The Protein Tyrosine Kinase-Dependent Pathway Mediates the Effect of K Intake on Renal K Secretion

Dietary K intake plays an important role in the regulation of K secretion: a decrease stimulates and an increase suppresses kidney expression of protein tyrosine kinase (PTK), which plays a role in regulating Kir1.1 (ROMK), which is responsible for K secretion in the cortical collecting duct (CCD) and K recycling in the thick ascending limb. Tyrosine phosphorylation of ROMK channels increases with low dietary K and decreases with high dietary K. Moreover, stimulation of tyrosine phosphorylation of ROMK1 enhances ROMK1 internalization and reduces the K channel number in the cell surface in the CCD.

Maintaining plasma K within a normal “narrow range” is essential for the function of heart, skeletal muscle, and neurons. The majority of K is located in the intracellular fluid, and K content in the extracellular volume is ~70 meq. Therefore, a K-rich diet can easily double the K content in the extracellular fluid and raise the plasma K to a life-threatening level. However, under physiological conditions, plasma K is kept within a normal range and is not significantly affected by daily K intake within a reasonable range. This is achieved by both extrarenal and renal K handling (2, 6). Skeletal muscle is the most important extrarenal tissue, acutely regulating K homeostasis by shifting K from the extracellular fluid to the intracellular volume following a K-rich meal (14). However, the kidney is concerned predominantly with long-term K balance and is responsible for the final excretion of K taken through the meal. Thus, under normal conditions, dietary K intake and renal K excretion are in good balance for the adult. On the other hand, if the K intake is restricted, K is shifted from intracellular volume to extracellular fluid. Also, renal K excretion decreases to <1% of the control level. This is achieved by both suppression of K secretion in the cortical collecting duct (CCD) and stimulation of K absorption in the inner medullary collecting duct (6, 25). A large body of evidence indicates that the effect of dietary K intake on renal K secretion is partially mediated by the regulation of ROMK (Kir1.1) channel activity (20).

Function and Location of ROMK

The ROMK channel is a member of the inwardly rectifying K channel family with two transmembrane segments (8). Unlike other Kir channels, ROMK is a weakly inwardly rectifying K channel and allows a substantial outward K current. This property is essential for the physiological function of ROMK channels because they are responsible for K recycling in the thick ascending limb (TAL) and for K secretion in the kidney CCD (6). FIGURE 1A is a model showing the function of ROMK channels in the TAL and the CCD.

The role of ROMK in the TAL is K recycling, which is essential for maintaining the activity of the Na-K-2Cl cotransporter. The importance of K recycling in mediating NaCl reabsorption in the TAL is best demonstrated by genetic studies in which a defective gene product encoding ROMK caused salt wasting, metabolic alkalosis, and dehydration (Bartter’s disease) (18). The importance of K recycling can be illustrated by at least three aspects. First, K exit across the apical membrane tends to hyperpolarize the cell membrane potential, which increases the driving force for Cl diffusion across the basolateral membrane. If Cl diffusion is diminished due to decreases in Cl electrochemical gradient, increases in intracellular Cl concentrations could lead to inhibition of the Na-K-2Cl cotransporter. Second, K recycling is partially responsible for the lumen-positive potential, which is the driving force for transepithelial Na, Mg, and Ca reabsorption. It has been estimated that as much as 50% of Na content is absorbed via a paracellular shunt (7). Third, K recycling provides an adequate supply of K for the Na-K-2Cl cotransporter and is especially important in the cortical TAL because Na concentration is at least ten times higher than luminal K concentration. Patch-clamp experiments have identified two types of K channels, 30 pS and 70 pS, in the TAL (21). However, it is possible that ROMK is an important component of both K channels in the TAL. This notion is supported by the finding that neither 70-pS nor 30-pS K channels have been found in the TAL from ROMK-null mice (12, 13).
The ROMK channel plays an important role in K secretion in the CCD. K secretion takes place by a two-step process: K enters the cell across the basolateral membrane by Na-K-ATPase and is then secreted into the lumen along its electrochemical gradient via apical K channels. There are two types of apical K channels, ROMK and Ca-dependent maxi K, in the apical membrane of the CCD. ROMK channels are mainly responsible for K secretion during low rates of flow because the maxi K channel has a low open probability under such conditions. However, when the flow rate is high, maxi K channels are also involved in K secretion (26, 27). However, it is generally agreed that ROMK is mainly responsible for renal K secretion under normal conditions.

ROMK channels in the kidney have three alternative splicing forms: ROMK1, 2, and 3 (3). ROMK1 is exclusively expressed in the CCD, whereas ROMK3 is expressed in the TAL. ROMK2 is expressed in both the TAL and the CCD. Therefore, ROMK1 is solely involved in K secretion in the CCD, and FIGURE 1B shows the schematic structure of ROMK1, which has three putative PKA, three PKC, and one protein tyrosine kinase (PTK) phosphorylation site. It is well established that the effect of hormones such as vasopressin on renal K secretion is partially mediated by stimulating ROMK phosphorylation (21).

**Dietary K Intake Regulates ROMK Channel Activity**

Although a variety of hormones and factors regulates renal K secretion, aldosterone and dietary K intake play a key role in the regulation of renal K excretion. The mechanism by which aldosterone regulates K secretion is not completely understood. It is possible that the aldosterone-induced stimulation of K secretion is the result of an increase in the driving force for K secretion rather than a direct stimulation of ROMK. We (unpublished observations) and others (16) have observed that infusion of aldosterone or application of DOCA failed to increase the apical ROMK channel activity in the CCD (16). In contrast, aldosterone significantly

**FIGURE 1.** Model of the TAL and CCD and schematic structure of ROMK. **A:** model illustrates the location of ROMK and the function of the K channels in the TAL and CCD. **B:** schematic structure of ROMK1 shows a characteristic 2 transmembrane segments and putative phosphorylation sites for protein kinase A (PKA), protein kinase C (PKC), and protein tyrosine kinase (PTK).
increased the number of epithelial Na channels (ENaC). Also, low Na intake has been shown to increase the ENaC number in the CCD but has no effect on the apical ROMK channel activity in the CCD. Thus it is likely that the effect of aldosterone on renal K excretion is mainly induced by stimulation of Na-K-ATPase and ENaC activities. However, it has recently been reported that serum- and glucocorticoid-regulated kinase (Sgk) stimulates ROMK export to the plasma membrane (9, 28). Because aldosterone increases Sgk expression, it is possible that aldosterone may have a direct effect on ROMK via Sgk. However, further experiments in the renal tubules are required to explore the role of Sgk in the regulation of ROMK.

Dietary K intake has a direct effect on the activity of ROMK in both TAL and CCD. It has been observed that the ROMK channel activity increases within 6 h after high K intake in the CCD (17). The effect of high K on ROMK channels is most likely mediated by stimulation of the previously “silent” ROMK channels rather than through stimulation of ROMK transcription, because mRNA levels of ROMK are not affected by high K intake (5). K restriction for 1 wk significantly decreases ROMK channel activity in the CCD compared with a “normal-K” diet (1.1% K). However, the difference between ROMK channel activity in the CCD from rats on a 0.7% K-containing diet and those on a K-deficient diet is not significant. Therefore, the different K content in a normal-K diet may be responsible for the discrepancy regarding the effect of low K intake on ROMK channel activity in the CCD. Moreover, K restriction causes hypokalemia and a low plasma K may inhibit Na-K-ATPase. Because ROMK channel activity is coupled to the Na-K-ATPase activity (21), it is possible that ROMK channel activity in the CCD is further suppressed by a diminished Na-K-ATPase activity during K restriction. Therefore, ROMK channel activity in the CCD from K-restricted animals should be lower than that measured in the split-open tubule because the experimental conditions may not mimic in vivo conditions. Although the TAL is not directly involved in renal K secretion, it could affect the final renal K excretion through the alteration in K absorption in the TAL. We observed that dietary K intake also has an effect on the ROMK channel activity in the TAL: low K intake suppresses and high K intake increases apical K channel activity. Thus K intake has an effect on ROMK channel activity in the TAL and the CCD. Because dietary K intake-mediated regulation of ROMK channel activity is not always mimicked by aldosterone, it is possible that the effect of K levels in the diet on ROMK channels is mediated by an aldosterone-independent mechanism.

Expression of PTK is Regulated by Dietary K Intake

In searching for the aldosterone-independent signaling that mediates the effect of K intake on ROMK channel activity and renal K secretion, it has been found that dietary K intake regulates PTK expression and activity. It has been observed that the activity of PTK is higher in the kidney from K-restricted rats than from those on a high-K diet (22). In addition, K restriction significantly increases the expression of c-Src and c-Yes, two representative members of Src family PTK (24). Also, immunostaining indicates that c-Src is expressed in the TAL and the CCD (11). Figure 2 is a confocal image showing that c-Src is expressed in tubules that have a positive ROMK staining in the cortex (Figure 2A) and in the outer medulla (Figure 2B). To further demonstrate that c-Src is present in the TAL and CCD, double staining was conducted using aquaporin 2 as an indicator of the CCD (Figure 2C) and Tamm-Horsfall protein as a marker of the TAL (Figure 2D), respectively. Figure 2, C and D are confocal images demonstrating that c-Src is highly expressed in the CCD and TAL. Although the mechanism by which low K intake stimulates PTK expression is not completely understood, we speculate that increases in superoxide levels in the kidney (Wang WH, unpublished observations) could be involved in mediating the effect of low K intake on PTK expression. In this regard, it has been reported that hormones such as angiotensin II and growth factors can stimulate superoxide production (1, 4).

ROMK is a Tyrosine-Phosphorylated Protein, and Tyrosine Phosphorylation of ROMK is Regulated by K Intake

Not only is the Src family PTK expressed in the TAL and CCD, but ROMK is also a substrate of PTK. A putative PTK tyrosine phosphorylation site is located in the COOH terminus of ROMK (tyrosine residue 337 for ROMK1). An in vitro tyrosine phosphorylation assay indicated that 32P is incorporated into purified ROMK1 protein in the presence of exogenous c-Src, whereas 32P incorporation is absent in ROMK1 mutant R1Y337A, in which tyrosine residue 337 is mutated into alanine. Also, c-Src is able to phosphorylate the synthesized peptide corresponding to amino acids 333–362 of the COOH terminal of ROMK1. This suggests that tyrosine residue 337 of ROMK1 is the site for tyrosine phosphorylation (10). Although direct evidence showing that ROMK2 and ROMK3 are substrates for PTK is absent, it is conceivable that ROMK2 and 3 should also be phosphorylated by PTK because
the putative PTK phosphorylation site is present in the COOH terminus of ROMK 2 and ROMK3. In addition, a strong tyrosine phosphorylation signal of ROMK is observed in the outer medulla of the kidney, where >90% of nephron segments are the TAL.

The possibility that PTK is involved in the regulation of K channel activity by K intake is further suggested by the finding that K restriction has significantly augmented the tyrosine phosphorylation of ROMK, whereas high K intake significantly reduced the tyrosine phosphorylation of the ROMK (10). Therefore, these data strongly suggest that K intake regulates ROMK channel activity via changing tyrosine phosphorylation levels of ROMK.

**Stimulation of Tyrosine Phosphorylation of ROMK1 Decreases the Channel Activity**

After establishing that low K intake increases PTK activity and tyrosine phosphorylation of ROMK, we...
investigated whether increases in tyrosine phosphorylation of ROMK1 inhibit channel activity because ROMK1 is exclusively expressed in the CCD and is responsible for K secretion. To stimulate the tyrosine phosphorylation of ROMK1, we coexpressed c-Src and ROMK1 in cells and studied the effect of PTK on ROMK1 channel activity. Coexpression of ROMK1 with c-Src significantly decreased ROMK1 current in *Xenopus* oocytes injected with ROMK1 and c-Src (15). Also, herbimycin A and genistein, inhibitors of PTK, were employed to study the effect of tyrosine phosphorylation on ROMK1 channel activity. The usefulness of herbimycin A as a tool for the study of tyrosine phosphorylation of ROMK is confirmed by the observation that herbimycin A decreases the tyrosine phosphorylation of ROMK1 expressed in human embryonic kidney cells (19). Inhibition of PTK with genistein or herbimycin A augmented the K current in the oocytes injected with ROMK1 and c-Src (17). The notion that increases in tyrosine phosphorylation decrease ROMK1 current is also supported by experiments in which inhibition of PTK increases ROMK channel activity in the CCD. Interestingly, increases in channel activity induced by inhibition of PTK is modest in rats on a normal-K diet and is progressively enhanced by prolonged K depletion. Maximal channel stimulation has been reached after rats were on a K-deficient diet for 5–7 days (24). The observation that prolonged K restriction enhances the effect of inhibiting PTK on ROMK1 is consistent with the finding that PTK expression induced by low K intake is at the highest level at 7 days.

If inhibition of tyrosine phosphorylation increases ROMK1 channel activity, suppression of protein tyrosine phosphatase (PTP) activity should have an opposite effect. To study the role of PTP in the regulation of ROMK1, phenylarsine oxide (PAO), an agent that blocks PTP, has been used to stimulate tyrosine phosphorylation of ROMK1. The notion that inhibition of PTP stimulates the tyrosine phosphorylation of ROMK1 is supported by experiments in which the tyrosine phosphorylation level of ROMK1 was doubled in human embryonic kidney cells treated with PAO (19). Also, the patch-clamp experiments have further demonstrated that PAO inhibits the activity of ROMK1 channels in oocytes injected with ROMK1 and c-Src (15) and in the isolated CCD (23). These results strongly suggest that the ROMK1 channel activity is regulated by tyrosine phosphorylation. Increases in tyrosine phosphorylation inhibit and decreases in tyrosine phosphorylation increase the ROMK1 channel activity.

**Tyrosine Phosphorylation of ROMK1 Stimulates Endocytosis**

The inhibitory effect of PTK on ROMK1 is not the result of changing the channel open or close kinetics because addition of exogenous c-Src failed to inhibit the activity of ROMK1 expressed in oocytes (15). A large body of evidence suggests that stimulation of tyrosine phosphorylation of ROMK1 may increase the internalization of the K channel. First, treatment of oocytes injected with ROMK1 and c-Src with a hypertonic solution that is known to block endocytosis abolished the inhibitory effect of PAO on ROMK1. Second, treatment with concanavalin A, an agent that has been shown to block the endocytosis of membrane protein, abolished the PTP-induced decrease in K current in oocytes.
injected with ROMK1 and c-Src (15). Third, confocal microscopy has revealed that the intensity of green fluorescent protein-tagged ROMK1 in the cell membrane diminished significantly in oocytes injected with c-Src and ROMK1 (15). Finally, biotin labeling has shown that PAO treatment in human embryonic kidney 293 cells transfected with ROMK1 and c-Src reduced the surface ROMK1 density (19). These results strongly support the view that stimulation of tyrosine phosphorylation of ROMK1 enhances the internalization of the K channel. The notion that stimulation of tyrosine phosphorylation of ROMK1 increases its internalization is also supported by experiments in which the ROMK location in the CCD was examined in the PAO-treated and untreated CCD tubules from rats on a high-K diet.

**FIGURE 3A** is a confocal image showing a clear apical membrane staining of ROMK in the CCD from rats on a high-K diet. In contrast, inhibition of PTP with PAO increases the ROMK staining in the intracellular compartment in the CCD (**FIGURE 2B**). An increase in the intracellular ROMK staining is also observed in the CCD from rats on a K-deficient diet, which stimulates PTK activity in the kidney.

**Conclusion**

**FIGURE 4** is a model of CCD illustrating the current understanding regarding the effect of K intake on renal K secretion. A high K intake increases aldosterone levels, which in turn stimulate K secretion by augmenting the driving force for K secretion. Also, high aldosterone may increase Sgk activity, which has been shown to enhance the delivery of ROMK to the plasma membrane in Xenopus oocytes (28). In addition, high K has an aldosterone-independent mechanism which stimulates ROMK channel activity in the CCD. In contrast, a low K intake increases PTK activity and stimulates tyrosine phosphorylation of ROMK in the CCD. As a consequence, ROMK channels in the CCD are internalized.

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Because of space limitations, it is not possible to cite all relevant papers. We apologize for this.

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