Quantification of K⁺ secretion through apical low-conductance K channels in the CCD

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Gray, Daniel A., Gustavo Frindt, and Lawrence G. Palmer. Quantification of K⁺ secretion through apical low-conductance K channels in the CCD. Am J Physiol Renal Physiol 289: F117–F126, 2005. First published February 22, 2005; doi:10.1152/ajprenal.00471.2004.—Outward and inward currents through single small-conductance K⁺ (SK) channels were measured in cell-attached patches of the apical membrane of principal cells of the rat cortical collecting duct (CCD). Currents showed mild inward rectification with high [K⁺] in the pipette (Kᵥ), which decreased as Kᵥ was lowered. Inward conductances had a hyperbolic dependence on Kᵥ with half-maximal conductance at ~20 mM. Outward conductances, measured near the reversal potential, also increased with Kᵥ from 15 pS (Kᵥ = 0) to 50 pS (Kᵥ = 134 mM). SK channel density was measured as the number of conducting channels per patch in cell-attached patches. As reported previously, channel density increased when animals were on a high-K diet for 7 days. Addition of 8-cpt-cAMP to the bath at least 5 min before making a seal increased SK channel density to an even greater extent, although this increase was not additive with the effect of a high-K diet. In contrast, increases in Na channel activity, assessed as the whole cell amiloride-sensitive current, due to K loading and 8-cpt-cAMP treatment were additive. Single-channel conductances and channel densities were used as inputs to a simple mathematical model of the CCD to predict rates of transepithelial Na⁺ and K⁺ transport as a function of apical Na⁺ permeability and K⁺ conductance, basolateral pump rates and K⁺ conductance, and the paracellular conductance. With measured values for these parameters, the model predicted transport rates that were in good agreement with values measured in isolated, perfused tubules. The number and properties of SK channels account for K⁺ transport by the CCD under all physiological conditions tested.

ROMK: epithelial sodium channels; high-potassium diet; aldosterone; cAMP: epithelial transport model

SMALL CONDUCTANCE (SK) channels are the most abundant K channel type observed in patch-clamp studies of the apical membrane of the mammalian cortical collecting duct (CCD) and connecting tubule (CNT) (6, 7, 34). These channels are believed to be encoded by the ROMK gene (16, 36), a conclusion that was recently directly confirmed by the absence of the channels in a ROMK knockout mouse strain (13). They are thought to be the predominant route for K⁺ secretion by the distal nephron (10). However, the ROMK knockout animals showed net secretion of K⁺ into the urine. They had high rates of K⁺ excretion without the hyperkalemia that would be expected from elimination of the major secretory system, implying that other pathways exist (12, 13). A quantitative assessment of the role of the SK channels in secretion is therefore useful. The properties of the SK/ROMK channels have been studied extensively both in the CCD and in homologous expression systems but typically these studies have measured the larger and more reproducible inward currents of these inwardly rectified channels. K⁺ secretion in the renal tubule, however, entails outward current flow. In addition, these outward currents will vary with luminal [K⁺] and apical membrane voltage, but these relationships have not been investigated systematically. To carry out a more quantitative analysis of the contribution of the SK channels, we measured outward K⁺ currents in cell-attached patches of principal cell apical membranes with various K⁺ concentrations in the pipette. These measurements have been combined with estimates of channel densities under different dietary and hormonal conditions and applied to a numerical model of ion transport in the CCD.

METHODS

Animals

Sprague-Dawley rats (Charles River Laboratories, Kingston, NY) weighing 100–150 g were fed either control rat chow (0.6% K, 0.4% Na, 0.9% Cl, 0.2% Mg, 0.9% Ca) or a matched high-K⁺ (5.2% K, 5.1% Cl⁻) diet (Harlan Teklad, Madison, WI) for 1–2 wk before experiments as indicated. After the animals were killed, kidneys were excised and thin sections were cut with a razor blade. CCDs were isolated with forceps under a dissecting microscope and split open with a fine needle. They were then attached, apical side up, to a small coverslip using Cell-Tak (Collaborative Biomedical Products, Bedford, MA), placed in a glass-bottom chamber on an inverted microscope, and superfused with bath solution at 37°C.

Patch Clamp

Principal cells in split-open CCDs were identified by their flat appearance and polygonal shape, and giga-ohm seals were formed on the luminal surface. Pipettes, made from hematocrit capillary tubes (VWR International, West Chester, PA) with three pull from a vertical pipette puller (model 700C, David Kopf Instruments, Tujunga, CA), were coated with Sylgard (Dow Corning, Midland, MI) and fire polished to yield tip resistances of 2–5 MΩ. Single-channel currents were recorded over a range of voltages between ~100 and +80 mV, although, over the life time of a given patch, it was not always possible to obtain data at all of these potentials. Whole cell recordings were obtained in a similar fashion using suction to break the apical membrane patch. Currents were recorded with an EPC-7 patch-clamp amplifier (Heka Elektronik, Lambrecht, Germany) and digitized with a Digidata 1332A interface (Axon Instruments, Union City, CA). Data were filtered at 1 kHz and analyzed with pCLAMP8 software (Axon Instruments).

For assessing the density of channels per patch under different conditions, channels were counted as described previously (6, 17, 19). For small channel densities, the number of current levels was ob-
served directly. For larger densities, the current level with all channels open was measured with a pipette potential of zero (equal to the bath potential) to minimize currents across the seal. The mean current level with all channels closed could not be observed directly but was estimated from other patches with no channels or a small number of channels. The number of channels was then estimated from the difference in these current levels divided by the single-channel current.

To ensure that the membrane areas being studied were comparable, we used paired pipettes (pulled from the same piece of glass tubing) for measurements of control and cAMP-treated tubules. This pairing was not possible to do in comparing animals on high-K and control diets. However, on the average the pipette resistance, and by inference the pipette diameter, was the same in the two groups (means ± SD 2.85 ± 0.41 MΩ for controls and 2.88 ± 0.39 MΩ for high-K animals). Channels were counted only in patches where the seal resistance was at least 1 GΩ.

**Solutions**

For assessing single-channel currents, the pipette solutions contained (in mM) 0, 5, 10, 20, 40, 60, 80, 100, or 140 K acetate, 10 HEPES, 2 CaCl₂, and 1 MgCl₂ with pH adjusted to 7.4 with KOH. The high bath solutions containing (in mM) 135 Na methanesulfonate, 5 KCl, 2 NaOH.

For assessing channel densities, the pipette solutions contained (in mM) 140 KCl, 10 HEPES, 2 glucose, 2 CaCl₂, and 1 MgCl₂ with pH adjusted to 7.4 with KOH. The high bath [K⁺] was used to depolarize the cell membrane to −90 mV so that an applied pipette potential, Vₚ, would result in a potential across the patch of −Vₚ.

For assessing channel densities, pipette solutions contained (in mM) 140 KCl, 10 HEPES, 2 CaCl₂, and 1 MgCl₂ with pH adjusted to 7.4 with KOH. Bath solutions contained (in mM) 140 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 2 glucose, and 10 HEPES adjusted to pH 7.4 with NaOH.

For whole cell clamp measurements, tubules were superfused with solutions containing (in mM) 135 Na methanesulfonate, 5 KCl, 2 CaCl₂, 1 MgCl₂, 2 glucose, 5 BaCl₂, and 10 HEPES adjusted to pH 7.4 with NaOH. The patch-clamp pipettes were filled with solutions containing (in mM) 7 KCl, 123 aspartic acid, 20 CsOH, 20 TEAOH, 5 EGTA, 10 HEPES, 3 MgATP, and 0.3 NaGDP with the pH adjusted to 7.4 with KOH. Amiloride-sensitive currents were measured directly. For larger densities, the current level with all channels opened was the same in the two groups (means ± SD 0.41 MΩ ± 0.3 MΩ for controls and 0.41 MΩ ± 0.3 MΩ for high-K animals). At 37°C, the activity of K⁺ in the pipette was denoted by K⁺,p.

**Correction of Pipette [K⁺] for Activity**

To assess K⁺ selectivity, we used the Nernst equation:

\[
E_K = \frac{RT}{F} \ln \left( \frac{[K^+]}{[Na^+]} \right)
\]

where \([K^+]_{ext}\) and \([Na^+]_{int}\) are the activity coefficients for the external and internal solutions, respectively. At 37°C,

\[
E_K = 61.5 \log (K_{Na})_{ext} + \text{constant}
\]

To calculate the activity coefficient, \([K^+]_{ext}\), we used the DeBeye-Hückel theory (4) for a dilute bi-ionic salt solution (ionic strength <0.3):

\[
\log f = Z^2/2(0.5)(w^{1/2})/(1 + w^{1/2})
\]

where \(w\) is the ionic strength, and \(Z^+\) and \(Z^-\) are the charges of the cation and anion of the salt. The value of \([K^+]_{ext}\) ranged from 0.934 for Kₚ⁺ = 4 to 0.735 for Kₚ⁺ = 134 mM. The activity of K⁺ in the pipette is denoted by Kₚ⁺ = \([K^+]_{ext}\)Kₚ⁺.

**Modeling**

A mathematical model of the CCD epithelium was based on the scheme shown in Fig. 7. Equations for the pathways shown were as follows:

**Apical Na channels.** Flux through the channels was assumed to follow the GHK equation:

\[
I_{Na,A} = P_{Na}(E_{Na,A} - V_i)
\]

where \(P_{Na}\) is Na permeability, \(Na^+_i\) = cytoplasmic Na concentration, \(Na^+_l\) = luminal Na concentration, and \(V_{i}\) = apical membrane voltage. **Apical K channels.** These were assumed to be ohmic and to follow the linear equation:

\[
I_{K,A} = G_{K,A}(E_{K,A} - V_i)
\]

where \(G_{K,A}\) is apical membrane K channel conductance, \(E_{K,A}\) = equilibrium potential for \(K^+\) = \(-(RT/F)\ln(K_B^+\int/K_A^+\int)\), \(K_B^+\int\) = cell K⁺ concentration, and \(K_A^+\int\) = luminal K⁺ concentration.

**Basolateral K channels.** These were also assumed to be ohmic and to follow the equation:

\[
I_{K,B} = G_{K,B}(E_{K,B} - V_i)
\]

where \(G_{K,B}\) is basolateral membrane K channel conductance, \(E_{K,B}\) = equilibrium potential for \(K^+\) = \(-(RT/F)\ln(K_B^+\int/K_A^+\int)\), and \(K_B^\int\) = interstitial (bath) K⁺ concentration.

**Na-K pump.** Na⁺ efflux through the pump was assumed to have cooperative kinetics according to the equation:

\[
I_{Na,P} = 3I_{pump,max}[1 + (K_{Na}/Na^+_i)^3]
\]

where \(I_{pump,max}\) = maximal pump current, \(K_{Na}\) = value of Na for half-maximal pump turnover rate = constant, K⁺ influx through the pump was assumed to be stoichiometrically related: \(I_{p,p} = 2/3 I_{Na,P}\).

**Paracellular conductance.** Assumed to be linear and nonselective:

\[
I_{par} = G_{par}(V_T)
\]

where \(G_{par}\) = paracellular conductance, and \(V_T\) = transepithelial voltage. The equations were solved using the additional constraints: \(I_{Na,A} = I_{Na,P}\) (cellular Na balance), \(I_{K,A} = I_{K,B} + I_{K,P}\) (cellular K balance), and \(I_{Na,A} + I_{K,A} = I_{par}\) (charge balance). The equations were solved using the “solver” function in Microsoft Excel. Positive currents denote cation fluxes in the reabsorptive (lumen to interstitium) direction. Negative currents denote cation fluxes in the secretory direction.
RESULTS

Conductance Through SK channels with Various Lumenal (Pipette) [K⁺]

Single-channel currents through SK channels were recorded from CCDs obtained from rats fed a high-K⁺ diet. Membrane voltages ranged from −100 to +60 mV and \( K_p \) was varied from 0 to 134 mM. Cell-attached patches containing a single SK channel with \( K_p \) of 134 and 4 mM are shown in Fig. 1A. At all voltages, the channel is mainly open with brief closures. Occasional longer closures, probably reflecting divalent cation block (2), were more prevalent at 4 mM \( K_p \). The noise amplitude of the open channel state is greater at +60 (outward current) than at −60 mV (inward current), presumably reflecting fast block by intracellular Mg²⁺ and polyamines at positive membrane potentials. Current-voltage (I-V) curves generated from these data (Fig. 1B) show reversal potentials near \( E_K \) for each \( K_p \), assuming a constant intracellular [K⁺] of 140 mM. Small deviations from \( E_K \) arise mainly from LIJs (see METHODS). Inward rectification decreases at lower \( K_p \), probably because of increased outward (Goldman) rectification which reduces inward but not outward currents. I-V curves for additional \( K_p \)'s from 0 to 134 mM are shown in Fig. 2. From this family of curves, a plot of reversal potential vs. log(\( K_p \)) was generated (Fig. 3A) where \( K_p \) represents K⁺ activity as described in METHODS. The slope of a linear, least-squares fit of these points was 56 ± 1 mV/decade consistent with high-K⁺ selectivity. Inward and outward slope conductances as a function of \( K_p \) were also derived from the family of I-V curves in Fig. 2. Inward conductance increased as \([K^+]_p\) was raised but saturated at values >40 mM (Fig. 3B). These data were fit with a hyperbola with \( G_{\text{max}} \) of 67 ± 3 pS and \( K_m \) of 20 ± 3 mM. We also measured the outward slope conductance at voltages just positive to the reversal potential (Fig. 3C). These conductances had a finite minimal value of about 50 pS in symmetrical 140 mM K⁺.

These are the conductances that are used to quantify K⁺ secretion in the model described below.

Regulation of Apical Membrane Transport Properties

We and others previously examined the effects of increasing dietary K⁺ intake on the density of SK channels in the rat CCD (17, 19, 33, 37, 38). High-K intake elevated channel density by two- to fourfold over a period of about 1 wk. It has also been shown that ADH, presumably acting through activation of adenylate cyclase and PKA, can activate the channels (1, 32). However, these effects have not been quantitatively compared and their interactions have not been explored.

SK channel densities were measured in cell-attached patches on principal cells of the CCD (Fig. 4). In most patches, the number of active channels could be counted easily from the number of current levels. In some patches, particularly with cAMP-treated cells, the number was 10 or more. Because the channels have a high \( P_o \), the state with all channels closed was never visited. In these cases, the number was...
derived from traces with zero voltage across the membrane seal such that the current level with all channels closed was close to zero. The number of channels was then calculated from the current level with all channels open divided by the single-channel current (see Fig. 4).

Under basal conditions, the majority of patches were silent and the average number of channels/patch was only 0.20 (Fig. 5). This was somewhat smaller than the density reported previously under similar conditions (17, 19, 35). Channel activity was substantially increased by treating the tubules for 5 min or longer with the membrane-permeant cAMP analog 8-cpt-cAMP. Under these conditions, most patches (67%) contained active channels and the mean density was 3.5 channels/patch. Feeding the rats a high-K diet for 7–10 days before the experiment also enhanced the number of conducting channels, in this case to 1.1/patch. Treatment of these tubules with 8-cpt-cAMP further increased channel number but the average density did not exceed that achieved with 8-cpt-cAMP-treated tubules from control animals. Thus the effects of K loading and cAMP on SK channel density do not appear to be additive.

For comparison, and because the information was needed for the modeling described below, we also measured the amiloride-sensitive current under whole cell clamp conditions as an estimate of apical Na channel activity (5) (Fig. 6). I-V relationships were obtained under conditions where K and Cl conductances were minimal. The change in current after addition of 10 μM amiloride to the bath (I_Na) was measured at a cell potential of −100 mV. As reported previously (5) for untreated tubules from control animals, I_Na was small and unmeasurable. Addition of 8-ctp-cAMP to the bath elicited a mean current of 170 ± 48 pA/cell. When tubules were taken from K-loaded animals, Na currents could be measured in the absence of cAMP (203 ± 57 pA). Adding the cyclic nucleotide to the bath increased I_Na further to 527 ± 126 pA, indicating that in the case of the Na channels the effects of K loading and cAMP are additive.

Modeling Na and K Transport in the Rat CCD

To compare the measured parameters with results from isolated, perfused tubules, we constructed a simple mathematical model of a transporting epithelium as shown in Fig. 7. The model includes apical Na⁺ and K⁺ channels, basolateral K⁺...
channels, a basolateral Na-K pump, and a paracellular conductance pathway that is assumed to be nonselective. This is a model of an epithelium made up entirely of principal cells. It neglects movements of protons and HCO₃⁻ and does not include intercalated cells. As such, it is greatly simplified compared with more complete numerical models of the CCD (39). However, it includes the major pathways for Na⁺ more complete numerical models of the CCD (39). However, it includes the major pathways for Na⁺ and K⁺ transport and for our particular purposes has the advantage that all of the parameters, with the exception of the paracellular conductance, have been measured using rat CCD in our laboratory.

We started with a model epithelium carrying out a “moderate” transport rate (see Fig. 7). We used an apical solution of 140 mM Na⁺ and 5 mM K⁺ to facilitate comparison with measurements on isolated, perfused tubules in which similar compositions of the luminal perfusates were used. We assumed an apical Na⁺ permeability (PNa) of 0.94 × 10⁻⁸ cm²/s, which corresponds to currents measured after a short (18 h) period of Na deprivation (5) (see APPENDIX for details and explanation of units). For the apical K⁺ conductance, we started with a K⁺ channel density of 0.4 channels/µm² (17) corresponding to a Gk,A = 440 nS/mm tubule (see APPENDIX). Maximal pump rates were set at 17 nA/mm, slightly higher than those measured under nonstimulated conditions (18), and KNa, the intracellular Na concentration required for half-maximal pump turnover, was assumed to be 10 mM. The basolateral K⁺ conductance under conditions of 5 mM peritubular K⁺ was taken to be 5,800 nS/mm (11). Paracellular conductance was assumed to be 300 nS/mm based on a resistance of 1 kΩ·cm². This epithelium transports Na⁺ and K⁺ at rates of 16.0 and −9.6 pmol·min⁻¹·mm⁻¹, respectively, through the transcellular pathway with a transepithelial voltage of −35 mV. In our convention, positive fluxes indicate reabsorption and negative fluxes represent secretion.

Both the apical PNa and the apical K⁺ conductance are known to be physiologically regulated. To assess how the fluxes varied with these parameters, we varied their values by factors of 2 and 4 above and below the basal levels. The results are shown in Fig. 8. Both Na⁺ and K⁺ fluxes depended on PNa, as expected. For Na⁺ the effect is simply an increase in the rate of entry across the apical membrane. For K⁺ the increase is mediated by electrical coupling, with increasing PNa depolarizing the apical membrane, increasing the driving force for K efflux. However, increasing PNa alone resulted in a subproportional increase in fluxes. Although intracellular Na⁺ increases,
bringing the Na pump closer to saturation, the major factor limiting the increase in Na\textsuperscript{+} reabsorption is the depolarization of the apical membrane, diminishing the driving force for Na\textsuperscript{+} entry into the cell. K efflux then becomes limited by the Na entry rate.

As the apical K\textsuperscript{+} conductance ($G_{K,A}$) decreases to zero, K\textsuperscript{+} transport vanishes, reflecting the absence of a transcellular pathway for K\textsuperscript{+}, while Na\textsuperscript{+} transport decreases toward a finite value. Raising $G_{K,A}$ increases both fluxes. The effect on K\textsuperscript{+} is direct, while that on Na\textsuperscript{+} is the result of hyperpolarization of the apical membrane. As with the case of $P_{Na}$, the increases in transport rates tend to saturate, i.e., they are subproportional to the rise in $G_{K,A}$.

The effect of increasing paracellular conductance is shown in Fig. 8C. Net Na transport was almost independent of $G_{par}$. This was because increasing $G_{par}$ hyperpolarized the apical membrane, increasing transcellular Na flux. This was more or less offset by an increase in Na backleak across the junctions. Increasing $G_{par}$ decreased K\textsuperscript{+} secretion, as K\textsuperscript{+} efflux across the apical membrane was diminished as the apical membrane was hyperpolarized.

The effect of modulating $G_{K,B}$ is shown in Fig. 8D. Increasing or decreasing $G_{K,B}$ by a factor of 10, while leaving all other input parameters unchanged, resulted in negligible alterations in Na\textsuperscript{+} and K\textsuperscript{+} fluxes. This results from the small net outward currents that go through the channels under basal conditions.

Fig. 7. Epithelial model of the CCD. Pathways included in the model are apical Na\textsuperscript{+} and K\textsuperscript{+} conductances (permeabilities), basolateral K\textsuperscript{+} conductance, Na-K pump, and a nonselective paracellular conductance. Parameters for the model in a state of moderate activation of Na\textsuperscript{+} and K\textsuperscript{+} transport are listed, along with the resulting flux and voltage predictions.

Fig. 8. Sensitivity analysis for Na\textsuperscript{+} and K\textsuperscript{+} transport in the epithelial model. Net transport rates are shown as functions of apical Na\textsuperscript{+} permeability ($P_{Na}$: A), apical $G_{K}$ (B), paracellular conductance (C), and basolateral $G_{K}$ (D). ■ Represent net Na reabsorption under each condition, plotted as positive fluxes. ● Represent net K secretion under each condition, plotted as negative fluxes.
and the large starting values of the conductance relative to the apical conductances.

Under baseline conditions, the direction of K flux across the basolateral membrane is outward (positive) and serves to recycle K brought into the cell by the Na pump. However, it has been suggested that under certain conditions, for example high-K diet and high aldosterone, the direction of this flux reverses resulting in K entry into the cell (23). We therefore tested whether our model would predict such a reversal. A reversal in direction of basolateral K⁺ flux was achieved by raising \( G_{K,A} \) alone (Fig. 9A). As \( G_{K,A} \) is increased up to fourfold, \( J_{K,B} \) reverses from +1.7 (outward flow) at baseline to -3.3 pmol·min⁻¹·mm⁻¹ (inward flow). The paracellular conductance also affects the predicted direction of K flux through the basolateral K channels. When \( G_{par} \) is decreased from baseline values, basolateral conductive K flux becomes inward (Fig. 9B). This is a consequence of a depolarization of the apical membrane voltage, increasing apical K⁺ secretion and decreasing Na⁺ entry as indicated in Fig. 8C. When the ratio of K efflux to Na⁺ influx exceeds 2:3, the assumed stoichiometry of the Na-K pump, K⁺ must enter the cell across the basolateral membrane to maintain a steady state.

We compared the known effects of a high-K diet and of aldosterone administration on the fluxes of Na⁺ and K⁺ predicted by the model. Both of these chronic conditions increase apical Na⁺ permeability, with the value obtained with aldosterone being about twice as large as that with high K (17, 20). Both maneuvers also increase the maximal pump fluxes, which are about three- and fourfold higher than the basal level with high-K diet and aldosterone, respectively (17, 18). K loading increases the density of apical SK channels about fourfold while aldosterone alone does not (20). The basolateral K⁺ conductance is enhanced by about twofold with a high-K diet (11). No comparable measurements are available for the aldosterone-treated condition, but we assume a similar increase as both cases are associated with a significant amplitication of basolateral membrane surface area (27, 28, 31). The other parameters are assumed to be unchanged.

The results of the simulation are shown in Table 1. The apical Na⁺ currents under our “basal” conditions (rats on a control diet with no treatment of the tubules) were essentially zero, leading to prediction of Na and K fluxes were also close to zero. Indeed, fluxes measured in isolated, perfused rat CCDs under these conditions were unmeasurably small (21, 30), although this may not pertain in vivo. Both aldosterone administration and a high-K diet are predicted to increase Na⁺ reabsorption as well as K⁺ secretion. Somewhat surprisingly, the predicted Na reabsorption rates are higher for the high-K diet than for aldosterone infusion. This is due to hyperpolarization of the apical membrane voltage, increasing the driving force for Na uptake. This will also decrease the driving force for K secretion, but this effect is more than compensated by the increased apical K conductance. The direction of K⁺ flow through the basolateral K⁺ channels is reversed under high-K conditions, reflecting the increased apical K⁺ conductance as described above, but remains in the outward direction with high aldosterone.

Finally, we asked whether the measured values of membrane properties could account for net ion fluxes measured in isolated, perfused CCDs. We first compared the predictions with the experimental data of Reif et al. (21) who measured net Na⁺ fluxes under conditions of high flux rates such that the luminal ion concentrations did not change appreciably. Table 2 shows the values of the key parameters chosen to simulate the various conditions and the predicted net fluxes for Na⁺. Agreement with the data of Reif et al. is remarkably good, given that no free parameters were used to generate the simulated values. K⁺ fluxes were not measured in that study. They were measured by Tomita et al. (30), although under conditions of relatively low perfusion rates such that the luminal K⁺ concentration did not change appreciably along the length of the tubule. We therefore recalculated the predicted fluxes under conditions of reduced luminal Na⁺ and increased luminal K⁺ using concentrations that were the means of those entering and leaving the tubules. As shown in Table 2, the agreement with measured values of Na⁺ reabsorption is reasonably good under all conditions, and the agreement with measured K secretion is satisfactory in the absence of ADH/cAMP. The measured net K fluxes in the presence of ADH were lower than those predicted from the model using data from cAMP-treated tubules, both under basal and mineralocorticoid-treated conditions.

**DISCUSSION**

**Single-Channel SK Currents**

We measured single-channel currents through SK channels as a function of voltage and \( K_p^+ \) (Fig. 2). Inward rectification was apparent at high \( K_p^+ \) but decreased as \( K_p^+ \) was lowered,
pressed in oocytes are increased by raising extracellular K.

Macroscopic outward currents through ROMK channel ev-
a model of the principal cell and used to estimate K
of open probability and channel number, was incorporated
through an SK channel. This information, along with estimates
of single-channel currents.

probably because of the superposition of outward, Goldman-
type rectification. Inward conductance increased hyperboli-
cally with \( k_0 \), with a \( G_{\text{max}} \) of 67 pS and an apparent \( k_0 \) of 20
mM. Outward conductance near the reversal potential also
increased with \( k_0 \), although it started with a finite value of 15
pS at \( k_0^- = 0 \). At large positive membrane voltages, outward
currents converged to the same values independent of \( k_0^- \).
Macroscopic outward currents through ROMK channel ex-
pressed in oocytes are increased by raising extracellular K\(^+\) (3,
22). Our results suggest that this probably reflects an increase
in the number of open channels rather than an increase in
single-channel currents.

For a given lumenal \([K^+]\) and apical membrane potential,
we can predict the single-channel current or conductance
through an SK channel. This information, along with estimates
of open probability and channel number, was incorporated into
a model of the principal cell and used to estimate K\(^+\) secretion
in the CCD under a variety of dietary and hormonal conditions
as discussed below.

**Activation of SK and Na Channels by cAMP and High-K Diet**

It is well known that SK channels can be activated by ADH
or its second messenger cAMP, as well as the cAMP-activated
kinase PKA (1, 32). It has also been shown previously that
feeding rats a high-K diet increases SK channel density (17, 19,
33, 37). In this study, we compared these two effects and looked
at interactions between them. We found that the effect of
cAMP added in vitro was larger than that of the high-K diet
alone and that the two effects were not additive. This suggests
that activation of the channels was near maximal with cAMP
but not with a high-K diet alone. A caveat to the interpretation
of these results is that in this set of experiments the densities in
both the control and the high-K group were about half those
reported previously by us and by others (17, 19, 33, 37, 38).
The effect was consistent; in one of five rats examined under
control conditions, the mean channel density in 10 patches was
0.6. In the other four, it was less than 0.1. We do not know the
reason for this low basal density, but it is possible that cAMP,
but not a high-K diet, was able to rescue the low activity. Thus
the relative effects of cAMP and a high-K diet might be
different in animals with a more typical basal density.

The finding that these two stimulatory maneuvers were less
than additive was unexpected. One interpretation is that the
two pathways converge at some point. cAMP presumably acts
through PKA-dependent phosphorylation. ROMK channels
themselves have phosphorylation sites and are targets for the
kinase (40). We do not believe that the effects of high-K intake
are mediated by cAMP/PKA; the mechanism of this activation
is not fully understood but may involve a reduction in PTK
activity (33). It is more likely that the two stimulatory effects
converge at a point more downstream in the signal transduction
pathways. We do not know what the putative point of converge-
ence of these two mechanisms might be.

K adaptation and cAMP treatment stimulated the whole cell
amiloride-sensitive conductance, presumed to reflect the activ-
ity of apical Na\(^+\) channels, to similar extents. In contrast to
the case of the SK channels, these two stimulatory effects were
additive in the case of the Na\(^+\) channels.

**Model Calculations**

The main goal of the modeling was to evaluate whether the
measured conductances of apical membrane channels, together
with the measured basolateral membrane conductance and pump
activity, could account for Na\(^+\) and K\(^+\) transport as measured in isolated, perfused CCDs. The model is simplified
to focus on these characteristics. We are most confident in
the parameters that can be measured under whole cell conditions:
the apical Na\(^+\) permeability, the basolateral K\(^+\) conductance,
and the Na-K pump current. The measurement of apical K\(^+\)
channel conductance is based on the single-channel properties
of the SK channels as well as on the SK channel density. The
latter depends on the estimate of the membrane area in a
cell-attached patch that is not known precisely but has been
estimated from the geometric properties of the patch pipettes
(19). We did not include a possible contribution of BK or
maxi-K channels in this model. The other parameter that has
not been measured directly is the paracellular conductance.
This was estimated from measurements of unidirectional Na\(^+\)
flux from bath to lumen (9, 21, 29).

The basic results of the model simulations were not surpris-
ing. Both Na\(^+\) reabsorption and K\(^+\) secretion depend strongly
on the apical Na\(^+\) and K\(^+\) conductances. The coupling between
the fluxes is electrical; increasing Na influx depolarizes the
apical membrane and increases the driving force for K efflux and
vice versa. Neither flux was strongly dependent on the
basolateral K\(^+\) conductance, reflecting the large size of this
conductance relative to those of the apical membrane under all
conditions. The fluxes were affected in opposite directions by
changes in paracellular conductance. Increasing this conduct-
ance hyperpolarized the apical membrane, increasing Na\(^+\)
influx and decreasing K\(^+\) efflux. The increased transcellular
Na\(^+\) flux was largely offset by a larger Na\(^+\) backflux through
the paracellular pathway.

The predicted changes in direction of K\(^+\) flux through the
basolateral channels were less intuitive. The reversal of flow
from the normal “recycling” mode to K influx resulted from

<table>
<thead>
<tr>
<th>Condition</th>
<th>( J_{Na} ) pmol/min (^{-1}) mm(^{-1} )</th>
<th>( J_{Na} ) pmol/min (^{-1}) mm(^{-1} )</th>
<th>( J_{K} ) pmol/min (^{-1}) mm(^{-1} )</th>
<th>( J_{K} ) pmol/min (^{-1}) mm(^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>-2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>cAMP</td>
<td>24</td>
<td>19</td>
<td>23</td>
<td>15</td>
</tr>
<tr>
<td>aldol</td>
<td>26</td>
<td>34</td>
<td>23</td>
<td>20</td>
</tr>
<tr>
<td>aldol + cAMP</td>
<td>89</td>
<td>110</td>
<td>61</td>
<td>58</td>
</tr>
</tbody>
</table>

*Data from Ref. 21. †Data from Ref. 30. Aldol, aldosterone.
conditions in which apical K efflux was larger than the basolateral K\(^+\) influx through the pump. This comes about when the apical K conductance is large or when the paracellular conductance is small. Both of these alterations depolarize the apical membrane and increase rates of K\(^+\) secretion. The model predicted a reversal in direction of K\(^+\) flux when the animals were on a high-K diet but not with chronic mineralocorticoid treatment. The latter conclusion is in contrast to studies in rabbit CCD where such a reversal has been documented (23). This difference may arise because mineralocorticoids increase apical K\(^+\) conductance in the rabbit CCD (25) but not in that of the rat (20, 26). In addition, chronic DOCA-treated rabbits may have a decreased paracellular conductance (24, 25), which, as discussed above, can lead to a reversal in basolateral K\(^+\) flux. We assumed no changes in paracellular conductance in the model calculations.

Measurements of Na\(^+\) and K\(^+\) fluxes in the rat CCD are limited. Reif et al. (21) measured Na fluxes with high perfusion rates that effectively kept the luminal ion concentrations clamped. Tomita et al. (30) measured net fluxes of Na\(^+\) and K\(^+\) with flows slow enough to allow changes in the luminal ion composition. In general, the measurements of individual components of the transport system, together with the simple model, account well for these measurements. The most obvious discrepancy is that our calculated K\(^+\) secretion rates were higher than the measured values in the presence of ADH/cAMP, as were the calculated ratios of Na\(^+\) reabsorption: K\(^+\) secretion. This could arise from different effects of cAMP, used in our experiments, and of AVP, which was used in the perfused tubule measurements. Alternatively, the differences could be explained by specific cAMP-dependent pathways for transport of Cl which would increase the fraction of transported Na\(^+\)/H\(^+\) neutralized by anion reabsorption rather than by K\(^+\) secretion. In any case, the measured density of SK channels can account for the measured K\(^+\) secretion rates in the rat CCD.

As mentioned earlier, ROMK \(-/-\) mice can secrete K\(^+\) and do not have hyperkalemia (12, 13), suggesting that other pathways for K\(^+\) secretion exist. However, our results indicate that such pathways are not required to describe K\(^+\) secretion in the isolated, perfused CCD. It is possible that the additional pathways are present in other segments of the nephron. Indeed, micropuncture data suggest that most K\(^+\) secretion takes place in the distal tubule before the tubular fluid enters the CCD (14). Recent modeling studies support this idea and indicate that the CCD might be a site of K\(^+\) reabsorption under most conditions (39). Another possibility is that the alternate pathways play a minimal role under the conditions tested but could become more important when the requirement for K\(^+\) excretion exceeds the capacity of the SK channels. This could occur in the ROMK \(-/-\) mice or with a high-K diet, a condition not yet studied in the rat with the isolated, perfused tubule approach.

APPENDIX

Choice and Calculation of Model Parameters

Apical P\(_{Na}^\text{apical}\). In control animals under basal conditions, we do not detect Na channel activity either by single-channel or whole cell analysis (5, 15). We have chosen for our “basal” condition the activity observed with a mild stimulation due to overnight Na deprivation (5). Under these conditions, the amiloride-sensitive current was 140 pA/cell with a cell potential of \(-100\) mV and 140 mM Na\(^+\) in the bath and nominally zero Na\(^+\) in the cell. Using the current form of the GHK equation (see METHODS), we calculate a permeability coefficient of 0.26 pA\(\cdot\)M\(^{-1}\)\(\cdot\)cell\(^{-1}\). This has been converted into units of \(9.4 \times 10^{-8}\) cm\(^2\)/s using the Faraday constant and a value of 340 principal cells/mm tubule (8), giving “permeability” per length of tubule. The units arise from this length normalization and because currents were measured per cell rather than per membrane area.

For other conditions, we used amiloride-sensitive currents of 170 pA (cAMP, Fig. 6), 203 pA (high-K diet, Fig. 6), 527 pA (high-K diet + cAMP, Fig. 6), and 340 pA for aldosterone (8, 15). We do not have measurements for aldosterone + cAMP, so we assumed that cAMP stimulated \(I_{\text{Na}}\) by the same factor as it did in high-K animals.

Apical G\(_{\text{Na}}\). Under control conditions, we estimated a K\(^+\) channel density of 0.4 channels/\(\mu^2\) (17, 19). We assume an apical surface area of 185 \(\mu^2/cell\) (11). The outward single-channel conductance is 20 pS with 4 mM K\(^+\) in the pipette (Fig. 1) and the open probability is about 0.9 (2). The basal G\(_{\text{Na},A}\) is therefore 1.3 nS/cell. Multiplying by 340 principal cells/mm we obtain 440 nS/mm.

We used 3.5 channels/\(\mu^2\) in cAMP-treated tubules from both control and high-K animals (Fig. 4) and assumed that the value was similar for aldosterone + cAMP. For the aldosterone-treated animals, we used the same value as control (17).

Basolateral Na-K pump. For maximal pump currents, we used measured values of 35 pA/cell (control), 140 pA/cell (aldo) and 100 pA/cell (high K) (17, 18). Multiplying by 340 principal cells/mm we obtain values of 11.9, 47.6, and 34.0 nA/mm tubule. For “basal” conditions, we increased the pump current from 35 to 50 pA/cell to allow the pump to keep pace with apical Na\(^+\) entry. We do not have data for the effects of cAMP treatment on pump currents. We also found that it was necessary to increase \(I_{\text{pump}}\) by \(-30\%\) to keep up with the increase in Na entry. We therefore assumed values of 50 pA/cell (cAMP), 150 pA/cell (aldosterone + cAMP), and 130 pA/cell (high K + cAMP). The exact value did not affect the overall transport rate very much as long as it was adequate to maintain Na\(^+\) entry rates. G\(_{\text{Na}}\) was assumed to be constant at 10 mM.

Basolateral K conductance. The basolateral K conductance was taken from Gray et al. (11) to be \(-17\) nS/cell with 5 mM K in the bath for control animals and \(-31\) nS/cell for high-K animals. These convert to 5.800 and 10.500 nA/mm. We assumed that the value with aldosterone was similar to that for high K and that cAMP did not change G\(_{\text{Kb}}\). This assumption does not add much uncertainty to the calculation as transport rates are quite weakly dependent on the basolateral conductance (Fig. 8D).

Paracellular conductance. Initial values for paracellular conductance were based on measurements of bath to lumen Na fluxes in isolated, perfused rabbit CCD (9, 29). We calculated the paracellular Na conductance using the GHK equation conductance and assumed the total paracellular conductance to be twice as large. This gave a conductance per principal cell of 0.88 nS or 300 nS/mm. For direct comparison with perfused rat CCD data, we used measurements of bath to lumen Na\(^+\) flux of 40 pmol/min \(\cdot\)mm\(^{-1}\) with a small transepithelial potential (21). This corresponds to a conductance of 7 nS/principal cell or of 2.4 \(\mu^S/mm.

GRANTS

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