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## Distal Potassium Handling Based On Flow Modulation of Maxi-K Channel Activity

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### Abstract

**Purpose of review**—Studies on the mechanisms of distal  $K^+$  secretion have highlighted the importance of the renal outer-medullary  $K^+$  (ROMK) and maxi-K channels. This review considers several human disorders characterized by hypo- and hyperkalemia, as well as mouse models of these disorders, and the mechanisms by which ROMK and maxi-K may be dysregulated.

**Recent findings**—Analysis of knockout mice lacking ROMK, a model for type II Bartter's syndrome, has shown a role for maxi-K in distal  $K^+$  secretion. Knockout mice lacking either the  $\alpha$  or  $\beta 1$  subunits of maxi-K also show deficits in flow-dependent  $K^+$  secretion. Analysis of transgenic and knock-in mouse models of pseudohypoaldosteronism type II (PHA2), in which mutant forms of with-no-lysine kinase 4 (WNK4) are expressed, suggests ways in which ROMK and maxi-K may be dysregulated to result in hyperkalemia. Modeling studies also provide insights into the role of  $Na^+$  delivery versus flow in  $K^+$  secretion.

**Summary**—The importance of both ROMK and maxi-K to distal  $K^+$  secretion is now well-established, but the relative role each of these two channels plays in normal and diseased states has not been definitively established. Analysis of human and animal model data can generate hypotheses for future experiments.

### Keywords

$Ca^{2+}$ -activated  $K^+$  channel; maxi-K; rat outer medullary  $K^+$  channel; distal  $K^+$  secretion; pseudohypoaldosteronism type II; Bartter's; diuretics; hypokalemia; hyperkalemia

### Introduction

Since the 1964 study of Malnic et al, in which the distal nephron was shown to play an essential role in the regulation of urinary  $K^+$  excretion [1], much progress has been made in defining the molecular physiology regulating distal  $K^+$  transport. Nevertheless, questions remain regarding the mechanisms by which renal  $K^+$  homeostasis is disturbed in a variety of disorders. This review will consider the role of the  $K^+$  channels ROMK (KCNJ1, Kir1.1, SK) and maxi-K (BK), in several clinical disorders, including diuretic use, Bartter's disease and PHA2, in light of our understanding from animal and human data.

## ROMK and Maxi-K: distal tubule potassium channels

ROMK is an inwardly rectifying  $K^+$  channel that has a high open probability and small conductance [2]. Maxi-K, which has a large conductance, has a low open probability at baseline, but can be activated by increases in intracellular  $Ca^{2+}$  and membrane depolarization [3]. Both ROMK and maxi-K are found in the apical membrane of thick ascending limb (TAL), distal convoluted tubule (DCT), connecting tubule (CNT), and cortical collecting duct (CCD). Much of the characterization of these two channels has been done in CCD due to its experimental accessibility, but increasing evidence points to the importance of late DCT and CNT [4,5]. In modeling experiments, the bulk of  $K^+$  secretion was shown to occur in CNT, with some contribution from the late DCT [6•]. In support of this, mice with a collecting duct-specific knockout of the  $\alpha$ -subunit of the epithelial  $Na^+$  channel (ENaC) are able to increase urinary  $K^+$  excretion in response to a  $K^+$  load as well as wild-type mice [7]. Since electrogenic  $Na^+$  reabsorption by ENaC provides the driving force for  $K^+$  secretion, this suggests that DCT and CNT can account for the bulk of  $K^+$  secretion.

### Flow-stimulated $K^+$ secretion

Flow dependence of distal  $K^+$  secretion was recognized decades ago [8-11]. Although this was initially thought to occur solely through ROMK, characterization of maxi-K suggested that this channel could also contribute. Hunter et al first reported the existence of calcium-activated  $K^+$  channels in the apical membrane of CCD [12], and in subsequent studies characterized the  $Ca^{2+}$ -sensitivity of the channels. They suggested that calcium-activated  $K^+$  channels may play a role in  $K^+$  secretion in conditions that increase intracellular  $Ca^{2+}$  [13]. Taniguchi and Guggino showed that membrane stretch increased the open probability of calcium-activated  $K^+$  channels in cell-attached patches [14]. Subsequent studies showed that increasing luminal flow in CCD can increase intracellular calcium [15,16], and that flow-dependent  $K^+$  secretion can be blocked by inhibitors of maxi-K [17,18]. These results established that maxi-K is responsible for flow-dependent increases in  $K^+$  secretion in isolated tubules.

### Role of maxi-K in type II Bartter's

In patients treated with loop diuretics, or those with Bartter's syndrome, the inhibition or decreased function of the sodium-potassium-chloride cotransporter (NKCC2) in TAL results in increased distal delivery of  $Na^+$ . Increased  $Na^+$  reabsorption by ENaC, with increased lumen-negative potential difference (PD), increases  $K^+$  secretion, effects which are potentiated by increased aldosterone levels due to volume contraction. Patients with type II Bartter's are hypokalemic despite mutations in ROMK [19], suggesting that another  $K^+$  channel must be mediating  $K^+$  secretion in these patients. Similarly, knockout mice lacking ROMK, in which there is a complete absence of small conductance  $K^+$  channel activity in TAL and CCD, have increased urinary  $K^+$  excretion compared to wild-type mice [20,21]. In addition, ROMK knockout mice have increased fractional excretion of  $K^+$  in response to hydrochlorothiazide and furosemide [22•], presumably due to  $K^+$  secretion through maxi-K. This was examined by Bailey et al [23], who showed that iberiotoxin, a maxi- $K^+$  inhibitor, decreased  $K^+$  secretion by about 50% in late distal tubules from wild-type mice, and abolished  $K^+$  secretion completely in tubules from ROMK knockout mice. This suggests that ROMK and maxi-K account for the entirety of  $K^+$  secretion in late distal tubule, and provides a mechanism by which  $K^+$  secretion can continue in the absence of ROMK. Interestingly, type II Bartter's patients can have transient neonatal hyperkalemia [24], perhaps due to a delay in the expression of maxi-K. In animal models, neither ROMK nor maxi-K is expressed at high levels at birth, but the appearance of maxi-K is delayed compared to ROMK [25].

### Maxi-K knockout mice have defects in flow-dependent K<sup>+</sup> secretion

The functional role of maxi-K in flow-stimulated K<sup>+</sup> secretion has been examined in two additional knockout mice. The maxi-K channel is composed of a pore-forming  $\alpha$ -subunit, as well as accessory  $\beta$ -subunits, of which four have been described [3,26]. The  $\beta$ 1 subunit, which enhances the voltage and Ca<sup>2+</sup> sensitivity of maxi-K, is expressed in connecting tubule [27]. In maxi-K  $\beta$ 1 knockout mice, urinary excretion of K<sup>+</sup> after volume expansion with intravenous fluids was blunted compared to wild-type mice [28]. In addition, flow-dependent K<sup>+</sup> excretion, as estimated by measuring urinary flow rates versus K<sup>+</sup> excretion in volume-expanded mice, was also reduced in the knockout mice [27].

In mice lacking the maxi-K channel  $\alpha$ -subunit, treatment with a V<sub>2</sub>-receptor antagonist increased urinary flow rate by an equal amount compared to wild-type mice. Whereas urinary K<sup>+</sup> excretion increased in wild-type mice, it did not increase in the knockouts [29], again suggesting a role for maxi-K in flow-dependent K<sup>+</sup> secretion. Interestingly, there was a marked upregulation of ROMK in  $\alpha$ -subunit knockout mice fed a high K<sup>+</sup> diet, and aldosterone levels were increased in the knockout mice on low K<sup>+</sup>, control, or high K<sup>+</sup> diets, presumably compensatory responses for the lack of maxi-K.

### Which is more important to distal K<sup>+</sup> secretion: fluid flow or Na<sup>+</sup> delivery?

In patients on diuretics or with Bartter's, increased distal flow is accompanied by increased distal delivery of Na<sup>+</sup>. This raises an interesting question as to the importance of fluid flow vs Na<sup>+</sup> delivery in flow-stimulated K<sup>+</sup> secretion. Good and Wright showed that the flow dependence of K<sup>+</sup> secretion is independent of luminal Na<sup>+</sup> concentration, except at very low luminal Na<sup>+</sup> concentrations [9,10]. In addition, flow dependence of K<sup>+</sup> secretion is seen even in the presence of amiloride [11]. Hence, increased distal flow, even without changes in distal Na<sup>+</sup> delivery and reabsorption via ENaC, may be sufficient to increase distal K<sup>+</sup> secretion. This can be explained by fluid flow stimulating apical Ca<sup>2+</sup> entry to activate maxi-K channels (Fig. 1). The results of Rieg et al [29], showing that K<sup>+</sup> secretion was increased by fluid flow induced by V<sub>2</sub>-receptor antagonist administration, which would not be expected to increase distal Na<sup>+</sup> delivery, further support this idea.

Distal Na<sup>+</sup> delivery, however, is indispensable for K<sup>+</sup> secretion as it provides the electrical driving for K<sup>+</sup> efflux via maxi-K or ROMK (Fig. 1). In distal micropuncture experiments, a decrease in luminal Na<sup>+</sup> concentration sufficient to result in decreased transepithelial voltage resulted in decreased K<sup>+</sup> secretion, even under high-flow conditions [10]. In other distal micropuncture experiments, rats treated with loop or thiazide diuretics, in which flow into both early and late distal tubule is increased, the increase in K<sup>+</sup> secretion along the distal tubule exceeded the increase in Na<sup>+</sup> reabsorption in this segment [30]. This suggests that both maxi-K activity stimulated by flow, as well as increased Na<sup>+</sup> reabsorption by ENaC with generation of a more lumen-negative PD, is likely contributing to the kaliuresis in diuretic-treated animals. Type II Bartter's patients are less hypokalemic than patients with Bartter's due to mutations in NKCC2 or the Kb subunit of the basolateral chloride channel [31], suggesting that both K<sup>+</sup> channels contribute to the hypokalemia seen in diuretic use (Fig. 1). Similarly, the effect of furosemide and hydrochlorothiazide on kaliuresis is blunted in ROMK knockout mice [22•].

### Lessons learned from pseudohypoaldosteronism type II

Patients with the autosomal-dominant disorder pseudohypoaldosteronism type II (PHA2) invariably have hyperkalemia, and many have hypertension, though the onset of high blood pressure can be delayed [32,33]. Early studies showed that urinary K<sup>+</sup> excretion was at or below that of daily K<sup>+</sup> intake, despite hyperkalemia, indicating a defect in urinary K<sup>+</sup> excretion [33-40].

PHA2 is caused by mutations in the related kinases WNK1 and WNK4 [41], and animal models have furthered our understanding of the pathophysiology of this disorder. Specifically, a transgenic mouse expressing a mutant allele of WNK4 (Q562E), corresponding to one of the mutations found in human PHA2, has been generated [42], as well as a knock-in mouse in which the wild-type WNK4 has been replaced by a mutant allele (D561A) corresponding to a second PHA2 mutation [43]. Like humans with PHA2, the mutant mice are hypertensive and hyperkalemic. The Q562E transgenic mice have a hyperplastic DCT, and the D561A knock-in mice have increased expression of NCC as well as increased phosphorylation and apical density of this transporter. These results suggested that overactivity of NCC could explain the phenotypes seen in the WNK4 mutant mice as well as in humans with corresponding mutations. Indeed, the hyperkalemia and hypertension seen in the Q562E transgenic mice was suppressed by deletion of NCC, and treatment of the D561A knock-in mice with hydrochlorothiazide also corrected hypertension, as well as increasing the urinary fractional excretion of  $K^+$ , with normalization of serum  $K^+$ . Patients with PHA2, at least those with mutations in WNK4, are also responsive to thiazide treatment, and it has been estimated that their sensitivity to thiazide is increased approximately six-fold compared to normal individuals [32,38].

### Mechanism of hyperkalemia in PHA2: role of NCC, ROMK, and maxi-K

A proposed mechanism to explain these patients' hyperkalemia, then, is that there is increased  $Na^+$  reabsorption in DCT due to increased NCC activity, with decreased distal delivery of  $Na^+$ , less negative luminal PD, and therefore less  $K^+$  secretion in CNT (Fig. 2). Indeed, increased surface expression of ENaC, seen in the CCD of the D561A knock-in mice, likely reflects a compensatory response to this reduced fluid flow and  $Na^+$  delivery. In modeling experiments, a decrease in distal  $Na^+$  delivery from 65 mM to 25 mM, if flow rate is kept constant at 6 nl/min, would be expected to decrease  $K^+$  secretion in CNT by about 50% [6••]. However, this mechanism alone will not lead to sustained hyperkalemia unless the expected compensatory increase in the function of ROMK and maxi-K is abolished. In PHA2, disease-causing mutations in WNK1 and WNK4 result in inhibition of ROMK activity via increased endocytosis [44-46]. The apparently normal level of ROMK in the apical membrane of distal nephron in PHA2-mimicking mice compared to controls [42,43] could be interpreted as a relative deficiency in surface ROMK considering that these mice have severe hyperkalemia. Second, although there is a compensatory increase in the expression of the maxi-K  $\alpha$ -subunit by ~3 fold [43],  $K^+$  secretion via this channel is likely below normal in the knock-in mice as a result of reduced fluid flow and  $Na^+$  delivery – that is, there is a functional deficiency in the activity of the channel (Fig. 2). However, the upregulation of maxi-K likely explains why maneuvers which increase distal flow, such as sodium sulfate infusion or treatment with hydrochlorothiazide, lead to brisk kaliuresis and improvement in hyperkalemia [34,36,38-40,43,47].

### $Na^+$ transport in PHA2 in steady-state

The argument has been advanced that changes in  $Na^+$  delivery or distal flow could not explain differences in tubular  $K^+$  excretion in PHA2 patients because they are in steady-state. However, steady-state in a patient with disease may not recapitulate a non-diseased state. For example, assume that in a normal individual there is reabsorption of 90% of filtered  $Na^+$  in proximal tubule and TAL, 6% in DCT, and 3% in the CNT and collecting duct, with 1% excretion in the urine (Fig. 3). In a patient with PHA2, if the primary defect is increased  $Na^+$  reabsorption by NCC in DCT, such that DCT reabsorption is now 12%, there may be a compensatory decrease in proximal and thick limb reabsorption to 86%, but if the final urinary excretion of filtered  $Na^+$  is still 1%, the connecting tubule and collecting duct will see only 2% of the filtered  $Na^+$  as compared to 4% in a normal individual. This reduction in  $Na^+$  (and water) delivery could be sufficient to explain the observed defects in

urinary  $K^+$  excretion. Ultimately, however, micropuncture experiments in WNK4 mutant mice are needed to resolve this issue.

## Conclusions

Despite advances in our understanding of the  $K^+$  secretory channels ROMK and maxi-K, the relative roles of these two channels in regulating tubular  $K^+$  secretion in health and disease have yet to be completely elucidated. A combination of pharmacologic studies, perhaps with novel drugs allowing inhibition of maxi-K and ROMK after systemic administration, physiologic investigations including patch-clamp and micropuncture studies, and genetic studies in which the channels are manipulated in a spatially and temporally restricted fashion, could help advance our understanding of these questions.

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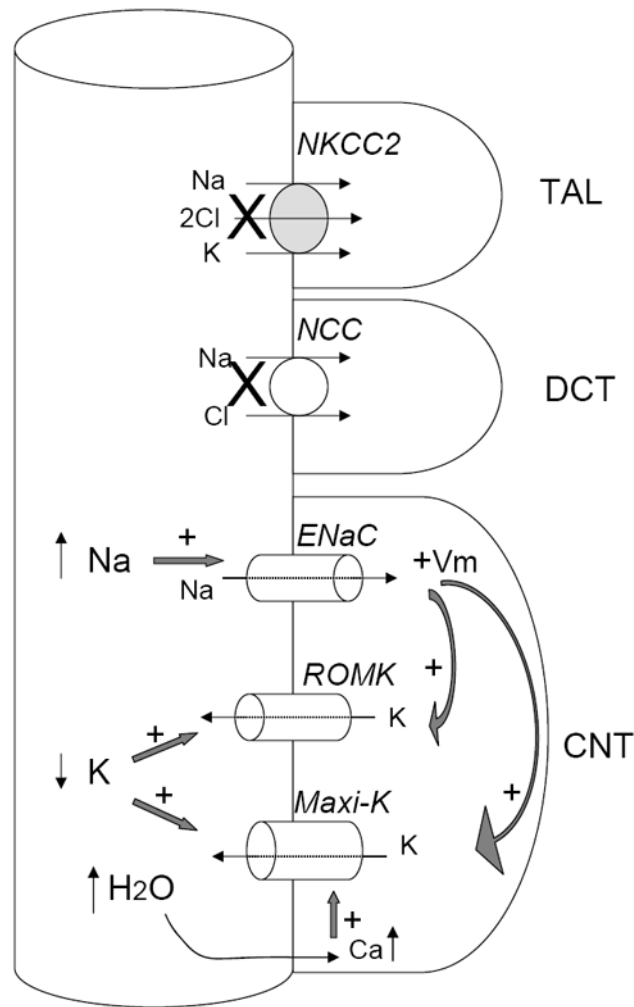
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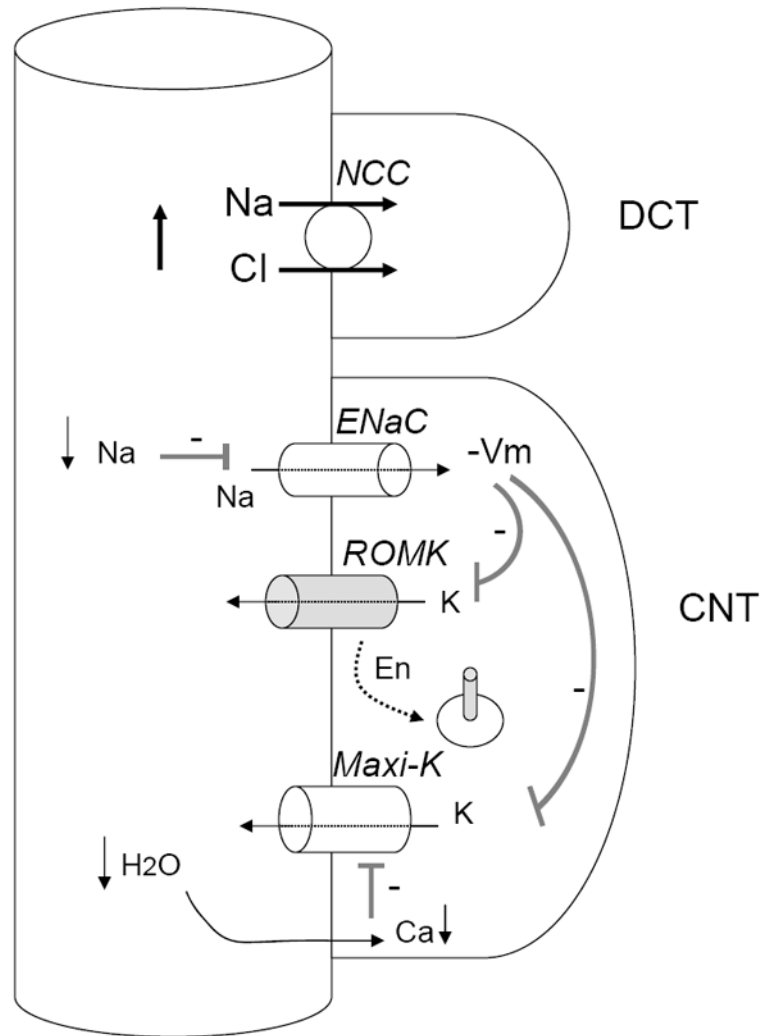
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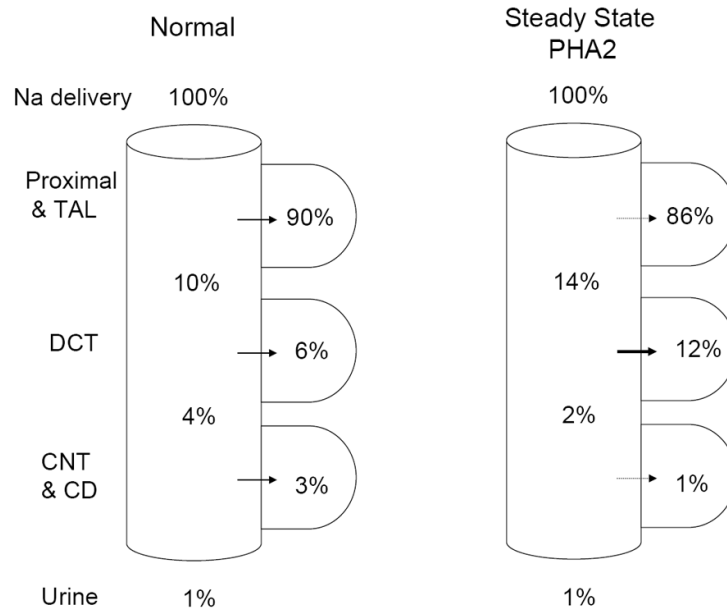
**Figure 1. Flow-dependent increase in K<sup>+</sup> secretion involves both ROMK and Maxi-K**  
 Decreases or loss of function of NKCC2 or NCC increase fluid and Na<sup>+</sup> delivery to CNT, the key site for K<sup>+</sup> secretion. Increased fluid flow decreases luminal K<sup>+</sup> concentration. Increased Na<sup>+</sup> delivery increases Na<sup>+</sup> reabsorption via ENaC and depolarizes apical membrane potential (indicated by “+V<sub>m</sub>”). Both factors increase K<sup>+</sup> secretion via ROMK and maxi-K. In addition, increased fluid flow stimulates Ca<sup>2+</sup> entry to activate maxi-K. Thus, both ROMK and maxi-K contribute to flow-dependent K<sup>+</sup> secretion at the low flow rate. The relative contribution by maxi-K is increased at the high flow rate. Maxi-K is also activated by membrane depolarization. The contribution from membrane polarization due to increased ENaC activity to the activation of maxi-K, however, is relatively small compared to that from increases in fluid flow and the rise in intracellular Ca<sup>2+</sup> concentration. See text for abbreviations.





**Figure 2. Three potential mechanisms for hyperkalemia in patients of PHA2 with WNK4 mutations**

First, overactivity of NCC decreases  $\text{Na}^+$  reabsorption via ENaC, hyperpolarizes membrane potential (indicated by “ $-V_m$ ”), and decreases  $\text{K}^+$  efflux via ROMK and maxi-K. Second, mutations in WNK4 that cause PHA2 inhibit ROMK by increasing its endocytosis (indicated by “En”). Third, reduced fluid flow to CNT decreases the opening of maxi-K. Because hyperkalemia causes upregulation of ROMK and maxi-K, the increase in the endocytosis of ROMK and “functional” inhibition of maxi-K (i.e., secondary to reduced  $\text{Na}^+$  and fluid delivery) are likely critical for sustained hyperkalemia.



**Figure 3. Hypothetical model for steady-state tubular  $\text{Na}^+$  handling in PHA2 patients with WNK4 mutations**

It is assumed that WNK4 mutations increase  $\text{Na}^+$  reabsorption in DCT from 6% (of filtered load) in control to 12% in the diseased state. Assuming that the compensatory decrease in  $\text{Na}^+$  reabsorption (in response to volume expansion and hypertension) occurs in the tubular segments proximal as well as distal to DCT,  $\text{Na}^+$  delivery to the CNT/CD will be reduced compared to the control (2% vs 4% in PHA2 vs control, respectively). It should be mentioned that the axial fluid profile does not parallel that for  $\text{Na}^+$  delivery because DCT lacks aquaporin-2.