Published in final edited form as:

Curr Opin Nephrol Hypertens. 2010 September ; 19(5): 463–470. doi:10.1097/MNH.0b013e32833c34ec.

Regulation and function of potassium channels in aldosterone-sensitive distal nephron

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Abstract

Purpose of review—K channels in the aldosterone-sensitive distal nephron (ASDN) participate in generating cell membrane potential and in mediating K secretion. The aim of the review is to provide an overview of the recent development regarding physiological function of the K channels and the novel factors which modulate the K channels of the ASDN.

Recent findings—Genetic studies and transgenic mouse models have revealed the physiological function of basolateral K channels including inwardly rectifying K channel (Kir) and Ca^{2+} -activated big-conductance K channels in mediating salt transport in the ASDN. A recent study shows that intersectin is required for mediating with-no-lysine kinase (WNK)-induced endocytosis. Moreover, a clathrin adaptor, autosomal recessive hypercholesterolemia (ARH), and an aging-suppression protein, Klotho, have been shown to regulate the endocytosis of renal outer medullary potassium (ROMK) channel. Also, serum-glucocorticoids-induced kinase I (SGK1) reversed the inhibitory effect of WNK4 on ROMK through the phosphorylation of WNK4. However, Src-family protein tyrosine kinase (SFK) abolished the effect of SGK1 on WNK4 and restored the WNK4-induced inhibition of ROMK.

Summary—Basolateral K channels including big-conductance K channel and Kir4.1/5.1 play an important role in regulating Na and Mg^{2+} transport in the ASDN. Apical K channels are not only responsible for mediating K excretion but they are also involved in regulating transepithelial Mg^{2+} absorption. New factors and mechanisms by which hormones and dietary K intake regulate apical K secretory channels expand the current knowledge regarding renal K handling.

Keywords

big-conductance K; c-Src; renal outer medullary potassium channel; voltage-gated K channel; with-no-lysine kinase

Introduction

K channels have been identified in both the apical and basolateral membrane of the ASDN, including distal convoluted tubule (DCT), connecting tubule (CNT), and cortical collecting duct (CCD). Although the ASDN reabsorbs only less than 10% of filtered Na load, the Na

absorption in the ASDN is finely controlled by hormone and dietary Na intake. Moreover, the ASDN is responsible for the K excretion and plays an important role in maintaining K homeostasis. The K channels in the baso-lateral membrane participate in generating cell membrane potential. Because Cl transport in the DCT and the Na transport in the CNT and CCD are electrogenic, alteration in the cell membrane potential is expected to affect Cl transport in the DCT and Na transport in the CNT and CCD. Thus, this review has summarized the recent development regarding the role of the several types of basolateral K channels in the ASDN in mediating membrane transport. Apical K channels including renal outer medullary potassium (ROMK) and Ca^{2+} -activated big-conductance K channels play a key role in mediating K secretion in the ASDN. Previous studies have demonstrated that with-no-lysine kinase (WNK) family kinase, SGK1, and SFK regulate ROMK channels and may play a role in regulating K secretion [1–3]. However, it is still not completely known how these signaling pathways work in concert to regulate renal K secretion in the ASDN. This review attempts to illustrate an integrated mechanism by which SGK1, WNK4, and SFK interact with each other to meet different physiological stimuli. Also, the review article summarizes the recent development regarding the role of the apical voltage-gated K channels in regulating Mg^{2+} transport in the DCT and the regulatory mechanism of big-conductance K channels and big-conductance K channel-dependent K secretion.

Basolateral K channels

Patch-clamp experiments, immunocytochemical and molecular biological methods have confirmed the expression of several inwardly rectifying K channels and Ca^{2+} -activated big-conductance K channels in the basolateral membrane [4–8]. Two lines of evidence suggest that Kir4.1/5.1 heterotetramers are the main type of K channels in the basolateral membrane in the ASDN: first the Kir4.1 protein is detected in the basolateral membrane of the DCT and the CCD [7,9]; second the patch-clamp experiments have shown that Kir4.1/Kir5.1 channels form the major K channels in the basolateral membrane of principal cells in the CCD [7].

Kir4.1 has been shown to interact with the Ca^{2+} -sensing receptor, which inactivates the K channels [10]. Because Kir4.1 participates in generating the cell membrane potential, activation of the Ca^{2+} -sensing receptor is expected to depolarize the membrane potential, thereby inhibiting transepithelial Na/Cl transport in the DCT. The importance of Kir4.1 in regulating membrane transport in the DCT is best demonstrated in patients with SeSAME syndrome characterized by seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance. The disease is caused by a defective gene product encoding KCNJ10 (Kir4.1) which is expressed in a variety of tissues including in the basolateral membrane of the ASDN. The renal phenotypes of SeSAME syndrome are hypokalemia, metabolic alkalosis, and hypomagnesemia [11^{••}]. Figure 1 is a cell model illustrating the role of Kir4.1/5.1 in regulating NaCl transport in the DCT. Activation of Kir4.1/5.1 is expected to increase the negativity of basolateral membrane, thereby providing the driving force for Cl exit across the basolateral membrane. In contrast, defective Kir4.1 depolarizes the basolateral membrane, thereby diminishing the driving force for Cl exit across the basolateral membrane. Consequently, delivery of NaCl to the CNT and CCD is increased and leads to enhancing Na absorption in the CNT and CCD in the expense of K waste,

resulting in hypokalemia and metabolic alkalosis. Also, decreased basolateral membrane potential impairs Mg^{2+} absorption in the DCT due to a compromised driving force for Mg^{2+} .

The big-conductance K channel is composed of a pore-forming α subunit (*Slo 1*) and an accessory β subunit [12,13]. Immunocytochemical staining shows that big-conductance K α and $\beta 1$ subunits are expressed in the basolateral membrane of principal cells in the CNT and CCD and their expression is stimulated by Na restriction. The physiological function of basolateral big-conductance K channels in principal cells is possibly responsible for hyperpolarizing the basolateral membrane, thereby increasing the driving force for Na absorption. The notion is supported by the observation that big-conductance K $\beta 1^{-/-}$ mice (*Kcnmb1*^{-/-}) have a higher Na clearance than that of wild-type mice in response to low Na intake [4]. The impaired function of basolateral big-conductance K channels in principal cells also affects the renal K secretion in *Kcnmb1*^{-/-} mice in response to a high K intake [14**]. *Kcnmb1*^{-/-} mice on a high K diet are hypertensive, volume expanded, and have reduced urinary K and Na clearances, an effect which can be treated with mineralocorticoids receptor antagonist. This suggests that the impaired K secretion results in high aldosterone levels, which stimulates Na transport in *Kcnmb1*^{-/-} mice.

Apical K channels

Molecular biological approaches and patch-clamp experiments have identified several types of K channels in the apical membrane including inwardly-rectifying K channel, Ca^{2+} -activated big-conductance K and voltage-gated K channels.

Renal outer medullary potassium channel

The ROMK channel is a member of K_{IR} and responsible for K secretion in the CNT and CCD under normal conditions and for K recycling across the apical membrane of the thick ascending limb. The structural information of ROMK is largely obtained from the X-ray crystallographic structure of a K channel from *Streptomyces lividans* [15]. A recent review has elegantly summarized the current understanding regarding the functional and structural relationship of ROMK channels [16]. ROMK channels are pH sensitive and a decrease in cell pH from 7.4 to 7.0 completely inhibits channel activity [17,18]. Moreover, the pH sensitivity of ROMK channels is controlled by extracellular K [19,20]. It has been shown that removal of extracellular K inactivates ROMK channels, which are closed by internal acidic pH. In the absence of extracellular K, intracellular alkalization fails to reactivate ROMK channels and adding extracellular K is required to reactivate ROMK channels. It has been suggested that internal pH gating and external K gating might act in the same amino acid (leucine residue 160 of ROMK2) [21]. Acidification induces conformation changes preventing the K access to the transmembrane pore from the cytoplasm [22]. Several studies have demonstrated that ROMK channels may interact with cystic-fibrosis-transmembrane-conductance-regulator (CFTR) or sulfonylurea receptor type 2B (SUR2B) and that such interaction is required for ATP sensitivity [23–25]. However, the role of CFTR in regulating ATP-sensitivity of ROMK channels is still not completely understood [26]. Because the regulation of ROMK channels by ATP required protein kinase A (PKA)-dependent phosphorylation of CFTR, ATP sensitivity of ROMK might be the result of the PKA-phosphorylation state of CFTR rather than direct ATP regulation [26].

Ca²⁺-activated big-conductance K channel

Big-conductance K channel activity has been detected at the apical membrane of both principal cells and intercalated cells of the CCD [27,28]. Real-time PCR analysis has demonstrated that a high K intake stimulates the mRNA transcription of big-conductance K α , β 2, and β 4 subunits but not β 1 in the rabbit CCD [29]. Because a low Na intake, which increases the aldosterone level, fails to mimic the effect of a high K intake on the mRNA level of big-conductance K α , β 2, and β 4 subunits, it excludes the role of aldosterone in regulating big-conductance K channel expression [30]. Although the role of big-conductance K channels in mediating the flow-stimulated K secretion has been firmly established [31–33], it is still not completely understood whether intercalated cells or principal cells are responsible for mediating the big-conductance K channel-dependent K secretion. The patch-clamp experiments have demonstrated that apical big-conductance K channel activity is higher in intercalated cells than in principal cells [27]. However, because intercalated cells have a low Na-K-ATPase activity, it is still arguable whether big-conductance K channels in intercalated cells significantly contribute to K secretion in the collecting duct [34]. A recent study performed in *Kcnmb4*^{-/-} mice in which the big-conductance K β 4 subunit is deleted suggests the role of big-conductance K channels in intercalated cells in mediating flow-dependent K secretion. Holtzclaw *et al.* [35] have demonstrated that renal K excretion was compromised in response to a HK intake in mice lacking the β 4 subunit, which is expressed in the apical membrane of intercalated cells in the CNT and CCD. Furthermore, immunocytochemical staining shows that a high K intake decreased intercalated cells size in wild-type but not in *Kcnmb4*^{-/-} mice. Moreover, the flow rate speed was higher in knock-out mice than in wild-type mice. It is suggested that the big-conductance K channel in intercalated cells plays a role in K secretion through decreasing the intercalated cell size and increasing the luminal volume of the CNT and the CCD, thereby creating conditions more favorable for K secretion.

Although low activity of big-conductance K in principal cells was observed in the split-open CCD, two lines of evidence suggest the possibility that big-conductance K channels in principal cells may be involved in mediating renal K secretion: first big-conductance K channel activity in principal cells is increased in the CCD from rats on a high K diet [28]; second big-conductance K channel activity is significantly augmented by inhibition of PKA, protein kinase A (PKC) [36], P38, and ERK [28]. However, the observation that the deletion of the big-conductance K channel α subunit does not affect the net K excretion in mice fed with a high K diet suggests that HK-induced stimulation of ROMK channel expression and a high plasma aldosterone level can compensate for deleting big-conductance K channels on K secretion [33].

Voltage-gated K channels

In the CNT and CCD, a double-pore K channel (KCNK1) is expressed in the apical membrane [37–40]. However, the physiological function of the double-pore K channels in the CNT or CCD is still not clear. In addition, immunostaining has demonstrated that voltage-gated K channels such as ERG1 and KCNQ1 are expressed in the apical membrane of the DCT and collecting duct [41,42]. A genetic study has revealed that voltage-gated K channels (KCNK1), which are expressed in the apical membrane of the DCT, play a role in

regulating Mg^{2+} transport in the DCT [43*]. A missense mutation in the Kv1.1 voltage-gated K channel caused human autosomal dominant hypo-magnesemia. Figure 1 is a model illustrating the possible role of Kv1.1 in regulating Mg^{2+} transport in the DCT. The activation of voltage-gated K channels is expected to hyperpolarize the apical membrane, thereby increasing the driving force for Mg^{2+} absorption through transient receptor potential cation channel 6 (TRPM6). Defective Kv1.1 channel function would impair the hyperpolarization of apical membrane potential, thereby decreasing the driving force for transepithelial Mg^{2+} transport through the TRPM6.

Regulation of renal outer medullary potassium channels

Although the regulatory mechanisms of renal outer medullary potassium channels (Kir1.1) have been extensively explored for a decade, recent studies have identified novel factors that modulate the function of Kir1.1.

Protein kinase A and protein kinase C

Stimulation of PKA-induced phosphorylation increases ROMK channel activity [44–46] either by enhancing the insertion of ROMK channels into plasma membrane [47,48] or by augmenting the effect of PIP2 [49]. The effect of PKC on the ROMK channel is complex because PKC has both stimulatory and inhibitory effects [50,51]. PKC-induced phosphorylation of ROMK channels is required for export of ROMK1 channels to the cell membrane [50]. However, stimulation of PKC decreased the sensitivity of ROMK channels to PIP2 [51], which activates ROMK channels [52,53].

WNKs and kidney-specific (KS)-WNK1

WNKs are serine/threonine protein kinases [54] and WNK1, 3 and 4 are expressed in the CCD [54–56]. A large body of evidence indicates that the WNK family plays an important role in the regulation of ROMK channels [1,55,57–60]. Expression of WNK1, 3 and 4 inhibits the ROMK channel activity and the effect of WNKs on ROMK is mediated by stimulation of clathrin-dependent endocytosis [1]. Intersectin, a scaffold protein containing two Eps15 homology domains and four or five tandem SH3 domains, is required for the interaction between WNK4 and clathrin [61]. In addition, ROMK binds directly to autosomal recessive hypercholesterolemia (ARH), a clathrin adaptor [62*]. WNK1 stimulates ROMK endocytosis possibly by facilitating ARH-dependent recruitment of ROMK into clathrin-coated pits. A kidney specific splice form of WNK1 (KS-WNK1), in which an alternative 5' exon replaces the first four exons of WNK1, is expressed in the CCD [63]. Unlike the long form of WNK1, KS-WNK1 lacks kinase activity and does not block ROMK channels. Moreover, KS-WNK1 can antagonize the inhibitory effect of WNK1 [58,59]. It has been reported that high K intake increases the expression of KS-WNK1 and accordingly attenuates the inhibitory effect of WNK1 on ROMK channels [58,59]. Thus, the alteration of the ratio between the long and short form of WNK1 may be an important mechanism by which a high K intake stimulates ROMK channel activity. K-restriction has been reported to decrease the expression of KS-WNK1 and increase long form WNK1. Consequently, the inhibitory effect of KS-WNK1 on WNK1 is diminished and WNK1-

mediated inhibition of ROMK channels is enhanced in the CCD from animals fed on a low K diet [58].

Aldosterone and serum-glucocorticoids-induced kinase

SGK1 has been reported to stimulate ROMK1 channels by facilitating the phosphorylation of ROMK channels at serine residue (Ser) 44, thereby enhancing the export of ROMK channels from the endoplasmic reticulum (ER) [3,48]. SGK1 also suppresses the effect of WNK4 on ROMK channels through the phosphorylation of WNK4 at Ser 1169 [60] and 1196 [64*]. Thus, the C-terminus containing Ser 1169 and 1196 was called the 'switch-domain' for renal K secretion. Moreover, WNK1 has also been reported to stimulate SGK1 through PI3 kinase [65]. WNK1-induced activation of SGK1 could also play a role in mediating the effect of a high K intake on K secretion. A large body of evidence suggests that SGK1 mediates, at least in part, the effect of aldosterone on renal K secretion [60,66–68]. This notion is supported by studies performed in SGK1 knock-out mice demonstrating that the phenotype of SGK1 deletion is similar to MR knock-out mice and displays compromised renal K secretion in response to a high dietary K intake [68]. However, the immunostaining study performed in SGK1 knock-out mice shows that apical staining of ROMK channels in the CNT and CCD is normal or even intensified [68], suggesting that SGK1 function can be compensated by an alternative mechanism. Thus, the stimulatory effect of aldosterone and SGK1 on K secretion may be the result of increasing Na transport.

Interaction of SFK, SGK1, and WNK4

ROMK channels are the substrate of the SFK, and tyrosine residue 337 of ROMK1 is a phosphorylation site of the SFK. The tyrosine phosphorylation of ROMK is regulated by dietary K intake: a low K intake increases whereas a high K intake decreases the level of the tyrosine phosphorylated ROMK [69]. Moreover, the expression of the SFK such as c-Src and c-Yes increased in the renal cortex and outer medulla obtained from rats on a K-deficient diet and significantly decreased by a high K intake [2]. Thus, it is possible that the SFK is involved in mediating the inhibitory effect of a low dietary K intake on the ROMK-like small-conductance K (SK) channels. Moreover, c-Src modulates the effect of SGK1 on WNK4 and abolishes the SGK1-induced phosphorylation of WNK4. Figure 2 is a model illustrating the role of the interaction among c-Src, SGK1 and WNK4 in regulating ROMK channels and K secretion. As discussed above, WNK4 inhibits ROMK channels and K secretion in the CNT and the CCD. SGK1 reversed the inhibitory effect on ROMK channels by stimulation of WNK4 phosphorylation in serine residues 1169 and 1196. However, activation of c-Src inhibits the SGK1-mediated phosphorylation of WNK4, thereby restoring the inhibitory effect of WNK4 on ROMK channels and K secretion [70**]. The c-Src-dependent modulation of SGK1–WNK4 interaction may play a key role in preventing K loss during volume depletion and in stimulating K secretion during a high K intake. Both volume depletion and K secretion are expected to increase SGK1 expression. However, a high K intake has been shown to suppress SFK activity whereas volume depletion does not inhibit SFK expression (Wang, unpublished observation). Consequently, although volume depletion stimulates SGK1 activity, high SFK activity blocks the effect of SGK1 and prevents K loss. The model can also explain the finding that a high K intake failed to stimulate ROMK channels in adrenalectomized rats [71]. However, the upstream signaling which stimulates

SFK is still not known. K restriction has been shown to stimulate renin and the angiotensin II system [72–74]. Moreover, micro-puncture studies have revealed that luminal perfusion of angiotensin II inhibited K secretion in the distal nephron [75]. Patch-clamp experiments have further demonstrated that angiotensin II down regulates ROMK channels in the CCD and that such inhibition could be demonstrated only in the CCD from K-restricted rats [76].

Klotho

Klotho is an aging-suppressing protein which is expressed in several organs, including the kidney. The application of the Klotho extracellular domain increases the surface expression of ROMK1 and enhances urinary K excretion [77*]. The mechanism by which Klotho increases the expression of ROMK in the plasma membrane is by eliminating terminal sialic acids from N-glycan of ROMK. Consequently, underlying disaccharide galactose-N-acetylglucosamine is exposed to galectin 1, a ubiquitous galactoside-binding lectin, which inhibits the clathrin-dependent endocytosis of ROMK.

CD63 and POSH (plenty of SH3)

CD63 is a tetra-spanning protein and coexpression of CD63 increases c-Src activity by stimulating c-Src phosphorylation in tyrosine residue 416, thereby enhancing the c-Src-induced inhibition of ROMK channels [78]. POSH is an E3 ubiquitin ligase and expression of POSH enhances the ubiquitination of ROMK, suggesting that POSH is involved in ubiquitination of ROMK channels [79].

Regulation of big-conductance K channels

Several recent investigations have identified that PKA, PKC and cytochrome P450-epoxygenase-dependent metabolites of arachidonic acid regulate Ca^{2+} -activated big-conductance K channels and K secretion in the collecting duct.

Protein kinase A and protein kinase C

A patch-clamp study has demonstrated that PKA and PKC inhibit big-conductance K channel activity in the principal cells of the CCD [36]. The role of PKA and PKC in regulating the flow-stimulated big-conductance K-dependent K secretion in the CCD is also shown in the isolated perfused rabbit CCD. PKA induced a tonical inhibition of big-conductance K channels or an associated regulatory protein in principal cells. However, PKA has no inhibitory effect on big-conductance K channels in intercalated cells. It is possible that big-conductance K channels in principal cells and intercalated cells may be the result of different splice variants which respond to PKA in different ways.

Cytochrome P450 (CYP) epoxygenase

Big-conductance K channels in the principal cells are activated by CYP-epoxygenase-dependent metabolism of arachidonic acid, an effect which is also mimicked by 11,12-epoxyeicosatrienoic acid (11,12-EET), a main product of CYP-epoxygenase-dependent metabolism in the kidney [80*]. Because a high K intake increased 11,12-EET levels in the CCD and stimulated the expression of CYP2C44 homolog, a main CYP-epoxygenase in the kidney, 11,12-EET may play a role in stimulating big-conductance K channel-dependent K

secretion during a high K intake. This notion is also supported by the finding that inhibition of CYP-epoxygenase abolished the flow-stimulated and big-conductance K channel-dependent K excretion in the isolated rabbit CCD [80*].

Conclusion

K channels in the ASDN play an important role in regulating NaCl and Mg^{2+} transport and K secretion. Defective basolateral KCNJ10 in the DCT results in hypokalemia and Mg^{2+} wasting in the kidney whereas defective basolateral Kv1 causes autosomal dominant hypomagnesemia. The several new proteins such as intersectin, Klotho, and ARH have been shown to regulate clathrin-dependent endocytosis of ROMK channels. Also, interaction among SFK, SGK1, and WNK4 can play a key role in stimulating K secretion during a high K intake and preventing K loss during volume depletion. The role of the big-conductance K channel in mediating K secretion has been firmly established; PKA, PKC and 11,12-EET are involved in regulating big-conductance K channel-dependent K secretion in the CCD.

Acknowledgments

This work is supported by the NIH grant DK54983 and P01 HL34300.

References and recommended reading

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

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 508–509).

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Figure 1. A cell scheme illustrating the role of apical voltage-gated K channel (Kv1) in regulating Mg²⁺ transport and the role of basolateral Kir4.1/5.1 in regulating Cl and Mg²⁺ transport under control conditions (left panel) and under pathological conditions (right panel)

The loss-of-function mutation of Kv1 decreases the negativity of the apical membrane, thereby diminishing the driving force for Mg²⁺ absorption. Defective Kir4.1 causes depolarization of the basolateral membrane, resulting in the inhibition of Cl exit across the basolateral membrane and Mg²⁺ entry across the apical membrane. The dotted line indicates a diminished function.

Figure 2. A cell scheme illustrating the role of WNK family kinase in regulating ROMK channels and the role of c-Src and SGK1 in modulating WNK4 function in the CCD

WNK1, WNK3, and WNK4 inhibit ROMK channels in the CNT and the CCD. SGK1 reversed the inhibitory effect on ROMK channels by stimulation of WNK4 phosphorylation in serine residues 1169 and 1196. However, activation of c-Src inhibits the SGK1-mediated phosphorylation of WNK4, thereby restoring the inhibitory effect of WNK4 on ROMK channels and K secretion. Also, KS-WNK1 inhibits the WNK1 effect on ROMK channels. The downward arrows and bars indicate the inhibitory effects.