymph (30). In the eye, Kir4.1 is expressed in retina Muller glia cells and in corneal epithelial cells, and it plays a role in the regulation of the extracellular K⁺ level and in regulating the healing process of cornea epithelial cells, respectively (14, 16). In the kidney, Kir4.1 interacts with Kir5.1 to form a 40-pS inwardly rectifying K⁺ channel in the basolateral membrane of the late thick ascending limb (TAL), distal convoluted tubule (DCT), connecting tubule (CNT), and initial collecting duct (CCD) (17, 41, 43). The importance of Kir4.1 in maintaining epithelial electrolyte transport in the DCT is convincingly documented by the report that patients with loss-of-function mutations of Kir4.1 in the kidney have metabolic alkalosis, hypomagnesemia, hypokalemia, and hypocalciuria (1, 28, 31). The aim of this short review is to provide an overview regarding the function and regulation of Kir4.1 in the TAL and distal nephron segments.

THICK ASCENDING LIMB

The TAL is responsible for absorption of 20–25% of the filtered NaCl, Ca²⁺, and Mg²⁺ and plays a key role in concentrating urine (9, 11). The basolateral K⁺ channels participate in generating the negative cell membrane potential, which provides the driving force for chloride (Cl⁻) exit through the basolateral Cl⁻ channels. Since Cl⁻ absorption in the TAL is completely through transcellular pathways, it is possible that the inhibition of basolateral K⁺ conductance should affect the transcellular Cl⁻ absorption. Patch-clamp experiments have identified two types of K⁺ channels in the basolateral membrane of the TAL: a 40-pS inwardly rectifying K⁺ channel and an Na⁺/K⁺ and Cl⁻–activated, 80–150-pS K⁺ channel (Kc₄.1 or slo₂.2) (24, 41). Because the 40-pS K⁺ channel was absent in the TAL of Kcnj10⁻/⁻ mice, this suggests that the 40-pS K⁺ channel is a Kir4.1 and Kir5.1 heterotetramer (41). The expression of Kir4.1 is not uniformly distributed along the mouse TAL, and it is highly detected in the late part of the TAL (41). Although Kir4.1 is also detected in human TAL, loss-of-function mutations of Kir4.1 in humans did not show the phenotype of Bartter syndrome, suggesting that the disruption of Kir4.1 has no significant effect on transport function in the TAL (1, 31). Studies performed in postneonatal day 9 Kcnj10⁻/⁻ mice demonstrated that disruption of Kir4.1 in mice leads to the stimulation of the 80–150-pS K⁺ channel expression, thereby compensating for the function of Kir4.1 (6). Therefore, the function of Kir4.1 in the TAL of mice is not dispens-
able. Because the disruption of Kir4.1 may lead to an increase in vasopressin release induced by volume contraction, it is suggested that an increase in vasopressin levels may be responsible for the stimulation of Na\(^+\)/H\(^+\) and Cl\(^-\)/H\(^+\)-activated K\(^+\)/H\(^+\) channels (6). However, new experiments are needed in adult Kcnj10 knockout mice to confirm this observation.

**DISTAL CONVOLUTED TUBULE**

The DCT is responsible for the reabsorption of 5–9% of the filtered Na\(^+\) and plays a role in the reabsorption of Ca\(^{2+}\) and Mg\(^{2+}\) (5). The DCT is generally divided into the early part (DCT1) and the late portion (DCT2). Whereas the thiazide-sensitive Na-Cl cotransporter (NCC) is expressed in the apical membrane of both DCT1 and DCT2, the channel activity of renal outer medullary K (ROMK) and epithelial Na\(^+\)/H\(^+\) channel (ENaC) is only detected in the apical membrane of DCT2, although ROMK immunostaining is also detected in DCT1 (36). The reabsorption of NaCl in the DCT1 is a two-step process. 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, whereas Cl\(^-\) exits the cell along its electrochemical gradient by basolateral Cl\(^-\) channels (ClC-kb) or the K/Cl cotransporter (KCC) (20, 22).

The basolateral K\(^+\) channels in the DCT participate in generating the cell membrane potential and are responsible for K\(^+\) recycling, which is important for sustaining the activity of the Na\(^+\)-K\(^+\)-ATPase (10). The patch-clamp experiments performed in the basolateral membrane of the DCT detected a 40-pS inwardly rectifying K\(^+\) channel (17). Because this 40-pS K\(^+\) channel is completely absent in Kcnj10\(^{-/-}\) and Kcnj16\(^{-/-}\) mice, it indicates that this 40-pS K\(^+\) channel is a heterotetramer of Kir4.1 and Kir5.1 (23, 43). Moreover, the disruption of Kir4.1 largely abolished the basolateral K\(^+\) conductance in the DCT, indicating that Kir4.1 plays a dominant role in determining the basolateral K\(^+\) conductance (43). The basolateral 40-pS K\(^+\) channel has been shown to be sensitive to cell pH, such that the K\(^+\) channel activity was inhibited by acidic pH (15). Moreover, Kir5.1 may play a role in the regulation of pH sensitivity of the Kir4.1/Kir5.1 heterotramer (25, 33). This notion is also supported in experiments performed on Kcnj16\(^{-/-}\) mice, in which the pH sensitivity of the basolateral 20-pS K\(^+\) channel, presumably a Kir4.1 homotramer, in the DCT is blunted (23). In addition, the calcium-sensing receptor (CaSR) has been shown to be colocalized with Kir4.1 in the DCT. Because the expression of CaSR decreased the surface expression of Kir4.1 in human embryonic kidney cells, it is suggested that CaSR may play a role in modulating the activity of the basolateral 40-pS K\(^+\) channel in the DCT (2, 13). Kir4.1 is a substrate of src-family protein tyrosine kinase (SFK), and coexpression of c-Src phosphorylates Kir4.1 at Tyr9 (42). The SFK-induced tyrosine phosphorylation of Kir4.1 plays a role in the stimulation of the basolateral 40-pS K\(^+\) channel in the DCT, because the inhibition of SFK suppressed the 40-pS K\(^+\) channel.
activity. The stimulatory effect of SFK on the basolateral 40-pS K⁺ channel requires the participation of caveolin-1, which is highly expressed in the basolateral membrane of the DCT, CNT, and CCD (37). The disruption of caveolin-1 inhibited the Kcnj10/16 activity in the basolateral 40-pS K⁺ channel in the mouse DCT (37).

Figure 1 is a scheme illustrating the physiological role of Kir4.1 in the DCT. It is possible that the basolateral Kir4.1/5.1 may control intracellular Cl⁻ concentrations ([Cl⁻]) in the DCT. For instance, inhibition of Kir4.1/5.1 depolarizes the negative cell voltage (V), thereby decreasing Cl⁻ exit across the basolateral membrane and increasing Cl⁻ in the DCT. This speculation is supported by the report that an increase in Cl⁻, induced by depolarization, is responsible for the inhibition of NCC activity in the cells expressing loss-of-function Kcnj10 mutants (35). Because an increase in Cl⁻ inhibits with-no-lysine kinase (WNK) and ste20 proline-alanine-rich kinase (SPAK) activity (26, 34), a decrease in SPAK activity reduces the phosphorylation of NCC. Since SPAK-induced phosphorylation has been shown not only to activate NCC but also to inhibit NCC degradation (19, 27, 29, 39), a decrease in the basolateral K⁺ conductance in the DCT leads to the suppression of NCC expression. Our previous experiments also showed that the membrane potential in the DCT was less negative in Kcnj10⁻/⁻ mice compared with their wild-type (WT) littermates (43). Thus it is conceivable that the activity of the basolateral K⁺ conductance in the DCT controls NCC activity through a Cl⁻-sensitive WNK pathway. In addition to Kir4.1, Cl channels and KCCs in the basolateral membrane could have an effect on the Cl⁻ concentration. Our previous study has demonstrated that Cl channel activity is tightly correlated with Kir4.1 activity, such that the inhibition of Kir4.1 decreased Cl channel activity (43). Thus the downregulation of Cl channels should amplify the effect of the Kir4.1 inhibition-induced increase in Cl⁻. On the other hand, WNK-regulated SPAK/oxidative stress response kinases have been shown to phosphorylate KCC, thereby inhibiting the KCC (3). Thus the inhibition of SPAK should activate KCC, thereby facilitating Cl exit and reducing Cl⁻. This may serve as a negative-feedback mechanism for controlling Cl⁻. Further experiments are required to explore the role of both Cl channels and KCC in the regulation of Cl⁻. Furthermore, it is essential to measure Cl⁻ in vivo or in vitro to validate this model.

CONNECTING TUBULE/CORTICAL COLLECTING DUCT

Immunostaining and electrophysiological experiments have shown that Kir4.1 is expressed in the basolateral membrane of the CNT and CCD (8, 15). Moreover, dopamine has been shown to inhibit Kir4.1/Kir5.1 directly in the CCD (40). Although Kir4.1 is expressed in the basolateral membrane of the CCD, the disruption of Kir4.1, however, causes a modest depolarization in the CNT/CCD, suggesting that Kir4.1 may not be the only type of K⁺ channel in the basolateral membrane of the CNT/CCD (32). Indeed, previous studies have identified three types of K⁺ channel activity in the basolateral membrane of the CCD (12, 38). We have also observed the activity of the 23- , 40-, and 60-pS K⁺ channels in the basolateral membrane of CNT and early CCD in WT mice (32). Since only 40 pS K⁺ channel was absent in the basolateral membrane of CNT/CCD in Kcnj10⁻/⁻ mice, it strongly suggests that the 23- and 60-pS K⁺ channels are not related to Kir4.1. Therefore, it is conceivable that the electrophysiological gradient for Na⁺ entry across the apical membrane should be maintained. Immunostaining and Western blot have also revealed that the disruption of Kir4.1 increased the expression of ENaC-β and γ subunits and also the cleaved form of ENaC-α in the kidney (32). However, because these experiments were performed in postneonatal day 9 Kcnj10⁻/⁻ mice, further experiments in adult Kir4.1 knockout mice are required to validate the finding. Furthermore, we speculate that the increase in ENaC expression in Kcnj10⁻/⁻ mice may be induced partially by the activation of the renin-ANG II-aldosterone system and vasopressin, which has been shown to stimulate ENaC activity (4). Thus the increase of Na⁺ absorption in the CCD compensates the Na⁺ wasting partially in the DCT, but it causes K⁺ wasting and hypokalemia.

SUMMARY

Studies performed in the global Kir4.1 knockout mice have firmly established the role of Kir4.1 in the regulation of NCC in the DCT. Moreover, since the disruption of Kir4.1 also inhibits the basolateral Cl⁻ channel activity, this indicates that the Cl⁻ should be involved in mediating the effect of Kir4.1 inhibition on NCC expression.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


