Renal potassium transport: mechanisms and regulation

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Giebisch, Gerhard. Renal potassium transport: mechanisms and regulation. Am. J. Physiol. 274 (Renal Physiol. 43): F817–F833, 1998.—The regulation of potassium metabolism involves mechanisms for the appropriate distribution between the intra- and extracellular fluid compartments and for the excretion by the kidney. Clearance and single-nephron studies show that renal excretion is determined by regulated potassium secretion and potassium reabsorption, respectively, in principal and intercalated cells of the distal nephron. Measurement of the electrochemical driving forces acting on potassium transport across individual cell membranes and characterization of several ATPases and potassium channels provide insights into the transport and regulation of renal potassium excretion.

principal cells; potassium secretion; potassium reabsorption; potassium channels

THE PHYSIOLOGICAL REGULATION of potassium, the most abundant cation in the body, requires achievement of two goals. First, high concentrations of potassium in the cytosol are necessary for many cells to function normally (130). Second, sizable concentration gradients of potassium across cell membranes are required for nerve excitation and muscle contraction. This condition can be met only by mechanisms that maintain low extracellular potassium concentrations. Accordingly, two internal milieus, intracellular and extracellular fluid, are continuously and separately controlled to safeguard potassium homeostasis.

An understanding of potassium homeostasis requires considering the special distribution of potassium in the body. Figure 1A shows that the vast majority of potassium is contained by cells; only a very small fraction, some 1–2% of total potassium, is located in the extracellular fluid (110, 130). The small extracellular pool can be greatly increased from two sources: external intake and internal redistribution. An intake of 35 meq, about one-third of the normal daily intake of potassium, would produce an increase of plasma potassium of about 2.5 meq/l, were that amount confined exclusively to the extracellular fluid. Such a rise in plasma potassium could have pronounced deleterious effects on neuromuscular and cardiac function. However, the apparent volume of distribution of an oral potassium load is greatly in excess of the extracellular fluid compartment: rapid redistribution into tissue stores, particularly those of muscle and liver, provide protection against unphysiological fluctuations of plasma potassium concentrations. The most important of the mechanisms that modulate the distribution of potassium between the extra- and intracellular compartment are listed in Fig. 1B (18, 110, 130).

The internal balance of potassium can also be severely disturbed whenever those mechanisms that retain large amounts of potassium within cells are compromised. Accordingly, interference with Na-K-ATPase activity or abnormal leakiness of cells to potassium, as may occur when there is extensive cell damage, always pose the threat of acute hyperkalemia by possibly lethal mixing between intra- and extracellular potassium pools (50, 110, 130).

The two mechanisms that counter changes in plasma potassium concentration are distinguished by different time courses: an excess or deficit of potassium in the extracellular fluid can be rapidly, within minutes, corrected by appropriate shifts into or out of body stores. The renal response is much slower, and renal corrections of disturbances in potassium balance require several hours (110, 130).

Approaches to the Study of Renal Potassium Excretion

Advances in our knowledge of the renal mechanisms of potassium excretion have depended on the application of a wide variety of techniques, summarized in Table 1. The modern period of investigation into modes of potassium transport by the kidney begins with the use of the flame photometer in the late 1940s and the application of clearance methods (7, 8, 97, 98). The comparison of the excreted with the filtered amounts demonstrated that the kidney tubules could secrete potassium, and secretion was strongly implicated even when potassium excretion was only a modest fraction of the filtered load. Early clearance studies also provided
Evidence for the presently accepted model of potassium excretion, according to which most of filtered potassium is reabsorbed in proximal nephron segments and potassium excretion in the urine depends on controlled secretion in the distal nephron (7, 8, 97, 98). Moreover, clearance studies also supported the view that potassium excretion was dependent on the availability of sodium in the urine. Factors such as adrenal hormones, systemic acid-base changes, diuretics, and potassium adaptation were shown to modulate potassium excretion by changes in the rate at which potassium was secreted by the renal tubules (7, 8).

With the revival of single-nephron studies in the 1950s, it became possible to localize the sites of potassium transport along the nephron directly and to gain further insight into the factors controlling potassium transport (86–90). It is interesting that the main conclusions about the localization and mechanisms of potassium transport derived from clearance studies were fully supported by micropuncture studies. Perfusion of tubules in vitro provided a refinement of single-nephron studies by providing information on potassium transport in those nephron segments not accessible to puncture (elements of the loop of Henle and collecting ducts). Such studies also allowed much more extensive manipulations of the intra- and extratubular environment and thus of luminal and peritubular factors controlling potassium transport (14, 42).

The application of electrophysiological methods in various nephron segments has been invaluable for defining the driving forces for potassium transport across the apical and basolateral cell membranes, as well as through paracellular pathways. Such studies, combined with measurements of intracellular potassium ion activities, made possible the precise localization of potassium transporters (ion pumps and ion channels) and thus the study of the mechanisms by which physiological or pathophysiological events alter potassium transport (37, 40, 130). Most recently, patch-clamp techniques have made it possible to define the properties of single potassium channels in both apical and basolateral membranes of tubule cells (38, 41, 103).

Morphological studies have also contributed significantly to our understanding of renal potassium transport. Structure-function relations have supported the thesis that principal tubule cells secrete whereas intercalated cells reabsorb potassium by demonstrating

Table 1. Approaches to the study of renal potassium excretion

<table>
<thead>
<tr>
<th>Approach</th>
<th>Description</th>
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<tbody>
<tr>
<td>1) Renal clearance studies</td>
<td>Comparison of excreted with filtered amount of potassium K secretion, Na/K exchange, regulation: hormones, acid-base disturbances, diuretics, adaptation, pathophysiology</td>
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<tr>
<td>2) Micropuncture and microperfusion</td>
<td>Measurement of net transport and unidirectional fluxes; localization of potassium transport along nephron, recycling of potassium, site of action of hormones, diuretics, and of potassium adaptation</td>
</tr>
<tr>
<td>3) Electrophysiology</td>
<td>Conventional and ion-sensitive microelectrodes, single-channel analysis by patch-clamp techniques; electrochemical potential of potassium across apical and basolateral membrane and paracellular shunt pathway; characterization of potassium channels in apical and basolateral cell membranes</td>
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<tr>
<td>4) Ultrastructure</td>
<td>Cell heterogeneity; electronprobe analysis; structure-function relation; immunohistochemistry of transporters (Na-K-ATPase and H-K-ATPase and K and Na channels)</td>
</tr>
<tr>
<td>5) Biochemical studies</td>
<td>ATPases, cell messengers</td>
</tr>
<tr>
<td>6) Molecular biology</td>
<td>Cloning of renal potassium channels, ATPases and receptors</td>
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cell-specific morphological changes of ultrastructure following potassium deprivation or potassium excess (63–65, 83, 128–130, 132–134). The combination of electron probe techniques with morphological studies has also been useful, because it allows measuring the ion content of single tubule cells in different states of transport. Rapid uptake of rubidium (a marker for potassium) occurs only into principal but not intercalated cells (3, 4). Application of immunochemical analysis of ATPase activity in single cells of the distal nephron fully supports the conclusion that principal but not intercalated cells are involved in sodium reabsorption and potassium secretion (109).

Biochemical studies on single collecting ducts have been essential for defining several types of ATPases in nephron segments that transport potassium. Relevant are studies on the adaptation of both Na-K- and K-H-ATPases in single tubules and the activation of specific pump isoforms in physiological and pathophysiological conditions (19–22, 36, 71, 171).

Finally, the advent of molecular biological techniques has further advanced our knowledge of the structure of several proteins that mediate potassium transport. Molecules such as Na-K- and K-H-ATPase as well as several renal potassium channels have been cloned (see Ref. 120) and have aided in the in-depth characterization of potassium transport.

Potassium Transport Along the Nephron

Studies on single tubules show that potassium excretion is mediated by three processes: glomerular filtration, reabsorption along the proximal tubule and the loop of Henle, and bidirectional transport (secretion and reabsorption) in the initial and cortical collecting tubule (Fig. 2). Potassium secretion was also demonstrated in isolated perfused collecting ducts in vitro (14, 28, 42, 106, 116, 117, 135).

Figure 2 shows that regulated secretion (in principal tubule cells) is the source of potassium excretion when potassium intake is normal or high, but potassium reabsorption (in intercalated cells) may replace secretion when reduced potassium intake requires a very low level of urinary excretion (37, 39–41, 86–90, 104, 173).

Cell Mechanisms of Potassium Transport

The direction and magnitude of potassium transport in the several nephron segments depend on the site-specific distribution of transporters in the membranes of tubule cells. Potassium movement may involve either transcellular or intercellular pathways. Figure 3 illustrates the mechanisms of potassium transport at four sites along the nephron. The transporters in the basolateral membrane are generally similar; different transport mechanisms mediate potassium movement in the apical membrane (41, 86, 88, 130).

Proximal tubule. Micropuncture and microperfusion of proximal tubules have provided the following picture of potassium reabsorption. First, the concentration of potassium varies little along the proximal tubule, and fractional rates of reabsorption are similar to those of sodium and water (37, 40, 86). Second, the transepithelial voltage is lumen negative in early loops and lumen positive further down the proximal tubule (35). Third, net potassium movement is strongly affected by sodium and water movement so that changes in fluid and potassium transport are closely coupled (11).

Two mechanisms, solvent drag and diffusion, have been suggested as the major driving forces for potassium reabsorption. Recent measurements of low reflection coefficients of potassium in perfused proximal convoluted tubule in vivo strongly suggest that solvent drag is an important driving force for potassium reabsorption (11, 165, 166) and that potassium ions, entrained in tubule fluid, are reabsorbed largely along the paracellular pathway. However, the observation that barium in the tubule fluid significantly lengthens the time of dissipation of imposed transepithelial potassium gradients suggests participation of a transcellular route of proximal potassium reabsorption by an unknown mechanism (147). Perfusion studies with several transport inhibitors in vivo have not provided...
The fact that the lumen of a large part of the proximal tubule is electrically positive with respect to the peritubular fluid provides a significant driving force for passive potassium reabsorption. Lowering of the potassium concentration in the interspace has also been suggested as an additional mechanism for potassium reabsorption by diffusion across the tight junction.

Urinary excretion of potassium is not directly controlled by changes in its reabsorption along the proximal tubule. However, secondary flow-dependent alterations in potassium transport along distal nephron segments occur when sodium delivery increases. Administration of mannitol, inhibition of reabsorption, high delivery rates of glucose, or reduced transport of bicarbonate (carbonic anhydrase inhibitors) are relevant examples. Distal potassium secretion may be suppressed when excessive reabsorption of sodium along the proximal tubule lowers distal sodium and fluid delivery.

Loop of Henle. This nephron segment includes several anatomically and functionally distinct nephron segments: the proximal straight tubule, the thin descending and ascending limbs, and the thick ascending limb. Thin segments are absent in cortical loops. Knowledge of potassium transport is based on both micropuncture of the tip of Henle's loop and on microperfusion of isolated perfused loop segments. The picture that has emerged suggests passive secretory entry of potassium into the medullary segment of the proximal straight tubule and the descending thin limb of Henle, as well as outward movement in the thin ascending limb. Both processes occur by diffusion, favored by the high potassium permeability and appropriate concentration gradients. Potassium entry into the thin descending limb is driven by the increasing potassium concentration along the corticomedullary axis. Conversely, as the potassium concentration in the interstitium declines toward the corticomedullary junction, potassium ions are passively reabsorbed. Passive potassium entry into the descending limb of Henle is part of the pathway of potassium recycling from medullary collecting ducts into the loop of Henle.

The mechanism of transport of potassium along the thick ascending limb of the loop in mammals and the diluting segment of amphibians is different from that in thin limbs. Potassium is reabsorbed under physiological conditions, but secretion has also been observed. Features of potassium reabsorption include two transport steps: apical uptake by secondary active, electroneutral Na-2Cl-K cotransport and passive exit across the basolateral membrane by diffusion through potassium channels or cotransport with Cl or HCO₃. A significant fraction of potassium reabsorption also passes through the paracellular pathway driven by the lumen-positive transepithelial potential difference. Apical potassium uptake is against a modest chemical gradient and depends on coupling to sodium that enters the cell along a large electrochemical gradient generated by the basolateral Na-K-ATPase activity.

A potassium conductance in the apical membrane is an essential component of potassium transport; it also maintains the activity of the Na-2Cl-K cotransporter. Potassium reabsorption may be replaced by potassium secretion when the Na-2Cl-K cotransporter is inhibited, for instance, by loop diuretics. Such reversal of the direction of potassium movement is best explained by diffusion from cell to lumen along a favorable electrochemical gradient, unopposed by cotransport-dependent potassium reabsorption. Potassium recycling across the apical membrane is required for the normal activity of the Na-2Cl-K cotransporter, because it provides an adequate supply of potassium ions. Removal of potassium from the lumen or inhibition of the apical potassium conductance leads to a sharp decline of sodium chloride concentration.
reabsoption (43, 45, 49, 53, 54, 150, 151). The apical potassium conductance is also essential for the generation of the lumen-positive potential difference, which provides a significant driving force for passive cation reabsorption along the paracellular transport pathway.

Of the known factors that regulate potassium transport in the thick ascending limb of Henle's loop, a low-potassium diet (141, 142), aldosterone, and vasopressin stimulate potassium reabsoption (47, 53, 127, 172), whereas potassium reabsoption is reduced by loop diuretics (43, 54, 58, 96), a high potassium intake (141, 142), and high plasma calcium (160). Arachidonic acid lowers potassium channel activity and would thus be expected to reduce potassium reabsoption (159). Prostaglandins are also involved in regulating Na-2Cl-K reabsoption, as demonstrated by the attenuation of the diuretic response to loop diuretics when prostaglandin synthesis is inhibited and reversal of this effect by placing prostaglandin E₂ (69, 70) in the lumen.

Initial and cortical collecting tubule. Regulated secretion of potassium in the initial and cortical collecting tubule plays a major role in urinary potassium excretion, but when potassium conservation is required, reabsoption can replace secretion (104, 130, 173).

Potassium secretion occurs in principal cells by active uptake across the basolateral membrane by Na-K-ATPase and passive diffusion across the apical membrane into the lumen (37, 40, 41, 86–90, 130). Besides diffusion along a favorable electrochemical gradient through potassium channels, it has been suggested that there is electroneutral cotransport of potassium with chloride in the apical membrane (12, 25–27, 41, 130, 143, 144). The cell model in Fig. 3 also includes apical sodium channels that permit sodium to enter principal cells passively and to depolarize the apical membrane. High concentrations of sodium in the lumen thus favor the electrochemical driving force for potassium diffusion into the lumen. Conversely, low concentrations of sodium curtail potassium secretion by producing hyperpolarization of the apical membrane which inhibits potassium secretion. The sodium channel blocker amiloride reduces potassium excretion by a similar mechanism (24, 36). In addition to its effect on the apical membrane potential, sodium affects potassium secretion by the requirement of the basolateral Na-K-ATPase for sodium. Enhanced delivery of sodium into principal cells increases the intracellular sodium concentration and stimulates basolateral sodium/potassium exchange. Accelerated potassium uptake favors its diffusion across the apical membrane.

The strong dependence of potassium secretion on distal sodium supply explains the observation that an increase in the delivery of sodium-containing fluid to the distal tubule increases potassium secretion (24, 39, 68, 84, 85, 130). Figure 4 shows that potassium secretion along the distal tubule rises in a flow-dependent manner and that the effect is modified by the dietary potassium intake. Figure 4 further shows that potassium secretion continues to increase, over the physiologic
Na-K-ATPase activity associated with an increase in basolateral potassium permeability induces a marked hyperpolarization of the cell potential with negativities significantly in excess of −100 mV (72, 113). These electrical effects lead to a reversal of the driving force for passive potassium transport across the basolateral membrane. As shown in Fig. 6, potassium ions in mineralocorticoid-treated animals now diffuse from peritubular fluid into the cytoplasm because the membrane potential exceeds the potassium equilibrium potential (72, 113). Increased potassium uptake thus takes place not only by accelerated active uptake via Na-K-ATPase but also passively through potassium conductive pathways (38, 72, 113).

Figure 5B demonstrates the role of aldosterone on potassium excretion (176). Stimulation by aldosterone results in an increase of the slope of the relation between plasma potassium and urinary potassium excretion. As a result, urinary excretion, as well as potassium balance, is maintained at progressively lower plasma potassium concentrations as circulating aldosterone levels rise (176).

The dependence of potassium secretion on sodium and flow poses problems, because obligatory coupling of potassium excretion to distal flow rate and sodium delivery could jeopardize potassium balance when flow rate into the distal tubule and collecting ducts changes independently of potassium balance. Figure 7 summarizes likely events that stabilize potassium excretion during changes in extracellular fluid volume despite fluctuations in distal flow rate (130). A fall in extracellular volume leads to increased reabsorption of sodium and fluid in the proximal tubule and would thus decrease distal potassium secretion because of the delivery of fluid and sodium to principal tubule cells declines. Potassium secretion should fall as a consequence of diminished sodium and fluid delivery. However, the contraction in extracellular volume also activates release of aldosterone, which stimulates distal potassium secretion. Therefore, changes in potassium

Fig. 5. A: relation between steady-state plasma potassium concentration and urinary potassium excretion in the dog as a function of dietary sodium intake (in meq/day). Dogs were adrenalectomized and given a fixed dose of aldosterone, and potassium content was varied at different sodium intake. (See Ref. 177.) B: relation between steady-state plasma potassium concentration and urinary potassium excretion as a function of aldosterone. Animals were adrenalectomized and given fixed doses of aldosterone, and potassium content of the diet was varied. [Reprinted from Zhoa et al. (178).]

Fig. 6. Schema of cortical collecting tubule cell with key sites of potassium transport. Depending on the magnitude of the electrical potential difference across the basolateral cell membrane, potassium ions may either leave the cell (recycle, A) or be taken up into the cell in parallel with pump-mediated potassium transport (B). [Modified from Giebisch (38).]

Fig. 7. Effect of extracellular fluid volume (ECFV) contraction on potassium secretion. Changes in potassium secretion may be minimized because of opposing actions of aldosterone and tubule flow rate on potassium secretion. [Reprinted from Stanton and Giebisch (130).]
excretion are minimized. As a consequence of these opposing effects, potassium excretion remains relatively constant. It is also known that potassium excretion remains fairly stable during changes in flow rate that result from variations in circulating vasopressin levels (130). It is reasonable to explain such stability of renal potassium excretion by postulating that the stimulating effect of enhanced distal delivery of fluid and sodium is offset by the fall in vasopressin, a hormone known to stimulate potassium secretion (30, 32, 117).

The situation is different when potassium balance is threatened by the synergy of factors that stimulate potassium secretion in principal tubule cells (130). Figure 8 illustrates the mechanisms responsible for the kaliuretic effects of many diuretics. Most diuretic agents promote an increase in sodium and fluid delivery to the distal tubule. At the same time, aldosterone release is activated as the extracellular fluid volume shrinks with the loss of sodium, with both factors acting synergistically to promote potassium secretion and kaliuresis. Similar increases of potassium secretion may occur in hyperkalemia with an increase in dietary potassium intake, when high flow rate due to inhibition of proximal sodium reabsorption and increased aldosterone act synergistically to promote kaliuresis. Enhanced potassium excretion may also occur in metabolic alkalosis when large amounts of bicarbonate reach the distal tubule fluid.

Potassium Reabsorption

The kidney's ability for potassium reabsorption in distal nephron segments was suggested by early observations that the concentration of potassium in the urine may fall below that in surrounding medullary structures. Microperfusion studies have confirmed net reabsorption of potassium along the perfused distal tubule in hypokalemic rats (104). Potassium reabsorption has been localized to the intercalated cells in the initial and cortical collecting tubule (63, 65, 83, 130, 132–134) and to cells of the collecting duct in the inner stripe of the outer medulla (170). It has been proposed that there is a component of potassium reabsorption in the initial collecting duct even during net secretion, because in microperfused tubules the steady-state transepithelial concentration gradients of potassium were always below that expected from the electrical potential difference (37, 40, 86, 87, 130). Accordingly, net secretion may be the result of simultaneous bidirectional secretory and reabsorptive potassium fluxes.

The discovery of potassium-activated ATPases in isolated collecting ducts has greatly advanced our knowledge of the molecular mechanisms of potassium reabsorption. Several isoforms of H-K-ATPases have been identified and shown to reabsorb potassium in exchange for hydrogen ions; they share some functional properties with gastric and colonic ATPases (13, 20–23, 71, 170, 171). Both functional (123–125) and morphological evidence (83, 128, 129) suggest that β-intercalated cells are an important, although perhaps not the exclusive, site of potassium/hydrogen exchangers. There are morphological changes in intercalated cells such as an increase in apical surface area and insertion of rod-shaped particles into the apical membrane in hypokalemia and in metabolic acidosis, both conditions in which potassium reabsorption and hydrogen ion secretion are increased (63, 65, 83, 128). These morphological changes suggest that potassium depletion induces recruitment of vesicles that contain potassium-absorbing transport ATPases from a subapical pool and their insertion into the apical membrane (129). Upregulation of this transport protein has been observed in potassium depletion (13, 20–23, 71, 171), and functional studies indicate stimulation by K⁺-H⁺ pump activity during metabolic acidosis (113, 114) and sodium deprivation (115).

Renal Potassium Channels

Two methods have greatly enhanced our understanding of the mechanisms of renal potassium transport. First, the introduction by Neher and Sakmann (103) of the patch-clamp technique has made it possible to study the localization and the properties of potassium channels along the nephron (38, 158, 162). Second, the cloning of renal potassium channels has provided new insights into the structure of potassium channels and their regulation. Table 2 and Fig. 9 summarize the role of potassium channels in renal electrolyte transport (38).

First, potassium channels are essential for generation of the cell-negative potential. A high concentration of potassium is maintained in the cytoplasm of tubule cells by the energy-dependent activity of the basolateral Na-K-ATPase (41, 130). Potassium channels provide a potassium conductance in the basolateral membrane and are thus responsible for the generation of the cell-negative diffusion potential, which provides an important driving force for the entry of positively

![Diagram](image)

**Fig. 8.** Effect of diuretics acting upstream of the initial collecting tubule on potassium secretion. Potassium secretion is stimulated by the synergistic effects of high urine flow rate and high aldosterone.

| 1 | Maintenance of negative potential of tubule cells |
| 2 | Regulation of volume of tubule cells (VRD) |
| 3 | Recycling across apical and basolateral cell membranes to supply potassium to Na-2Cl-K cotransport and Na-K-ATPase |
| 4 | Potassium secretion in initial and cortical collecting tubule |

Table 2. Function of renal potassium channels
charged ions and cotransporters across the apical membrane of tubule cells (41).

Second, the regulation of the volume of renal tubule cells is strongly dependent on the activity of potassium channels in both apical and basolateral membranes (38, 112, 130). Renal tubule cells share with many body cells the ability to reduce their volume when swelling occurs as a result of entry of solutes or dilution of their extracellular environment. Such volume regulatory decreases depend on the activation of potassium channels (66, 67, 112). Activation of potassium channels, with a simultaneous increase of anion conductance, allows potassium ions to leave the cell along a favorable electrochemical gradient and to restore cell volume toward its initial set point. Stretch-activated potassium channels are found in all nephron segments, but their mode of activation differs: direct activation by membrane stretch or indirect activation by an increase in the concentration of cell calcium resulting from stimulation of cation-selective channels (112). The latter mechanism is present in apical membranes of proximal tubule cells and involves large-conductance, calcium-activated potassium channels. These channels are also stimulated by membrane depolarization (66, 67, 112).

Third, potassium recycling across the apical membrane of the thick ascending limb of Henle's loop, as well as through the basolateral membrane of tubule cells, depends on several subtypes of potassium channels. Apical potassium channels in the thick ascending limb are important for the regulated supply of potassium to the Na-2Cl-K cotransporter and the generation of the lumen-positive potential, as demonstrated by the inhibition of sodium chloride absorption by blockers of potassium channels (43–45, 158, 162). Potassium channels in the basolateral membrane are inhibited by millimolar concentrations of Mg-ATP (5, 6) and by acidification of the cytosol (67, 95) and activated by cell swelling and application of negative pressure to excised membrane patches (112). Several studies have provided evidence that the ATP-sensitive potassium channels in the basolateral membrane of proximal tubule cells link the potassium conductance to the activity of the Na-K-ATPase (5, 6, 140, 167; see below). Acidosis inhibits potassium channels in the basolateral membrane, an effect consistent with the decrease of the potassium conductance in acidosis (75).

Thick ascending limb of the loop of Henle. Figure 10 summarizes our present knowledge of the factors that regulate potassium channel activity in the cells of the thick ascending limb (9, 44, 152, 158, 162, 164). Two channels with high open probability and conductances of 30 and 70 pS are located in the apical membrane and are subject to regulation by changes in pH and the concentrations of ATP, protein kinase A (PKA) and protein kinase C (PKC) (152). In addition, a large-conductance potassium channel, not shown in Fig. 10, activated by calcium and membrane depolarization, has been observed in cultured thick ascending limb cells (48). Because it has a low open probability, it is unlikely to contribute importantly to the apical potassium conductance; it may be activated by cell swelling and thus play a role in cell volume regulation.

Changes in cell calcium concentration modulate both types of apical potassium channel indirectly by activating PKC (160). The apical potassium channels are also inhibited by acidic pH and millimolar concentrations of

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**Fig. 9. Localization of ATP-sensitive potassium channels along the mammalian nephron. [Reprinted from Wang et al. (158).]**
ATP, cAMP and PKA mediate vasopressin-induced enhancement of channel activity. These results account for the previous observation that vasopressin increases the apical potassium conductance. Such PKA-induced activation of apical potassium channels increases sodium chloride reabsorption by enhancement of potassium recycling across the apical membrane (52, 53). Figure 10 shows that arachidonic acid is also an inhibitor of apical potassium channels, an effect mediated by the cytochrome P-450 metabolite 20-HETE (159). The inhibition of apical potassium channels that follows activation of basolateral calcium receptors has been found to be an effect of this arachidonic acid metabolite (160), and it has been suggested that P-450 metabolites thus link changes in extracellular calcium to apical sodium chloride reabsorption by modulating recycling of potassium.

Cortical collecting tubule. Figure 11 illustrates the factors that regulate the apical and basolateral potassium channels (158). Two types of apical potassium channels have been found in rat and rabbit principal tubule cells: not included in Fig. 11 is a large single-conductance potassium channel with low open probability. This channel is activated by increase of cell calcium and by membrane depolarization and is inhibited by tetraethylammonium (33, 59, 153, 158). Its low open probability makes it unlikely that it plays a role in potassium secretion, although it may be involved in cell volume regulation.

There is now general agreement that a low-conductance, inwardly rectifying potassium channel with high open probability mediates potassium secretion across the apical membrane of principal cells in the initial and cortical collecting tubule (34, 38, 41, 52, 79, 108, 119, 153, 158, 162). In contrast to the large-conductance channel, the low-conductance channel has a significant rubidium conductance (108), consistent with the observed secretion of rubidium by perfused cortical collecting tubules (116). The low-conductance potassium channel has a high open probability in physiological conditions of normal potassium intake. Recruitment of additional channels increases apical potassium conduc-

tance in response to such factors as vasopressin, cell alkalization, or adaptation to high potassium intake (15, 107, 153, 163). It is of interest that an increase in potassium intake and aldosterone have separate but additive effects on enhancement of apical potassium channel density (107). The apical potassium channel is highly sensitive to acidification of the cytosol, and channel activity declines markedly when cell pH is reduced from 7.4 to 7.0 (16, 28, 163). This is consistent with the effects of metabolic acidosis, which decreases potassium secretion in distal tubules (130).

An important property of apical secretory potassium channels is their sensitivity to changes in ATP. Patch clamp studies have shown that low concentrations of ATP (in the submillimolar range) are required for phosphorylation and for activation of channels, whereas millimolar concentrations of ATP inhibit channel activity (156, 158, 163). ATP-induced channel inhibition can be attenuated by ADP, cAMP-dependent PKA, and by an increase in pH. Secretory potassium channels are stimulated by vasopressin as evidenced by patch-clamp studies that show involvement of CAM via a PKA-dependent pathway (15). These results are consistent with observations in perfused distal tubules in vivo and cortical collecting ducts in vitro in which vasopressin stimulated potassium secretion (32, 117).

The apical potassium channel is indirectly inhibited by raising intracellular calcium concentrations (153, 156, 163). This effect can be demonstrated in cell-attached membranes when the calcium concentration of principal cells is artificially increased. In contrast, exposure of inside-out patches to high cytosolic calcium...
does not change channel activity (163). Several lines of evidence suggest that PKC and calcium-calmodulin-dependent kinases are stimulated by calcium and are involved in channel inhibition (73, 74). Conditions known to lower apical potassium channel activity and potassium secretion, such as inhibition of basolateral Na-K-ATPase activity (156; see below) or cyclosporin (78), involve calcium-induced changes in PKC activity. Other factors that modulate apical potassium channels also include direct inhibition by arachidonic acid (155) and interaction with the cytoskeleton (154).

The rapid decrease of channel activity that often follows membrane excision (channel “run-down”) can be delayed or abolished by deletion of Mg or by phosphatase inhibitors (73). This suggests that the balance between phosphorylation and dephosphorylation processes alters apical potassium channel activity. The demonstrated presence of Mg-dependent and -independent membrane-bound phosphatases is in accord with this inference (73).

Figure 11 also includes information on the regulation of basolateral potassium channels (158). The technical problems of studying the basolateral membrane have been resolved by selective removal of intercalated cells so as to expose lateral membranes of principal cells, or by collagenase treatment (119, 158). Several channels with differing single channel conductance have been identified (55, 80, 118, 119, 151, 158). Of special interest is their sensitivity to inhibition by cell acidification and their stimulation by NO and cGMP (55, 151). Other features of these channels are lack of inhibition by ATP and direct blockade by high cell calcium (55, 151).

Several lines of evidence indicate that NO is involved in the regulation of ion transport in principal cells. First, it is inferred that NO stimulates basolateral potassium channels, because inhibition of nitric oxide synthase reduces channel activity, whereas NO donors or cGMP stimulate channels (151). Second, NO-induced cGMP formation hyperpolarizes the membrane potential and secondarily augments apical sodium entry (81). On the other hand, the sodium channel blocker amiloride downregulates basolateral potassium channels by a mechanism involving changes in cell calcium and NO (80). Reduction of apical sodium entry and a fall in cell sodium decreases calcium by stimulating basolateral
Na\(^+\)/Ca\(^{2+}\) exchange. Since the synthesis of NO is Ca\(^{2+}\) dependent, a fall in NO and cGMP reduces basolateral potassium channel activity.

Active sodium extrusion across the basolateral membrane and apical and basolateral potassium channels are linked by two mechanisms (41) summarized in Figs. 12 and 13. Such relations are important because they prevent disturbances in cell volume and cell ion content when there are changes in net sodium reabsorption (51, 76, 121, 156).

First, transport-induced changes in cell ATP concentrations regulate basolateral ATP-sensitive potassium channels in proximal tubule cells (5, 6, 41, 60, 140, 156). Direct measurements of cell ATP indicate that stimulation of basolateral Na-K-ATPase lowers ATP; pump inhibition has the opposite effect. Measurements of basolateral membrane potentials and of potassium channel activity in perfused proximal tubules show that upregulation of ATPase activity, achieved by adding glucose or amino acids to the lumen, increases potassium channel activity. In contrast, pump inhibition, because it reduces ATP consumption and increases cell ATP, closes channels. Such coupling between Na-K-ATPase and basolateral potassium channels has been demonstrated in proximal tubule cells (167) but, as indicated in Fig. 12, may also operate in the thick ascending limb or in cortical collecting ducts. A similar mechanism could also link basolateral Na-K-ATPase activity and apical secretory potassium channels, because these channels are inhibited by ATP and could respond to transport-related alterations in cell ATP.

A second mechanism, shown in Fig. 13, involves transport-related changes in cell calcium (41, 156). In the illustrative example, the effects of inhibiting basolateral Na-K-ATPase increases cell sodium and curtails extrusion of calcium by the basolateral Na/Ca exchange. The rise in cell calcium that follows activates PKC, which blocks apical potassium channels. This calcium-dependent coupling mechanism between basolateral Na-K-ATPase and apical potassium channels involves changes in cell sodium. In contrast, pump-related alterations in consumption of ATP may occur without change in cell sodium, if basolateral pump stimulation is matched by increased sodium entry across the apical membrane.

It should be noted that additional mechanisms may relate basolateral pump activity with potassium channels (Fig. 11). The observation that basolateral potassium channels are also activated by membrane hyperpolarization (151) is consistent with the reported voltage dependence of the basolateral membrane conductance in amphibian collecting ducts (57). In view of the electrogenic nature of the basolateral Na-K pump, voltage-related conductance changes provide an additional mechanism for coupling between Na-K-ATPase activity and potassium channels.

Physiological coupling mechanisms between apical and basolateral transport. A striking example of the complex interaction between apical and basolateral transporters in principal tubule cells is the transport stimulation by aldosterone (1, 30, 99, 107, 111, 145, 146). Typical of that hormone response is the redundancy of mechanisms that control and correlate the activities of the basolateral Na-K pump and apical sodium and potassium channels. Figure 14 provides a summary of possible mechanisms. First, aldosterone rapidly stimulates Na-K-ATPase turnover in the membrane; this is followed by delayed activation of genomic pump expression involving de novo synthesis, targeting, and insertion of Na-K-ATPase molecules into the basolateral membrane. Second, aldosterone augments the basolateral potassium permeability. Both of these actions, electrogenic Na-K pump and potassium chan-
nel stimulation, increase the cell-negative potential (72, 113, 115). A third effect of aldosterone is its stimulation of both sodium and potassium channels in the apical membrane. Recruitment and insertion of Na-K-ATPase into the basolateral membrane mediated by indirect mechanisms such as the increase in cell sodium have also been reported (2). Some of the mechanisms discussed above, such as changes in cell calcium, NO, and ATP, are likely mediators of the parallel activation of basolateral and apical transport processes.

It should be emphasized that potassium secretion will be stimulated optimally only during simultaneous upregulation of both potassium and sodium channels. If aldosterone were only to increase opening of potassium channels, then the resulting increase of apical potassium permeability would tend to hyperpolarize the apical membrane and reduce the electrochemical driving force for passive potassium transport from cell to lumen (137). Stimuli of potassium secretion such as aldosterone (90, 105, 114), a high-potassium diet under conditions of constant aldosterone levels (101), and most likely metabolic alkalosis (131) increase both potassium and sodium conductance in the apical membrane and thus prevent attenuation of the driving force for potassium secretion.

Molecular Structure of Renal Potassium Channels

New insights into the mechanism of potassium secretion have been provided by cloning of several potassium channels from renal tissue. They fall into several categories and include isoforms of voltage- and cGMP-gated potassium channels, which share structural elements with Shaker channels (15), and low-conductance, inwardly rectifying potassium channels (56, 168, 178). These channels are of interest because they share many properties with native potassium channels in the apical membrane of cells in the thick ascending limb and in principal cells. No information is yet available on the molecular structure of basolateral potassium channels and of renal stretch- and Ca-activated channels.

Figure 15 shows the structural model of ROMK2 and includes the amino acid sequence of the NH2 termini of alternatively spliced products of the ROMK gene (52, 56). This channel protein has two membrane-spanning regions (M1 and M2) flanking an H5-like region, similar to the channel pore of voltage-gated potassium channels. The alternatively spliced channel proteins form functional channels and are differentially expressed along the nephron. ROMK2 is most widely distributed (thick ascending limb and cortical collecting duct), ROMK1 is specifically expressed in collecting ducts, and ROMK3 expression is limited to the medullary and cortical thick ascending limb and distal convo-
luted tubule (10, 174). The question of heteromultimeric complex formation is not resolved, but interactions of ROMK channels with the cystic fibrosis transmembrane conductance regulator (CFTR) confer increased sensitivity to inhibition by glibenclamide, a well-known potassium channel blocker, on the potassium channel, suggesting subunit interactions (93).

ROMK channels share many properties with the low-conductance potassium channels in the TAL and cortical collecting tubule. These include single channel conductance, weak inward Mg-dependent rectification, cation selectivity, inhibitor sensitivity, inhibition by low internal pH and by arachidonic acid. Loss of channel activity (“run-down”) is also observed in excised patches and after treatment with protein phosphatases. The latter two effects can be reversed by cAMP and low concentrations of ATP. Lack of sensitivity to external tetraethylammonium but inhibition by higher concentrations of Mg-ATP have also been observed in ROMK2 (38, 41, 52, 158, 162). Site-directed mutagenesis experiments have identified specific amino acids that are essential for pH sensitivity (16, 29), and three serine residues that mediate cAMP-mediated phosphorylation have been identified (175). Any two of these phosphorylation sites are required for channel activity and induce different kinetic modification of ROMK2 activity (82, 175). Figure 15 also shows a region of the channel protein that contains a PO4 binding motif that is essential for pH sensitivity (16, 29), and three serine residues that mediate cAMP-mediated phosphorylation have been identified (175). Any two of these phosphorylation sites are required for channel activity and induce different kinetic modification of ROMK2 activity (82, 175). Figure 15 also shows a region of the channel protein that contains a PO4 binding motif that was shown to be involved in Mg-ATP binding and channel inhibition. Mutations in the COOH terminus of the ROMK molecule have been identified in Bartter’s syndrome and are likely to initiate loss of function of the channel molecule (94). Figure 16 is a model of the secretory potassium channel molecule that includes factors known to regulate channel activity.

The challenge of future work will be to characterize further the behavior of potassium channels in physiological and pathophysiological conditions, to investigate the interaction of messengers with the channel protein, and to elucidate the way channels are synthesized and targeted to specific membrane sites. It is hoped that cloning efforts will provide insights into the molecular structure of potassium channels not yet identified, especially in the basolateral membrane. Such reductionist approaches taken with a physiological perspective should advance our knowledge of the mechanisms underlying renal potassium transport and its regulation.

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