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An ATP-regulated, inwardly rectifying potassium channel from rat kidney (ROMK)

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Potassium channels exhibit a wide functional diversity making them well suited for their broad roles in renal (and other) cells [1, 2]. Potassium channels can be classified into two broad groups based on their functional/biophysical properties: the delayed or outward rectifiers that are activated by depolarizing potentials and the inward rectifiers that include the classical (strongly) inwardly rectifying K^+ channel and the more weakly inwardly rectifying ATP-sensitive potassium (K_{ATP}) channels [1, 3–7]. The inward rectifiers are characterized by a lack of significant gating by voltage and by their ability to conduct potassium more readily in the inward than outward direction. The classical (strong) and K_{ATP}-type of inward rectifiers have been identified in a variety of excitable and nonexcitable cells. The strong inward rectifiers appear to function in maintaining the resting membrane potential and in regulating excitability (such as in cardiac muscle cells). ATP-sensitive potassium channels, on the other hand, open and close in response to cellular metabolic events and may serve important roles in some cells during ischemia. Renal K_{ATP} channels, while sharing many of the properties and characteristics of K_{ATP} channels found in other tissues (such as pancreatic β-cell and cardiac muscle cells [3]), lack sensitivity to TEA, have a much lower sensitivity to sulfonylureas (such as glyburide, a high affinity inhibitor of K_{ATP} channels found in heart and β-cells), and require higher (that is, mM) concentrations of ATP to inhibit channel activity [1, 7].

In the kidney, the apical (K^+ secretory) K_{ATP} channel serves a number of important roles in renal electrolyte transport [1]. In the thick ascending limb of Henle (TAL; both medullary, MTAL, and cortical, CTAL, segments; Fig. 1), K_{ATP} channels are the dominant conductance in apical plasma membranes and provide a crucial K^+ efflux pathway for potassium entering cells via the apical Na^+:K^+:$2Cl^-$ cotransporter [1]. This recycling of potassium ensures that an adequate supply of luminal potassium is provided for efficient function of the Na^+:K^+:$2Cl^-$ cotransporter [1]. In addition, this channel mediates the apical component of a transcellular (basolateral-to-apical) current flow that returns to the basolateral side via the paracellular pathway predominantly as a sodium current [8]. This provides for one-half of the net transepithelial movement of sodium [9]. Two types of inwardly rectifying and ATP-sensitive K^+ channels have been identified on apical membranes of TAL segments by patch clamp [10, 11]. Wang and coworkers [10] found a 20 to 30 pS K^+ channel in rabbit TAL that had a high open probability (Po), was inhibited by ATP (mM) and not sensitive to TEA (referred to as the "low conductance" channel). On the other hand, a different K_{ATP} channel was identified on apical membranes of rat TAL by Greger and coworkers [11–13]; this channel also had a high Po and was ATP-sensitive but had a higher unitary conductance of ~70 pS, was highly sensitive to reductions in cytosolic side pH (50% reduction in Po by a 0.2 pH unit decrease), and exhibited sensitivity to quinine or quinidine, TEA and Ca^{2+} (referred to as the "intermediate conductance" channel). Recently, Wang found both the low (~30 pS) and intermediate (~72 pS) conductance K_{ATP} channels in the same patches of rat TAL apical membranes [14]. He also confirmed that the intermediate conductance K_{ATP} channel is sensitive to quinidine and acidic pH while the low conductance channel is insensitive to quinidine. In addition, the low, but not the intermediate, conductance channel is inhibited by high (~250 μM) gliburide. These studies demonstrate that there are two distinct channel types in apical membranes of TAL and that these channels can be distinguished by single channel conductances and their sensitivities to channel inhibitors.

A functionally similar, if not identical, low conductance, inwardly rectifying K_{ATP} channel has been identified in apical membranes of principal cells in the cortical collecting duct (CCD) where it mediates K^+ secretion into urine (Fig. 1) [1, 7, 15, 16]. The K_{ATP} channel in rat principal cells is dually regulated by ATP: high MgATP concentrations reversibly block channel activity ($K_{1/2}$ = 0.6 to 1.0 mM) while lower concentrations of MgATP are required to maintain channel activity [1, 7, 17, 18]. The mechanism for ATP-mediated block of the principal cell K_{ATP} channel is unclear at present but may represent direct binding of nucleotide to the channel itself with a resulting change in channel conformation to the closed state and/or to altering the activity of the channel by regulating the phosphorylation of the channel itself, or some other protein involved to modulating channel activity. The stimulatory effect of low ATP concentration, however, clearly relates to regulation of channel activity by phosphorylation-dephosphorylation processes [18]: (i) channel activity rapidly diminishes (run-down) on patch excision unless the cytosolic face is exposed to low concentrations of MgATP; (ii) generally the catalytic subunit of cAMP-dependent protein kinase, PKA, is also required for channel maintenance and PKA, and together with MgATP can restore channel activity after run-down; (iii) nonhydrolyzable ATP analogues cannot maintain or restore channel

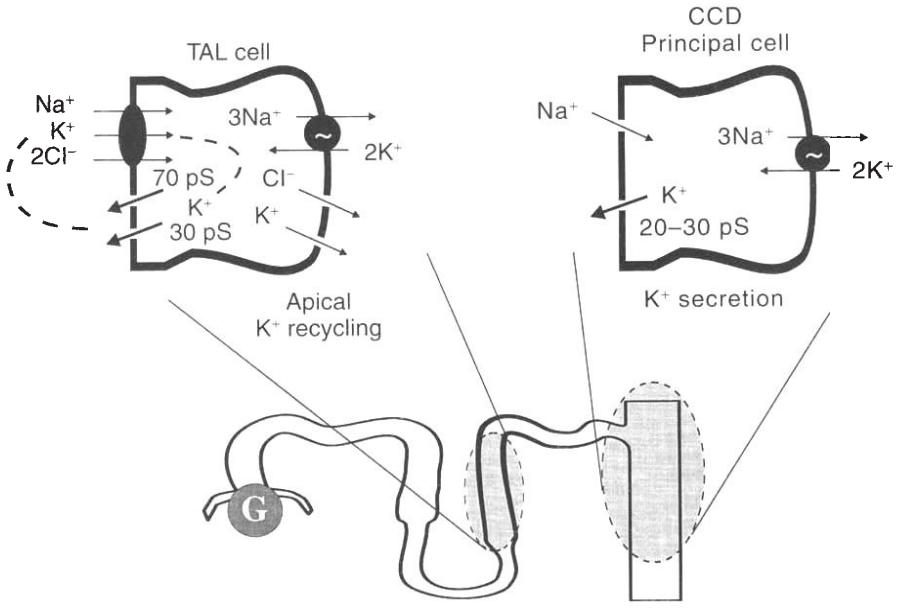

Fig. 1. *Model for potassium recycling across apical membranes of the thick ascending limb and for potassium secretion in principal cells from the cortical collecting duct in rat kidney.* See text for discussion.

activity; (iv) in patches in which channel activity is maintained by MgATP alone, the PKA inhibitor (PKI) reversibly reduces channel activity, providing evidence for an important role for endogenous PKA; and (v) PKC reversibly inhibits channel activity and antagonizes the stimulatory effect of PKA, a process that is Ca^{2+}-dependent [19]. In further studies Wang and Giebisch [17, 18] demonstrated that the ratio of ATP to ADP and cell pH are also important regulators of the small conductance K_{ATP} channel in the apical membranes of principal cells. K_{ATP} channel activity in rat principal cells is also inhibited by activation of protein kinase C [19] or calcium-calmodulin-dependent kinase II [20] or by arachidonic acid [21].

Much less is known about the regulation of the apical K_{ATP} channels in the TAL than in the CCD; however, we previously suggested that AVP (presumably cyclic AMP-dependent activation of PKA and subsequent phosphorylation of the channel or an associated regulatory protein) activated the K^+ conductance of the apical membrane in mouse MTAL [22, 23]. Reeves and coworkers have provided more direct evidence for this [24]. They showed that Ba^{2+}-sensitive, voltage-dependent $^{86}Rb^+$ influx in membrane vesicles from rabbit outer medulla was activated by cAMP-dependent protein kinase. Wang [14] has confirmed this effect of cAMP-PKA by showing activation of the low conductance K_{ATP} channel in cell attached patches by AVP or cAMP and in excised patches by the catalytic subunit of PKA.

Finally, it should be noted that large conductance (maxi-K^+), Ca^{2+}-activated K^+ channels have been identified in apical membranes of both TAL [25, 26] and CCD [1, 27–29]. These voltage-gated channels are normally quiescent but can be activated by μM cytosolic Ca^{2+}, are inhibited by TEA (more sensitive to TEA than the intermediate conductance K_{ATP} channel), and are insensitive to ATP. Since $K^+(Rb^+)$ secretion and the transepithelial voltage in the CCD are not blocked by luminal TEA [16, 28], it is generally thought that this apical maxi-K^+ channel is not directly involved in K^+ secretion by this nephron segment. The maxi-K^+ channel may function, however, as a K^+ efflux pathway during cell swelling [1, 7, 30].

An inwardly rectifying, ATP-regulated K^+ channel, ROMK, from rat kidney: A member of a family of inwardly rectifying potassium (IRK) channels

Cloning and molecular diversity

We recently cloned a cDNA, ROMK1 [31], encoding a ~45 kD channel protein with a novel channel topology (Fig. 2). Subsequently, other inwardly rectifying K^+ channels have been isolated [31–39] and form a new and rapidly expanding family of potassium channels (IRK family; Fig. 3). The ROMK1 channel protein has two potential membrane-spanning helices (M1 and M2; Fig. 2) making it quite distinct from voltage-gated or ligand-gated ion channels [40, 41], and this topology is a characteristic feature of these IRK channels. ROMK1 is expressed in kidney and several other tissues, for example, brain, spleen, lung and eye [31, 42].

Recent studies have demonstrated that the ROMK gene contains introns within the coding region [43–45], an unusual feature for a mammalian K^+ channel. Alternative splicing of ROMK exons yields several different transcripts: ROMK2a, ROMK2b and ROMK3 (Fig. 2). All of the proteins produced by these alternatively spliced transcripts form functional K^+ channels when expressed in *Xenopus* oocytes [36, 46]. The alternative splicing occurs at both the 5′-coding and 3′-non-coding regions. We do not know whether the alternative splice variation and use of different polyadenylation signals which lead to varying lengths of the 3′UTRs (untranslated regions) have functional or regulatory significance for the ROMK isoforms (mRNA half-life, efficiency of translation, etc.). The longer ROMK2b transcript may account for the ~3 kb mRNA observed on Northern blot analysis of rat kidney cortex and outer medulla RNA [31], and thus ROMK2b may represent the most abundantly expressed ROMK2 transcript in the rat kidney. In contrast, the 5′ end splicing alters both the length and amino acid sequence of the initial segment of the NH_2-terminus of ROMK channels (Fig. 2) [46]. In this regard, Brown and coworkers [47] by exchanging the NH_2- and COOH-termini between ROMK1 [31] and IRK1 [32] have demonstrated

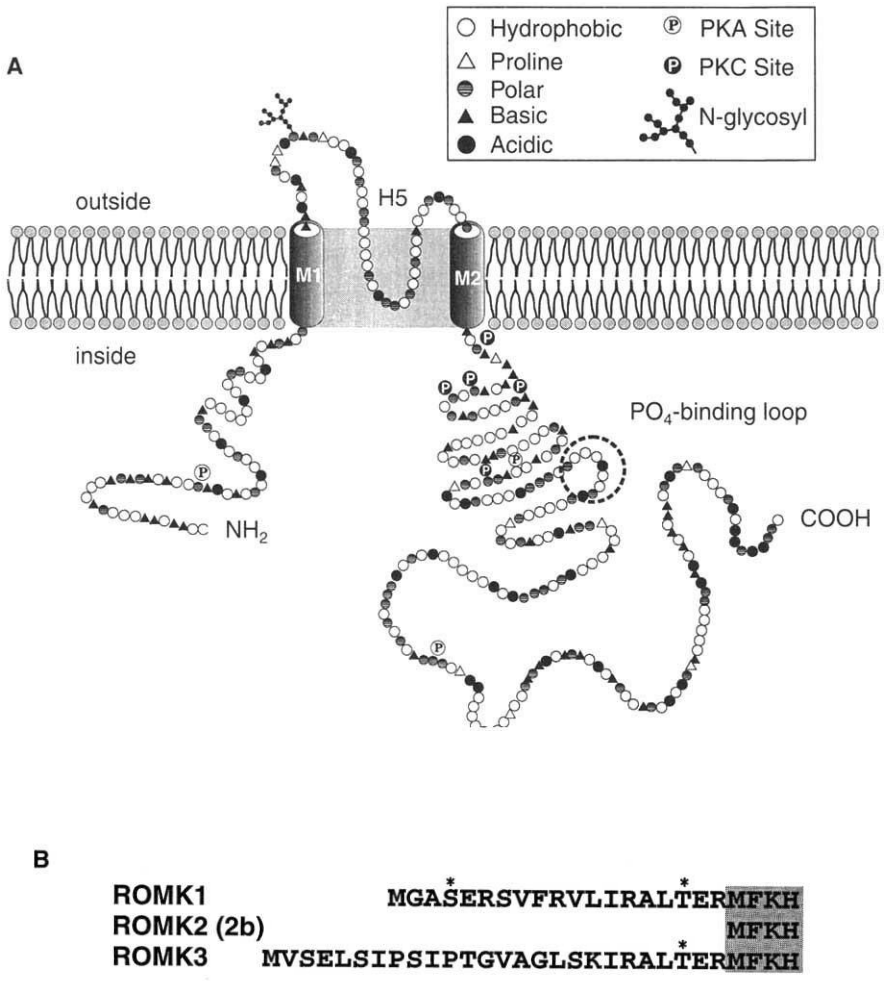

Fig. 2. *Topology model (A) of ROMK2 and the amino acid sequences of the different NH_2-termini of the alternatively spliced products of the ROMK gene (B).* In A, symbols represent individual amino acid residues and the cylinders represent membrane-spanning helices. The dashed circle in the COOH-terminus represents the location of the PO_4-binding loop or Walker A site proposed to be involved in MgATP binding. Potential protein kinase A and protein kinase C phosphorylation sites, and the single N-glycosylation site (N-glycosyl) are indicated. In B, amino acid sequences are show by single letter codes; the asterisk indicates potential protein kinase C phosphorylation sites.

the importance of these regions in determining the pore properties of these inwardly rectifying K^+ channels. Thus the relatively hydrophilic termini of these channels may form part of, or be closely associated with, the channel pore. The role of the NH_2-terminal variations on the ROMK isoforms in altering channel function and regulation by phosphorylation-dephosphorylation processes is currently under active investigation [48, 49].

We have also recently determined the nephron localization of the ROMK channel isoforms (Fig. 4) [45, 46]. It is evident that ROMK isoforms are differentially expressed along the nephron. ROMK2, which encodes the channel with the shortest NH_2-terminus (Fig. 2), is the most widely distributed of the ROMK transcripts (Fig. 4). The ROMK1 transcript is specifically expressed in collecting ducts (CCD, OMCD, and initial IMCD; Fig. 4) and ROMK3 is expressed in the earlier nephron segments (MTAL, CTAL and DCT; Fig. 4). Thus, all of these loop and distal nephron segments, except the OMCD, apparently express two different ROMK isoforms: ROMK2 and one of the isoforms (ROMK1 or ROMK3) encoding a channel protein with a longer NH_2-terminus. Whether ROMK channels form heteromultimeric complexes in some of these nephron segments, and if so, whether these multimeric channels have distinct functional and regulatory properties is under investigation.

The distribution of ROMK channel isoforms is consistent with the possibility that ROMK may represent, or form a major subunit of, the low-conductance secretory KATP channel observed in TAL and principal cells. Preliminary studies have suggested a predominant apical location using a ROMK-specific polyclonal antibody consistent with a role for this channel in K^+ secretion.

Preliminary studies suggest that ROMK2b has a single channel conductance that is not significantly different from ROMK1 (that is, 30 to 40 pS), a finding in accord with that for ROMK2 [36]. Thus, ROMK1 or ROMK2 (ROMK2b) does not appear to encode the moderate conductance (70 pS) K_{ATP} channel found in the rat TAL [11, 14], although we have not examined the possible role of ROMK2-ROMK3 heteromeric complexes in forming this higher conductance K_{ATP} channel. In this regard, however, it will be interesting to determine the distribution of two recently cloned inwardly rectifying K^+ channels with limited homology to ROMK (a pH-sensitive K^+ channel isolated from a rabbit collecting duct cell line [34] and a rat cardiac IRK channel [33]) which have single channel conductances of ~70 pS and are apparently expressed in kidney.

In a recent study, Hurst et al [50] examined the basic properties of the apical K^+ channel in rabbit macula densa cells and found a number of similarities between this channel and the apical K_{ATP} channels in CTAL and CCD (such as unitary conductance, non-voltage dependency, and pH sensitivity). They suggested, however, that this was a different channel based in large part on the lack of channel inhibition by disodium ATP (ATP^{-4}), that is,

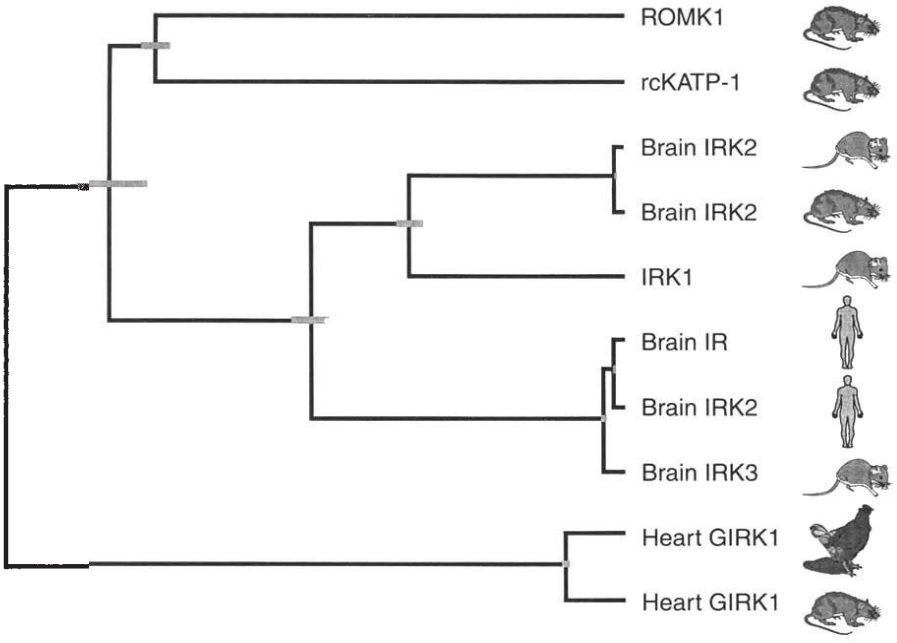

Fig. 3. *Family tree of inward rectifying potassium (IRK) channels.* Species from which the channel was cloned are indicated. Amino acid sequences for comparison were obtained from references [31–39] and the sequence databases.

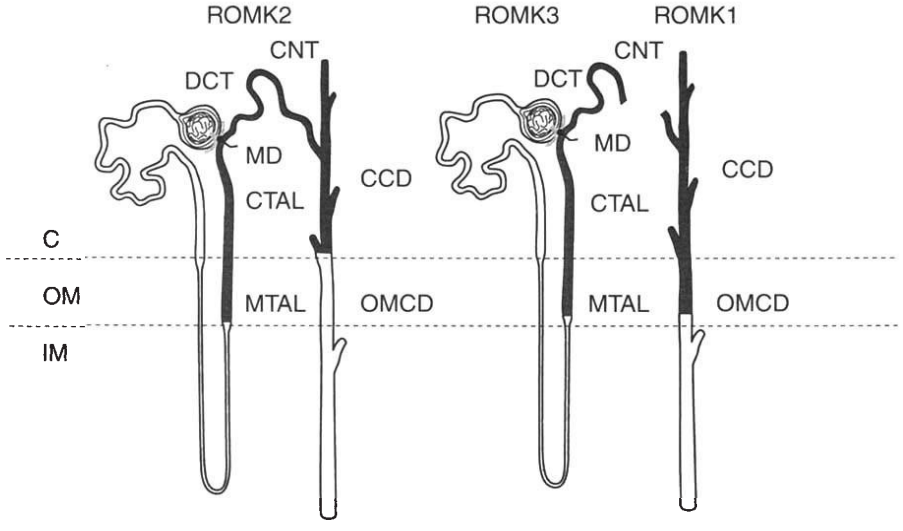

Fig. 4. *Differential expression of ROMK channel isoforms (ROMK1, ROMK2 and ROMK3) along the rat nephron.* Adapted from [46].

it appeared to lack ATP sensitivity. In this regard, Wang and Giebisch [18] and McNicholas et al [51] have shown for the apical K_{ATP} channels in principal cells can be reversibly inhibited by $MgATP^{-2}$, but are little affected by ATP^{-4}. Therefore it would be important to examine this issue in macula densa cells. At the present time, it seems reasonable to suggest that ROMK may be involved in formation of the apical K^+ channel in macula densa cells. Finally, the expression of ROMK transcripts in DCT and OMCD suggests that this IRK contributes to K^+ transport (secretion?) in these nephron segments. In this regard, Sansom et al [52] recently observed a low-conductance (~36 pS) in the mouse IMCD3 cell line with properties similar to that in rat TAL and principal cells.

Function of ROMK channels and correlation with structure

ROMK K^+ channels exhibit many of the ion permeation and regulatory properties of the low conductance K_{ATP} channel observed on patch clamping of apical membranes of thick ascending limb [10, 14] and principal cells [15, 17, 18]. ROMK channels expressed in *Xenopus* oocytes have the following properties: a unitary conductance of 30 to 40 pS in symmetrical 140 to 150 mM KCl [31, 49]; high K^+:Na^+ selectivity ($K^+ > Rb^+ > NH_4^+ > Na^+, Li^+$) [31, 36]; weak inward rectification resulting from block by internal Mg^{2+} and/or a polyamine such as spermine or spermidine [53–55] (Fig. 5); sensitivity to external Ba^{2+}, but not TEA^+ [31, 36] (Fig. 5); marked sensitivity to "cytosolic" side reductions in pH [56]; modulation by arachidonic acid [57]; "run-down" or loss of channel activity in excised patches in the absence of MgATP that involves dephosphorylation by a protein phosphatase (PP2c in *Xenopus* oocytes) [49]; reactivation of channels after run-down by re-exposure to MgATP and the catalytic subunit of protein kinase A [49]; and sensitivity (inhibition) by higher concentrations of MgATP [48, 56].

The ROMK channels conserve an amino acid segment between

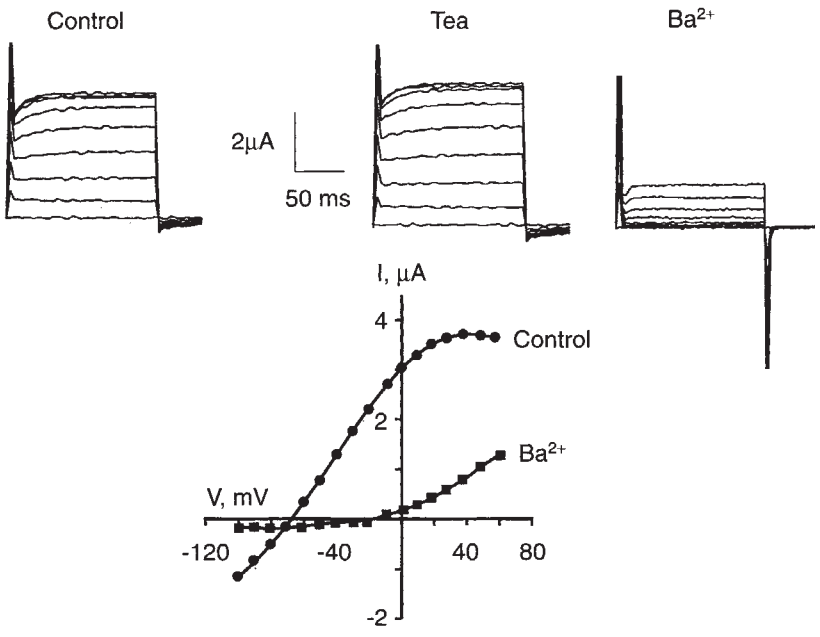

Fig. 5. *Whole cell current profiles (upper panels) and current-voltage (I-V, lower panel) relationships of the ROMK2 channel expressed in Xenopus laevis oocytes in control (5 mM external K^+) conditions or in the presence of tetraethylammonium (15 mM TEA) or barium (10 mM Ba^{2+}).* Symbols are for the I-V curve: ● in 5 mM K^+; ■ in the presence of 10 mM Ba^{2+}.

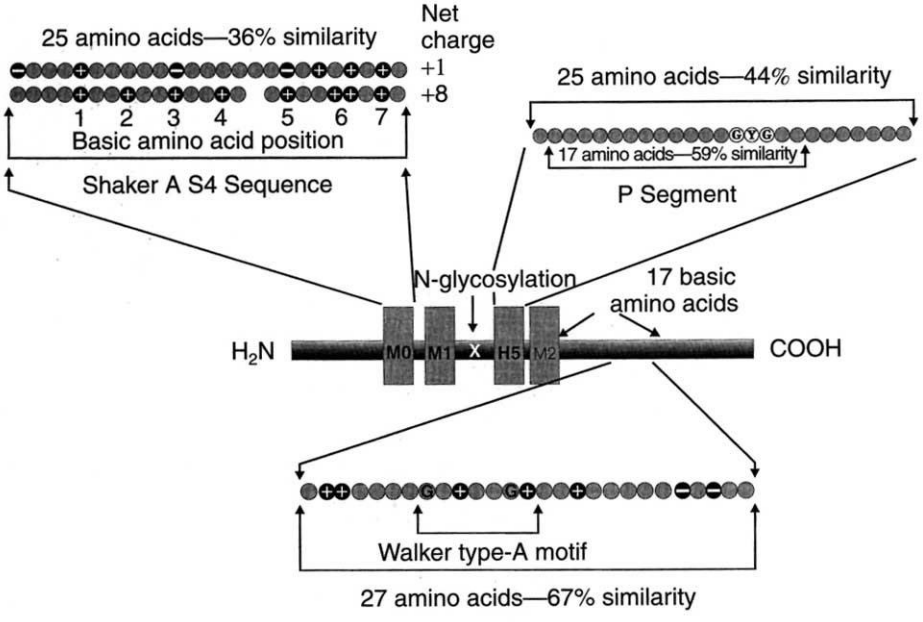

Fig. 6. *Schematic representation of the regions of the ROMK channel based on similarity to defined regions in other channels or proteins.* Circles represent individual amino acid residues; positive and negative signs indicate basic or acidic residues, respectively; single letter amino acid codes are shown for some residues. See text for discussion.

the two putative membrane spanning helices that is homologous to the pore-forming H5 region of voltage-gated K^+ channels (59% similarity; Figs. 2 and 6). This is of considerable importance, not only because it suggests that ROMK1 and voltage- (cyclic nucleotide-) gated channels evolved from a common ancestral gene, but also supports an important role for the H5 region in forming the channel pore [58–62]. In addition, this region contains the Gly-Try-Gly triplet which is conserved in channels with high K^+ selectivity [40, 63]. Of considerable interest, Taglialatela et al [47] have recently suggested that the COOH-terminus of IRKs may also importantly contribute to pore formation in the inward rectifier-type K^+ channels.

A characteristic of the low conductance, secretory K_{ATP} channel found in apical membranes of principal cells in the cortical collecting duct is a lack of sensitivity to external TEA^+ [1, 5, 15, 17, 28] which is also a feature of the K^+ secretory channel in this nephron segment [15, 28]. Consistent with a possible role of ROMK isoforms in K^+ secretion in kidney, this channel exhibits no significant TEA^+ sensitivity (up to 15 mM) when expressed in *Xenopus laevis* oocytes (Fig. 6). The presence of an aromatic amino acid, phenylalanine, at position 148 within the H5 region might be expected to impart significant sensitivity to external TEA^+ in ROMK channels since site-directed mutagenesis studies in *Shaker* channels have shown that substitution of an aromatic

amino acid at a similar position confers a high sensitivity to external TEA^+. We have argued, however, that the basic amino acid, arginine (R), at position 147 of ROMK1 might interfere with TEA^+ binding and explain the lack of high TEA^+ sensitivity in the ROMK1 channel [31].

An amphipathic region, MO, which is adjacent to M1 on the NH_2-terminal side of the ROMK1 channel protein (Figs. 2 and 6), exhibits a low level of similarity to the S4 region of voltage-gated ion channels. ROMK channels, however, lacks the typical S4 arginine-rich segment and the basic amino acid at every third position thought to provide the voltage sensor for *Shaker* channels. The net charge of +1 on ROMK1 versus +8 for the *Shaker* A type channel would be consistent with the virtual voltage insensitivity of ROMK1 channel (Fig. 6).

Inward rectification is also a characteristic of the apical secretory K_{ATP} channels in kidney as well as other members of the IRK family of channels. As described in the beginning of this review, IRKs exhibit either strong (such as, IRK1 [32]) or weak (such as, ROMK channels; Fig. 6; and other K_{ATP} channels) inward rectification. Several recent reports have begun to identify important amino acids residues or segments critical for both types of rectification [47, 64, 65]. Tagliatela et al [47] have shown that the $COOH^-$, but not NH_2^-, terminus is involved in determining inward rectification. In addition, Lu and MacKinnon [64] and Wible et al [65] have both shown that a single negatively charged amino acid residue (aspartic acid) in the second membrane-spanning helix of IRK1 is required for strong rectification, and that this basic residue markedly enhances the sensitivity for internal block by Mg^{2+}. In fact, mutating the asparagine in M2 of ROMK1 (Fig. 2) to aspartic acid changes ROMK1 gating from the weak- to the strong-type.

In view of the effects of ATP on renal K_{ATP} channel function, the finding of a potential nucleotide-biding site on ROMK1 provides support for the possibility that the ROMK channel might function as an ATP-sensitive K^+ channel. As discussed above, preliminary results suggest that ROMK2 is, indeed, sensitive to MgATP [48, 56]. A segment following the membrane-spanning M1 region contains a Walker A site motif [GXG(H in ROMK)XXGK] within a so-called PO_4-binding or P loop that has been linked to ATP binding in many other proteins [66] (Figs. 2 and 6). A larger, 27 amino acid region containing the Walker type A site, also exhibits significant similarity to the catalytic subdomain I of ERBB3, a member of the epidermal growth factor (EGF) receptor tyrosine kinase family (Fig. 6) [31]. Based on this potential ATP-binding segment and the presence of several potential protein kinase A and protein kinase C phosphorylation sites in the COOH-terminus of ROMK channel proteins, we have suggested that this region may be involved in regulation of ROMK by ATP (both by ATP-binding and ATP-dependent phosphorylation processes). We are currently looking into this possibility.

Summary

With the cloning of ROMK [31] and IRK1 [32], a new family of inwardly rectifying K^+ channels has been identified. ROMK channel isoforms are highly and differentially expressed in distal nephron segments of the mammalian kidney. These channels exhibit many of the characteristics of the low conductance, ATP-sensitive K^+ channels found in apical membranes of TAL, macula densa, and principal cells that are involved in potassium secretion. Thus ROMK channel isoforms appear to be involved in the formation of these secretory K_{ATP} channels. Further characterization of these channels should provide further evidence for their role in the secretory K_{ATP} channels and new insights into the function and regulation of these channels.

Acknowledgments

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