Regulation of the ROMK channel: interaction of the ROMK with associate proteins

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Wang, WenHui. Regulation of the ROMK channel: interaction of the ROMK with associate proteins. Am. J. Physiol. 277 (Renal Physiol. 46): F826–F831, 1999.—The ROMK channel plays an important role in K recycling in the thick ascending limb (TAL) and K secretion in the cortical collecting duct (CCD). A large body of evidence indicates that the ROMK channel is a key component of the native K secretory channel identified in the apical membrane of the TAL and the CCD. Although the ROMK channel shares several key regulatory mechanisms with the native K secretory channel in a variety of respects, differences in the channel modulatory mechanism are clearly present between the ROMK channel and the native K secretory channel. Therefore, it is possible that additional associate proteins are required to interact with the ROMK channel to assemble the native K secretory channel. This notion is supported by recent reports showing that cystic fibrosis transmembrane conductance regulator (CFTR) and A kinase anchoring proteins (AKAP) interact with the ROMK channel to restore the response to ATP sensitivity and protein kinase A stimulation. This review is an attempt to summarize the up-to-date progress regarding the interaction between the ROMK channel and the associate proteins in forming the native K secretory channel.

adenosine 5’-triphosphate-sensitive potassium channel; cystic fibrosis transmembrane conductance regulator; protein kinase A; A kinase anchoring protein

IT IS WELL ESTABLISHED THAT ion channels are associated with a variety of molecules to achieve the physiological function of the ion channels (54). In addition to coupling to auxiliary subunits that modulate the biophysical properties of the pore-forming subunit, the ion channels have been shown to interact with scaffold proteins and cytoskeleton. The interactions are essential for the channel regulation and targeting (54). For instance, the interaction between nitric oxide synthase and the N-methyl-D-aspartate (NMDA) receptor in postsynaptic membrane (6, 7, 9) is achieved by PDZ-domain-containing scaffold proteins, which are colocalized with neuronal nitric oxide synthase (12, 13) and involved in clustering the NMDA receptor (70).

The ROMK channel is an inward-rectifying K channel expressed mainly in the kidney (28, 35). Immunocytochemical studies and in situ hybridization revealed that the ROMK channel is present in the apical membrane of the thick ascending limb (TAL), distal tubule, and collecting duct (44, 71). The ROMK channel plays an important role in K recycling in the TAL and K secretion in the cortical collecting duct (CCD) (69). Although it is well established that the ROMK channel is the key component of the native K secretory channel identified in the apical membrane of the TAL and the CCD, the difference in terms of response to ATP and protein kinase A (PKA) stimulation between the ROMK channel and the native K secretory channel is apparent. Therefore, this review is focused on exploring the possibility that the associate proteins are required to assemble the native low-conductance K secretory channel.

PHYSIOLOGICAL ROLE OF THE ROMK CHANNELS

Extensive studies in the past several years have convincingly demonstrated that the ROMK channel is the key component of the renal low-conductance K secretory channel in the apical membrane of the TAL and the CCD (69). First, the ROMK channel has a channel conductance, open probability, and channel kinetics similar to those observed in the native tissue (47). Second, the location of the ROMK channel protein is consistent with the distribution of the native K secretory channel (44, 71). Finally, the mechanism by which the ROMK channel is regulated is reminiscent of the native K secretory channel (69).

The ROMK channel serves at least two cell functions: K recycling in the TAL, and K secretion in the CCD (23,
69). K recycling across the apical membrane of the TAL is important for NaCl reabsorption and the diluting mechanism in the TAL (25, 26). Three functions are served by K recycling across the apical membrane of the TAL (22). First, K recycling hyperpolarizes the cell membrane potential and, accordingly, provides the driving force for Cl diffusion across the basolateral membrane. Second, K recycling is essential for the lumen-positive potential, which is the driving force for the transepithelial Na reabsorption. Third, K recycling provides an adequate supply of K for the Na-K-Cl cotransporter. The importance of the ROMK channel in maintaining the normal function of the TAL is best demonstrated by genetic studies in which mutated gene products encoding the ROMK channel led to abnormal salt transport in human kidneys, i.e., Bartter’s syndrome (22, 58). Also, the ROMK channel plays a key role in K secretion in the CCD (69). The K secretion takes place by K entering the cell across the basolateral membrane via Na-K-ATPase and then secreting into the lumen through the apical K channels. It is believed that the ROMK channel provides the major route for K movement across the apical membrane of the CCD. This notion was supported by the recent report that mutations of the ROMK channel caused a defective renal K secretion in the neonatal human kidney (57).

**ATP REGULATES THE NATIVE K SECRETORY CHANNEL**

The K secretory channel is an ATP-sensitive K channel. Patch-clamp studies demonstrated that millimolar concentrations of Mg-ATP and sulfonylurea agents, such as glyburide, inhibit the channel activity (63). However, nonhydrolyzable ATP analogs failed to inhibit the channel activity at millimolar concentrations (64, 68). In addition to ATP, ADP can also block the low-conductance K channel. The ATP-induced channel inhibition can be relieved by a typical ATP-sensitive K channel opener, such as chromakalim (65). In contrast to the classic ATP-sensitive K channel in the pancreatic tissue, renal ATP-sensitive K channel has low affinity to the ATP and sulfonylurea agents (48). The ATP sensitivity of the K secretory channel was modified by several factors including PKA activity, intracellular pH, and ADP (69). Increases in PKA activity and ADP relieved the ATP-induced inhibition. The apical low-conductance K channel is exquisitely sensitive to changes in cytosolic pH (53, 64). Reduction of pH from 7.4 to 7.0 completely abolished the channel activity. In addition, a decrease in cytosolic pH sharply enhanced the sensitivity of the channel to ATP inhibition.

The physiological role of the ATP regulation is not completely understood. It is possible that ATP may link the activity of the apical K channel to the turnover rate of the basolateral Na-K-ATPase. In the proximal tubule, it has been demonstrated that the stimulation of Na transport by luminal application of substrates such as glucose and amino acids increases the activity of the basolateral ATP-sensitive K channel (11, 60). Presumably, stimulation of the Na transport increases the turnover rate of the Na-K-ATPase, and, accordingly, more ATP is consumed. A decrease in ATP concentration in turn activates the ATP-sensitive K channel. However, it is not clear whether the same mechanism regulates the renal ATP-sensitive K channel in the TAL and the CCD.

**CFTR AND ATP SENSITIVITY OF THE ROMK CHANNELS**

Studies performed by McNicholas et al. (43) have demonstrated that ROMK2 is sensitive to ATP. Addition of 5 mM Mg-ATP inhibited the activity of ROMK2 in inside-out patches, and the effect was reversible. As in the native low-conductance K secretory channel (66), ROMK2 is more sensitive to Mg-ATP than to Na-ATP. However, in comparison to the native counterpart, ROMK2 has a lower ATP sensitivity than that of the native K secretory channel in the CCD. Moreover, ROMK2 is not sensitive to sulfonylurea agents (41, 42). This suggests that additional associate proteins are needed to construct the native ATP-sensitive K channel in the TAL and the CCD. Cloning and characterization of sulfonylurea receptor (SUR) sheds light on the possible structure of the renal ATP-sensitive K channel found in the CCD and the TAL. SUR1 was first cloned by Aguilar-Bryan et al. (2) and later demonstrated interaction with an inward-rectifying K (IRK6.2) channel to form the ATP-sensitive K channel in the pancreatic tissue (5, 30). Studies performed by Ashcroft and colleagues (61, 62) have further illustrated that the ATP binding site is located in the COOH terminus of IRK6.2 and that SUR1 is the receptor for both sulfonylurea agents and the ATP-sensitive K channel openers (5, 15, 29). After SUR1 had been identified, SUR2, with low affinity to sulfonylurea agents, had been found to be expressed in cardiac, skeletal, and smooth muscle cells (1). However, both SUR1 and SUR2 are not expressed in the kidney (1). Since cystic fibrosis transmembrane conductance regulator (CFTR), a member of the ATP-binding cassette transport family including SUR1 and SUR2 (20), is expressed in the kidney, several groups have explored the possibility that CFTR may couple to the ROMK channels to form the functional renal low-conductance ATP-sensitive K channel. McNicholas et al. (41) have reported that when CFTR is coexpressed with ROMK2, application of sulfonylurea agents blocks the activity of ROMK2 channel. The studies carried out by the same group have further suggested that a functional CFTR-NBF1 (“nucleotide binding fold”) is required for CFTR-ROMK2 interaction (42). The observation that coupling the ROMK channel with CFTR is essential for restoring the response to sulfonylurea agents has also been reported by Ruknudin et al. (51). Moreover, they used the oil-gating technique to study the ATP regulation of ROMK1 and found that adding millimolar concentrations of Mg-ATP had no significant effect on channel activity. However, when CFTR was coexpressed with ROMK1, the K channel became sensitive to ATP to an extent similar to that observed in the native tissue (51), suggesting that CFTR may be required to increase the sensitivity to ATP. However, it is still not completely understood whether the ATP binding site is also located in the COOH terminus of the
ROMK channels. There are at least two possibilities to explain why the ATP-induced inhibition of ROMK1 can be observed only when ROMK1 is coexpressed with CFTR: 1) CFTR is directly involved in forming ATP binding sites; and/or 2) CFTR may significantly increase the ATP affinity to ROMK1. Since it has been shown that the ATP-binding site of the pancreatic ATP-sensitive K channel is located in the COOH terminus of IRK6.2 (17, 61, 62), it is mostly likely that the role of CFTR is to increase the sensitivity of the ROMK channel to ATP. Interestingly, several recent studies have reported that the membrane contents of phosphatidylinositol 4,5-bisphosphate (PIP2) modify the sensitivity of the pancreatic ATP-sensitive K channel to ATP (8, 10, 55). Adding PIP2 to the bath facing cytosolic side of inside-out patches attenuates the ATP-induced inhibition of the pancreatic ATP-sensitive K channel (10, 55). It is not clear whether the different sensitivity to ATP between the ROMK channel and the native K secretory channel is also partially induced by a different concentration of PIP2, since membrane PIP composition and loss of PIP2 in excised patches may vary among the different cells.

REGULATION OF THE NATIVE K SECRETORY CHANNEL BY PROTEIN KINASES

PKA has been shown to stimulate the native K secretory channel in both the TAL and CCD (68). Several studies have demonstrated that vasopressin stimulates the K secretion in the collecting duct, since the effect of vasopressin can be mimicked by cAMP, suggesting a role of cAMP-dependent pathway in regulating the apical K secretory channels (67). This notion is supported by patch-clamp experiments in which vasopressin and cAMP increased the number of active low-conductance K channels in the CCD (14). That PKA plays a key role in stimulating the low-conductance K channel was confirmed by single channel analysis (14). Inhibition of channel phosphorylation by removal of Mg-ATP from the bath solution completely decreased channel activity in inside-out patches. On the other hand, addition of Mg-ATP and cAMP restored rundown channel activity (68). That PKA-induced phosphorylation is essential for maintaining channel activity is further supported by observations that application of the inhibitory peptide of PKA blocked the low-conductance K channel (68). Furthermore, balance between such phosphorylation and dephosphorylation processes is strongly suggested by the presence of MgF2-dependent and Mg2+-independent phosphatases in the apical membrane of principal cells (34).

Ca2+ has been found to play a key role in linking the apical K conductance (52, 66) and Na transport to the activity of Na-K-ATPase (18, 19, 38, 52, 56). Inhibition of Na-K-ATPase decreased the open probability of the apical K secretory channel in the CCD, and the effect of inhibiting Na-K-ATPase depends on the presence of Ca2+. The effect of Ca2+ on the apical K secretory channels is indirect, since the inhibitory effect of Ca2+ can only be observed in intact cells but not in inside-out patches (66). Exploration of the underlying mechanism showed that PKC is involved in mediating the inhibitory effects of high concentrations of Ca2+ (66).

These observations suggest that channel activity is influenced by kinase-produced phosphorylation that either stimulates or inhibits the channel: PKA-induced phosphorylation increased, whereas PKC decreased, the apical K channel. This notion is further confirmed by studying the amino acid sequence of the ROMK channel.

AKAP AND REGULATION OF THE ROMK CHANNEL BY PKA

All isoforms of the ROMK channel have three putative serine phosphorylation sites for PKA and at least two putative PKC phosphorylation sites (28). Biochemical studies have convincingly demonstrated that all three serine sites can be phosphorylated in vitro (72). A large body of evidence suggests that PKA-induced phosphorylation is essential for maintaining channel activity and deletion of two serine PKA phosphorylation sites has been shown to inhibit the activity of ROMK channels (39, 41, 43, 72). However, the effect of PKA on ROMK1 could only be observed in excised patches (41), but not in intact cells, when ROMK1 was expressed in oocytes. In contrast, we observed the effect of PKA on the native low-conductance K channel in both cell-attached and inside-out patches (14, 67). We postulated that the absence of the membrane-bound PKA in oocytes may be responsible for failure of response of ROMK1 to stimulation of PKA. Association of PKA with cell membrane requires the presence of A kinase anchoring protein (AKAP), which has a binding
site for the regulatory subunit of PKA and two target- ing domains for the cytoskeleton (46, 50). Thus we hypothesized that the lack of response to stimulating PKA was the result of the absence of AKAPs in the oocyte membranes. By using the overlay assay with 32P-labeled regulatory subunit of type II PKA (RII), we confirmed that RII binding protein was absent in oocyte membrane (3). In contrast, the RII binding protein was clearly identified in the membrane obtained from renal cortex (3). This suggests that the AKAP is present in the kidney. Several studies on a variety of ion channels have recently shown that AKAPs are required for the effect of PKA (21, 31, 49). AKAPs are abundant proteins associated with the cell cytoskeleton and are involved in the localization and/or functional modulation of PKA in a variety of cell membranes (45, 50). Although AKAPs do not share high level homology among themselves, they do have similar structural requirements. For instance, the AKAPs have two or three target domains that are responsible for binding to cytoskeleton and have one binding domain for the regulatory unit of PKA (46, 50). AKAP79 and AKAP15 have been shown to be involved in the regulation of L-type Ca channels in cardiac myocytes and skeletal muscle (21, 24, 31).

The role of the AKAP in mediating the effect of PKA on the ROMK channel was demonstrated by experiments in which coinjection of cRNA encoding AKAP79 restored the response of the ROMK1 to either forskolin or cAMP (3). Although AKAP79 is mainly identified in neuronal tissues and is not found in the kidney (32), successful restoration of the response to the PKA stimulation in oocytes coexpressing ROMK1 and AKAP79 may be attributed to the fact that the ROMK channel is also expressed in the brain (33). In addition to AKAP79, coexpression of ROMK1 with AKAP75 (27, 36), an analog of AKAP79, mimics the effect of AKAP79 and restores the response of ROMK1 to forskolin (4).

Three lines of evidence indicate that the effect of forskolin is the result of stimulation of the cAMP-dependent PKA signal transduction pathway. First, the effect of forskolin can be mimicked by a membrane-permeant cAMP analog. Second, the effects of cAMP and forskolin are not additive. Third, pretreatment of oocytes with N-[(3-(4-bromophenyl)-2-propenyl)-aminoethyl]-5-isoquinolinesulfonamide (H-89), an agent which inhibits PKA, abolished the effect of forskolin. Figure 1 summarizes the results showing the effect of stimulating PKA on ROMK1 in the presence and in the absence of AKAP79. It is apparent that AKAPs are required for mediating the effect of stimulating PKA.

The PKA anchoring is important to control the spatiotemporal resolution of PKA-mediated phosphorylation of the ROMK channels. In addition, AKAP may also help to maintain a high local concentration of cAMP and to facilitate phosphorylation of the target proteins. Recently, Dong et al. (16) have cloned a specific AKAP from the lung. Since this AKAP is also expressed in the renal tubules, the AKAP is named as AKAP-KL. Moreover, immunocytochemical studies revealed that the AKAP-KL is expressed only in the apical membrane of the renal tubules, suggesting the presence of membrane-bound PKA in the renal tubule. Although the tubule segments expressing the AKAP-KL are not well defined, the presence of the AKAP in the kidney strongly suggests that AKAPs may play an important role in mediating the effect of the cAMP-PKA-dependent signal transduction pathway.

FUTURE DIRECTIONS

Recent studies from several laboratories have convincingly shown that the additional associated proteins are required to interact with the ROMK channel to restore the properties of the native K secretory channel. Although CFTR has been shown to interact with the ROMK channel to form a functional renal ATP-sensitive K channel, it is not known whether the native low-conductance ATP-sensitive K channel is composed of CFTR and the ROMK channels. Alternatively, the ROMK channel may interact with a renal specific SUR to form the native ATP-sensitive K channel. Interestingly, a new member of the SUR family, SURx, has been cloned from the rat kidney (59). The SURx shares a high homology with SUR2 in cardiac myocytes, and RT-PCR analysis further reveals that SURx is expressed in the TAL and the CCD. It will be interesting to see whether the renal SURx is involved in assembling the native K secretory channel with the ROMK channel.

Although the importance of AKAP in mediating the effect of stimulating PKA has been demonstrated, further experiments are required to identify the renal AKAP that interacts with the ROMK channel in the TAL and the CCD. In addition to AKAP-KL, it was recently reported that AKAP15, which regulates L-type Ca channels in the skeletal muscle, is also expressed in the kidney (24). It will be of interest to determine which renal AKAP is associated with the ROMK channel and modulates the channel activity in the native tissue.

Recently, it has been suggested that receptors of the activated PKC (RACK) are needed to mediate the effect of PKC (37). We have demonstrated that PKC inhibited ROMK1 channel activity in inside-out patches (40). However, stimulation of PKC failed to inhibit the activity of ROMK1 expressed in oocytes. It is possible that a RACK-like protein is required to restore the response to PKC stimulation.

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