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Regulation of ROMK (Kir1.1) channels: new mechanisms and aspects

Wen-Hui Wang

Department of Pharmacology, New York Medical College, Valhalla, New York

Abstract

This brief review attempts to provide an overview regarding recent developments in the regulation of ROMK channels. Studies performed in ROMK null mice suggest that ROMK cannot only form homotetramers such as the small-conductance (30-pS) K channels but also construct heterotetramers such as the 70-pS K channel in the thick ascending limb (TAL). The expression of ROMK channels in the plasma membrane is regulated by protein tyrosine kinase (PTK), serum and glucocorticoid-induced kinase (SGK), and with-no-lysine-kinase 4. PTK is involved in mediating the effect of low K intake on ROMK channel activity. Increases in superoxide anions induced by low dietary K intake are responsible for the stimulation of PTK expression and tyrosine phosphorylation of ROMK channels. Finally, a recent study indicated that ROMK channels can be monoubiquitinated and monoubiquitination regulates the surface expression of ROMK channels.

The ROMK channel plays an important role in K recycling in the thick ascending limb (TAL) and K secretion in the connecting tubule (CT) and cortical collecting duct (CCD) (16,19,34,43,45,61). Although maxi-K channels are also possibly involved in the regulation of K secretion in the CCD (66,67), it is generally agreed that ROMK channels are mainly responsible for K secretion under normal conditions. The regulation of ROMK channels has been extensively studied, and a recent review has provided extensive coverage regarding the regulatory mechanism of ROMK in the past (16). Thus this review is meant to emphasize the newest developments in the field, which may not have been included in previous reviews. Several studies have identified new amino acids that are involved in the regulation of pH sensitivity of ROMK1 channels in addition to lysine residue 80 (14). Also, it has been shown that changes in extracellular K concentrations can affect the sensitivity of ROMK channels to cell pH (9,49). Although these topics are important to an understanding of the function of ROMK channels, they are beyond the focus of the present review.

ROMK IS A KEY COMPONENT OF THE APICAL K CHANNELS IN THE TAL

Apical K channels play an important role in K recycling, which is essential for maintaining the normal function of the Na-K-Cl cotransporter in the TAL (16,61). Patch-clamp experiments demonstrated that two types of K channels, a 30- to 40- and a 70- to 80-pS, are expressed in the apical membrane of the TAL (3,59,62). It is well established that the 30-pS K channel is related to ROMK because it has similar biophysical properties and regulatory mechanisms to that in native tubules (61). However, it is not clear whether ROMK is also involved in forming the apical 70-pS K channel. The observation that the loss-of-function

mutations of ROMK result in severe salt wasting and metabolic alkalosis (Bartter's disease) (34,51) indicates strongly that ROMK is an important component of both the 30- and 70-pS K channels in the TAL. Because 70-pS K channels could contribute as much as 60–70% of apical K conductance in the TAL of the rat kidney (36), it is hard to imagine that loss-of-function mutations of ROMK could impair the NaCl transport in the TAL if ROMK is only responsible for the construction of the 30-pS K channels. This puzzle is partially resolved by the patch-clamp experiments performed in the TAL from ROMK null mice in which neither 70- nor 30-pS K channels are detected. In addition, ROMK null mice have demonstrated a typical phenotype of Bartter's syndrome: severe salt wasting, metabolic alkalosis, and hypokalemia (34). This suggests strongly that the 70-pS K channel is a heterotetramer including ROMK and an unidentified subunit. Alternatively, deletion of the ROMK gene could indirectly suppress the expression of the 70-pS K channel. However, the first possibility is further suggested by the observation that high K intake doubled the number of the 70-pS K channels while reducing the number of the 30-pS K channels in the TAL from both wild-type and ROMK (+/-) mice (35). It is possible that high K intake facilitates the formation of the 70-pS K channels by decreasing the formation of the 30-pS K channels. This is achieved by increasing reconstruction between ROMK and other unidentified subunits (Fig. 1) to form the heterotetramer (16). The conductance of the heterotetramer K channel could be different from that of the homotetramer. Relevant to this is the acetylcholine-activated K channel, which is composed of GIRK and cardiac inwardly rectifying K (CIR) channels. Expression of CIR alone in oocytes produces a K channel with single-channel conductance of 15 pS, whereas the coexpression of CIR and GIRK forms an acetylcholine-activated K channel with a single-channel conductance of 37 pS (26). It is possible that the 70-pS K channel may be formed by ROMK and another inwardly rectifying K channel. However, the nature of the unidentified subunit of the 70-pS K channel remains to be determined.

The abundance of ROMK channels in the TAL is regulated by hormones and Na diet. It has been shown that infusion of vasopressin into Brattleboro rats for 7 days increased the apical labeling of ROMK in the TAL (13). The role of vasopressin in the regulation of ROMK channel expression is further indicated by the finding that restriction of water intake can also significantly increase ROMK expression in the TAL. Also, ROMK expression is upregulated in the TAL in response to high Na intake (13). This suggests that ROMK channels and Na-K-Cl cotransporters are possibly coordinately regulated in the TAL by vasopressin and Na intake.

NEW ASPECTS OF THE EFFECTS OF PKC AND CFTR ON ROMK

Regulation by PKC

ROMK1 channels have three putative PKC phosphorylation sites: serine residues (Ser) 4, 181, and 201 (19). In vitro phosphorylation has shown that ROMK1 can be phosphorylated by PKC on Ser4 and Ser201 (29). Moreover, stimulation of PKC has been shown to inhibit ROMK channel activity in native tubules (28,60). However, the K current in oocytes injected with ROMK1, S4D or ROMK1, S4D/S201D in which the serine residue was mutated to aspartate was either unchanged or increased, suggesting that the PKC-induced inhibition of ROMK1 may not be the result of direct phosphorylation. This possibility was further suggested by the finding that stimulation of PKC with phorbol ester reduced phosphatidylinositol 4,5-bisphosphate (PIP₂) content in oocyte membrane and inhibition of PKC abolished the phorbol ester-induced decrease in PIP₂ (74). Because PIP₂ has been shown to maintain ROMK channels in an open state (20,33), it is possible that decreases in PIP₂ concentration induced by stimulating PKC may contribute to the inhibition of ROMK channel activity. In addition, PKC has been shown to regulate the surface expression of ROMK and endocytosis of ROMK induced by stimulation of PTK (29,54). We

demonstrated that mutation of Ser4 and Ser201 to alanine significantly suppressed the surface expression of ROMK1 in oocytes (29). This suggests that direct phosphorylation of ROMK1 by PKC is important for the surface expression of K channels in oocytes. On the other hand, we also observed that inhibition of PKC blocked the internalization of ROMK1 induced by stimulation of PTK (54). This seeming discrepancy may be the result of different PKC targets. It is possible that the role of PKC in mediating the internalization of ROMK channels may be achieved by targeting factors other than ROMK. Relevant to this hypothesis is the observation that PKC is involved in actin reorganization (5). Thus the complexity of PKC's effects on ROMK may be induced because PKC regulates ROMK channel activity by direct and indirect mechanisms.

Regulation by CFTR

The role of CFTR in the regulation of ROMK channel activity has been demonstrated previously. CFTR has been shown to play an important role in mediating sulfonylurea agent-induced inhibition and ATP sensitivity (38,48), although it has also been reported that ROMK channels have an intrinsic sensitivity to sulfonylurea agents (24). A recent study suggests that CFTR may play a role in mediating the interaction between ROMK and the epithelial Na channel (ENaC). Previous studies have shown the interaction between ENaC and CFTR such that stimulation of CFTR reduced ENaC activity (57), whereas coexpression of ENaC increased CFTR expression (25). Recently, it also has been reported that CFTR may link the activity of ENaC to ROMK. In the presence of CFTR, expression of ROMK channels was augmented in oocytes injected with ENaC (25). Also, the PDZ domain has been shown to facilitate the interaction between ROMK and CFTR (70). In contrast, coexpression of ENaC had no effect on ROMK expression in the absence of CFTR. This suggests that CFTR synchronizes the activity of ENaC and ROMK. However, whether such interaction takes place in the native tubule is not clear and needs to be examined.

SUPEROXIDE IS INVOLVED IN THE REGULATION OF ROMK CHANNEL ACTIVITY

ROMK1 is exclusively located in the CT and CCD (4) and plays an important role in K secretion. A large body of evidence indicates that the number of ROMK channels in the CCD is varied such that high K intake increases (44,46), whereas low K intake decreases, ROMK channel numbers in the CCD (2). The response of rats to low K is very sensitive because decreases in K content from 1.1 to 0.7% significantly reduced ROMK channel activity in the CCD to an extent not significantly different from that in rats on a K-deficient diet. This could be the reason the previous study failed to detect a significant difference in ROMK channel activity in the CCD between rats on a K-deficient diet and "normal K diet (0.7%)" (64). Several lines of evidence strongly suggest that the PTK-dependent signal transduction pathway plays a key role in mediating the effect of low K intake on ROMK channel activity. First, low K intake significantly increases the expression Src-family PTK such as c-Src and c-Yes in the kidney (64). Second, the Src family PTK is expressed in principal cells of the CCD and CT (32). Third, low K intake enhances whereas high K intake diminishes the tyrosine phosphorylation of ROMK (30). Finally, inhibition of PTK increases whereas blockade of protein tyrosine phosphatase (PTP) decreases the ROMK channel activity in the CCD (63,64). Studies with confocal microscopy have further indicated that stimulation of PTK-induced phosphorylation of ROMK1 facilitates the internalization (55,56). These data suggest that low K intake stimulates the activity of Src family PTK which, in turn, enhances the tyrosine phosphorylation of ROMK channels in the CCD. As a consequence, ROMK channels are internalized and the density of ROMK in the apical membrane decreases.

Although the role of PTK in mediating the effect of low K intake on ROMK channel activity has been established, the upstream signaling which regulates PTK activity is not completely understood. A recent study suggests the possible role of superoxide in the regulation of expression of Src family PTK. Using a chemiluminescence technique (68), it was found that low K intake significantly increased the concentration of superoxide anions in the renal cortex and outer medulla (2) and that the superoxide production induced by low K intake was blunted by tempol, a superoxide dismutase mimetic (27). The role of superoxide in mediating the effect of low K intake on PTK expression was further suggested by observations that treatment of M1 cell, a mouse principal cell line, with hydrogen peroxide significantly increased the expression of c-Src. The effect of hydrogen peroxide on c-Src expression was abolished by either cycloheximide or actinomycin A. This indicates that the stimulatory effect of hydrogen peroxide on c-Src expression is the result of increased transcription and translation. Indeed, Western blot analysis revealed that low K intake increased the phosphorylation of c-Jun on Ser73, an indication of activation of c-Jun. The view that increases in superoxide anions may stimulate transcription of PTK was also suggested by experiments in which treatment of M1 cells with hydrogen peroxide significantly increased the serine phosphorylation of c-Jun. Moreover, tempol treatment completely abolished the effect of low K intake on c-Jun activation and c-Src expression in the kidney of rats fed a K-deficient diet.

The possibility that superoxide is involved in the regulation of ROMK channel activity and renal K excretion is supported by several lines of evidence (2). First, lowering superoxide production in the kidney by tempol decreased the tyrosine phosphorylation of ROMK in the renal cortex and outer medulla and increased the ROMK channel activity in the CCD. Second, metabolic cage studies showed that the renal K excretion in the tempol-treated rats was 10 times higher than those in rats on a K-deficient diet alone. Third, animals receiving tempol developed a more severe hypokalemia than those without tempol treatment. Figure 2 is a model of principal cell illustrating the mechanism by which low K intake inhibits ROMK1 channel activity. Low K intake increases the production of superoxide, which, in turn, stimulates PTK expression. As a consequence, tyrosine phosphorylation of ROMK channels in the CCD increases and the ROMK channel is endocytosed. In addition to stimulation of PTK expression, superoxide and the related product have also been shown to stimulate PTK (6,42) or inhibit the activity of PTP (10,37). Thus it is possible that superoxide may be able to acutely regulate ROMK channel activity through modulation of PTK and PTP interaction. Indeed, we have observed that acute application of hydrogen peroxide inhibited ROMK channel activity in the rat CCD and that the effect was blocked by herbimycin A (unpublished observations), indicating that the effect of hydrogen peroxide on ROMK channel activity is due to stimulation of PTK activity. Although the role of superoxide in mediating the effect of low K intake on renal K secretion has been suggested, the mechanism by which low K intake stimulates superoxide production is still not clear. Because the major source of superoxide is from activation of NAD(p)H oxidase (12,15,39,40), it would be interesting to determine the role of NAD(p)H oxidase in the regulation of renal K secretion.

REGULATION OF ROMK CHANNEL TRAFFICKING

A large body of evidence indicates that ROMK channel activity is regulated by endocytosis and exocytosis. ROMK channels have been shown to interact with clathrin via AP-2 protein (73). Recently, it has been demonstrated that syntaxin 1A interacts with the COOH terminus of ROMK and that coexpression of syntaxin 1A inhibits ROMK channel activity in oocytes (58). Also, several new mechanisms including monoubiquitination, serum and glucocorticoid-inducible kinase (SGK), and with-no-lysine-kinase 4 (WNK4) have been discovered in the regulation of ROMK expression in plasma membrane.

Monoubiquitination

Ubiquitination plays an important role in the regulation of protein degradation and recycling by attaching ubiquitin molecules to lysine residues of substrate protein (8,17,47).

Ubiquitination can further be classified into monoubiquitination by adding only one or two ubiquitin molecules to the substrate protein or polyubiquitination by attaching more than four ubiquitin molecules. The polyubiquitinated protein is subjected to degradation, whereas monoubiquitinated proteins are targeted to internalization and possibly recycling to the cell membrane (11,18,50). A large body of evidence indicates that polyubiquitination plays an important role in the regulation of cell signaling and ENaC (52,53). However, the role of monoubiquitination in the regulation of membrane transporters and ion channels is not clear. Recently, it has been shown that ROMK channel activity could be regulated by monoubiquitination (31). First, immunoprecipitation of ROMK from renal tissue or from cell culture revealed a 50-kDa protein band that was also recognized by an ubiquitin antibody. Second, mutation of lysine residue 22 to arginine (R1K22R) in the NH₂ terminus of ROMK1 increased ROMK channel activity. Third, no ubiquitinated ROMK can be detected in oocytes injected with a ROMK1 mutant, R1K22R. Moreover, the biophysical properties of R1K22R such as channel conductance and open probability are the same as that of wt ROMK. Thus an increase in ROMK channel activity must be the result of augmentation of membrane expression of ROMK1 channels. This view is also supported by the observation that a higher biotin-labeled ROMK1, an indication of membrane expressed ROMK1, was observed in cells transfected with ROMK1 mutant than those of ROMK1.

The physiological importance of monoubiquitination in the regulation of ROMK channel is still not clear. Because ROMK channel activity in the CCD decreases in response to stimulation of PTK, it would be interesting to determine whether monoubiquitination is required for the PTK-induced internalization of ROMK1 in the CCD. Figure 3 is a model of a cell illustrating a possible role of ubiquitination in the regulation of ROMK trafficking. Stimulation of PTK has been shown to activate ubiquitin ligase activity (23). It is possible that PTK could enhance the ubiquitination of ROMK and accordingly internalization.

SGK1

Aldosterone has been shown to stimulate glucocorticoidinducible kinase 1 (SGK1) levels (41), which are possibly involved in mediating the effect of aldosterone on ENaC activity (1,7). SGK1 was first thought to have no effect on ROMK channel activity (7). As a matter of fact, ROMK was used as a negative control to demonstrate that the stimulatory effect of SGK1 on ENaC was specific. However, three separate studies have later demonstrated that SGK1 can also increase the activity of ROMK in the presence of Na/H exchangeregulating factor (NHERF) (70–72). It is possible that NHERF is served as a scaffold protein required for the stimulatory effect of SGK1 on ROMK. However, the role of NHERF, specifically NHERF2 in mediating the SGK effect, is controversial. Yoo et al. (71) observed that SGK could stimulate ROMK channel without NHERF. Moreover, they observed a modest stimulation of ROMK surface expression by NHERF alone. They suggested that NHERF is required for the effect of SGK on ROMK only when abundance of one of them is low. The mechanism of the SGK1 effect may be through stimulation of the serine phosphorylation of ROMK1. Confocal microscopy and cell surface antibody-binding assay have further shown that stimulation of SGK-induced phosphorylation increases the expression of ROMK1 channels in plasma membrane. The phosphorylation site of ROMK1 induced by SGK is located on Ser44 in the NH₂ terminus, which is also a putative PKA phosphorylation site (69) because mutation of Ser44 to alanine abolished the effect of SGK (71). Also, Yoo et al. (71) suggested that effect of SGK on ROMK activity in oocytes depends on the basal level of ROMK phosphorylation such that the stimulatory effect of SGK could be blunted when the basal phosphorylation level of ROMK is high. The role of SGK on K secretion is also

demonstrated in SGK null mice in which renal K secretion is impaired (21). However, it is still not known whether the stimulatory effect of SGK on renal K secretion is the result of increased ENaC activity that, in turn, augments the electrochemical gradient for K secretion or due to a direct stimulation of ROMK channel insertion. Confocal microscopy has shown that ROMK expression in the apical membrane of the TAL and CCD is actually increased rather than decreased in SGK null mice. This suggests that the role of SGK in stimulating ROMK insertion may not be essential or can be replaced by other kinases such as cAMP-dependent protein kinase A. A possible role of SGK is to regulate renal K secretion in response to a daily dietary K intake. It has been shown that a high dietary K intake for 12 h stimulates SGK expression in the kidney (71). Interestingly, it has been shown that ROMK channel activity significantly increased in the CCD from rats on a high-K diet for only 6 h (46). Thus SGK may regulate ROMK channel activity in the CT and CCD in response to a daily variation in dietary K intake.

WNK4

WNK4 is a serine-threonine kinase and has been identified in both distal convoluted tubule (DCT) and the CCD. Wild-type WNK4 has been shown to suppress the expression of the Na-Cl cotransporter in the plasma membrane of the DCT and, accordingly, to inhibit Na absorption (65). The gene product encoding inactivated WNK4 causes pseudohypoaldosteronism type II, a disease with characteristics of hypertension and low aldosterone levels. WNK4 is also expressed in the CCD, and expression of WNK4 in oocytes decreased the expression of ROMK1 (22). However, the inhibitory effect of WNK4 on ROMK1 does not require the kinase activity because coexpression of inactivated WNK4 also resulted in an inhibition of the expression of ROMK1 in *Xenopus laevis* oocytes. The physiological role of WNK4 may be that it serves as a switch between Na transport and K secretion. The WNK4-induced inhibition of Na transport in the DCT is expected to increase Na delivery to the CCD and enhance Na absorption, which leads to augmentation of the driving force for K secretion. However, because WNK4 inhibits ROMK channel activity, increases in electrochemical gradient for K secretion would have a diminished effect on renal K secretion. Mutation of WNK4 would release the inhibition of the Na-Cl cotransporter and lead to stimulation of Na transport in the DCT. Moreover, inactivated WNK4 further decreases the surface expression of ROMK, reduces K secretion in the CCD, and causes hyperkalemia. However, it is possible that decreases in Na delivery to the CCD may also be responsible for the diminished K secretion in patients with pseudohypoaldosteronism type II.

SUMMARY

Figure 3 is a model of the principal cell, illustrating the possible mechanisms by which ROMK channel expression in plasma membrane is regulated by WNK4, SGK, and ubiquitin. Stimulation of PTK decreases, whereas activation of SGK1 increases, the expression of ROMK. Also, increased WNK4 expression should diminish ROMK channel activity.

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Fig. 1. Model of cell in the thick ascending limb showing the role of ROMK channels in forming the 30- and 70-pS K channels and the effect of a low- and high-K diet on the formation of the two K channels.

Fig. 2.
Model of principal cell showing the mechanism by which low K intake inhibits ROMK channel activity in the cortical collecting duct.

Fig. 3. Model of principal cell demonstrating the effect of PTK, ubiquitination, with-no-lysine-kinase 4 (WNK4), and serum and glucocorticoid-induced kinase (SGK)1 on the expression of ROMK channels in plasma membrane. Y and U, tyrosine phosphorylation of ROMK, respectively.