A mathematical model of rat ascending Henle limb. II. Epithelial function

Alan M. Weinstein¹ and Thomas A. Krahn²

¹Department of Physiology and Biophysics, Weill Medical College of Cornell University, New York, New York; and ²Department of Medicine, Warren Alpert Medical School of Brown University, Providence, Rhode Island

Submitted 27 April 2009; accepted in final form 13 November 2009

Weinstein AM, Krahn TA. A mathematical model of rat ascending Henle limb. II. Epithelial function. Am J Physiol Renal Physiol 298: F525–F542, 2010. First published November 18, 2009; doi:10.1152/ajprenal.00231.2009.—A mathematical model of ascending Henle limb (AHL) epithelium has been fashioned using kinetic representations of Na⁺/K⁺/2Cl⁻ cotransporter (NKCC2), KCC4, and type 3 Na⁺/H⁺ exchanger (NHE3), with transporter densities selected to yield the reabsorptive Na⁺ flux expected for rat tubules in vivo. Of necessity, this model predicts fluxes that are higher than those measured in vitro. The kinetics of the NKCC and KCC are such that Na⁺ reabsorption by the model tubule is responsive to variation in luminal NaCl concentration over the range of 30 to 130 mM, with only minor changes in cell volume. Peritubular KCC accounts for about half the reabsorptive Cl⁻ flux, with the remainder via peritubular Cl⁻ channels. Transcellular Na⁺ flux is turned off by increasing peritubular KCl, which produces increased cytosolic Cl⁻ and thus inhibits NKCC2 transport. In the presence of physiological concentrations of ammonia, there is a large acid challenge to the cell, due primarily to NH₄⁺ entry via NKCC2, with diffusive NH3 exit to both lumen and peritubular solutions. When NHE3 density is adjusted to compensate this acid challenge, the model predicts luminal membrane proton secretion that is greater than the HCO₃⁻-reabsorptive fluxes measured in vitro. The model also predicts luminal membrane ammonia cycling, with uptake via NKCC2 or K⁺ channel, and secretion either as NH₄⁺ by NHE3 or as diffusive NH₃ flux in parallel with a secreted proton. If such luminal ammonia cycling occurs in vivo, it could act in concert with luminal K⁺ cycling to facilitate AHL Na⁺ reabsorption via NKCC2. With physiological ammonia, peritubular KCl also blunts NHE3 activity by inhibiting NH₃ uptake on the Na-K-ATPase, and alkalinizing the cell.

NKCC; KCC; NHE3; sodium transport; potassium transport; ammonia transport by AHL. The isotonicity restriction is not critical if the model focus is restricted to cortical AHL or to simulations of isolated tubule perfusions in vitro, but becomes problematic if one would like a model that can be extended to a medullary tubule in vivo. Whether the absence of acid/base transport is an important omission is uncertain: For tubules perfused in vitro, rates of HCO₃⁻ and NH₄⁺ transport are an order of magnitude less than for Na⁺ and Cl⁻ (19). Nevertheless, medullary ammonia concentrations are in a range at which they can drive substantial rates of Na⁺-NH₄⁺-2Cl⁻ transport across NKCC2.

In the present work, a more comprehensive AHL epithelial model is developed, which includes more robust kinetic representations of the important solute transporters, NKCC2 and KCC4, and model equations which allow lifting the isotonicity restriction (58). The model is used to examine the efficacy of variations in peritubular K⁺ concentration to modulate NKCC2 Na⁺ entry, and thus act as a controller of AHL Na⁺ reabsorption (42). The model also includes HCO₃⁻ and ammonia, along with their transporters, and specifically a kinetic representation of luminal NHE3. It is quickly apparent that medullary ammonia concentrations can drive substantial luminal NH₄⁺ uptake via NKCC2, and provide a constant acid challenge to the AHL cell. The calculations with ammonia bring to the fore a problem of rationalizing a proportional cellular response to this acid challenge, with the relatively low rates of transepithelial transport of HCO₃⁻ and NH₄⁺ that have been measured in vitro. The epithelial model developed here is targeted toward a simulation of rat AHL, and configuration as a tubule with medullary and cortical components.

MODEL FORMULATION

The model AHL follows the scheme that has been used previously for a tubule with a single cell type (e.g., Ref. 57). The model is formulated both as an AHL epithelium, with specified luminal and peritubular conditions, or as a tubule, in which luminal and peritubular concentrations vary axially. Figure 1 shows both diagrams, in which cellular and lateral intercellular (LIS) compartments line the tubule lumen. In this paper, only epithelial calculations are presented; in the companion paper (59), tubule models are explored. Within each compartment the concentration of species i is denoted Ci(t), where α is lumen (M), interspace (E), cell (I), or peritubular solution (S). Within the epithelium, the flux of solute i across membrane αβ is denoted Jαβ(i) (mmol·s⁻¹·cm⁻²), where αβ may refer to luminal cell membrane (MI), tight junction (ME), lateral cell membrane (IE), basal cell membrane (IS), or interstitial basement membrane (ES). Along the tubule lumen, axial flows of solute are designated FM(i) (mmol/s). The 12 model solutes are Na⁺, K⁺, Cl⁻, HCO₃⁻, CO₂, H₂CO₃, HPO₄²⁻, H₂PO₄⁻, NH₃, NH₄⁺, H⁺, and urea, as well as two impermeant species within the cells, a nonreactive anion and
cytosolic buffer. This is the minimal set of solutes needed for representation of acid excretion.

To formulate the equations of mass conservation with multiple reacting solutes, consider first an expression for the generation of each species within each model compartment. Within a cell or interspace, the generation of \( i \) [\( s_a(i) \)] is equal to its net export plus its accumulation

\[
s_t(i) = J_{IE}(i) + J_{IS}(i) - J_{ME}(i) + \frac{d}{dt}[V_t C_t(i)]
\]

\[
s_E(i) = J_{ES}(i) - J_{ME}(i) - J_{IE}(i) + \frac{d}{dt}[V_E C_E(i)]
\]

where \( V_a \) is the compartment volume (cm\(^3\)/cm\(^2\)). Within the tubule lumen, solute generation is appreciated as an increase in axial flux, as transport into the epithelium, or as local accumulation.

\[
s_M(i) = \frac{\partial F_M(i)}{\partial x} + B_M[J_{ME}(i) - J_{MI}(i)] + \frac{\partial}{\partial t}[A_M C_M(i)]
\]

where \( B_M \) is the tubule circumference, and \( A_M \) is the tubule cross-sectional area. It should be noted that with this formulation, generation terms \( s_a(i) \) for both cell and interspace have dimension mmol·s\(^{-1}\)·cm\(^{-2}\) epithelium, whereas for the tubule lumen the dimension is mmol·s\(^{-1}\)·cm\(^{-1}\). Although it is not necessary to represent luminal fluxes (Eq. 3) in the epithelial calculations of this manuscript, similarity to the solute generation equations for cell and interspace (Eqs. 1 and 2) is evident, and the formulation of mass conservation equations is identical. With this notation, the equations of mass conservation for the nonreacting species (Na\(^+\), K\(^+\), Cl\(^-\), and urea) are written

\[
s_a(i) = 0
\]

where \( \alpha = E, I, \) or M.

For the phosphate and ammonia buffer pairs, there is conservation of total buffer:

\[
s_a(HPO_4^{2-}) + s_a(H_2PO_4^-) = 0
\]

\[
s_a(NH_3) + s_a(NH_4^+) = 0
\]

Similarly, within the cell, there is conservation of total impermeant buffer, represented by a species with a single protonation site, Buf\(^-\) and HBuf. Although peritubular PCO\(_2\) will be specified, the CO\(_2\) concentrations of the cells, interspace, and lumen are model variables. The relevant reactions are

\[
H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons H_2O + CO_2
\]

where dissociation of H\(_2\)CO\(_3\) is rapid, and assumed to be at equilibrium. Since HCO\(_3^-\) and H\(_2\)CO\(_3\) are interconverted, mass conservation requires

\[
s_a(HCO_3^-) + s_a(H_2CO_3) = V_a[(k_d C_a(CO_2) - k_d C_a(H_2CO_3))]
\]

for \( \alpha = I \) or E, while for the tubule lumen,

\[
s_M(HCO_3^-) + s_M(H_2CO_3) = A_M[k_d C_M(CO_2) - k_d C_M(H_2CO_3)]
\]

In each compartment (\( \alpha = I, E, \) or M), conservation of total CO\(_2\) is expressed

\[
s_a(HCO_3^-) + s_a(H_2CO_3) + s_a(CO_2) = 0
\]

Corresponding to conservation of protons, is the equation for conservation of charge for all the buffer reactions (including cytosolic impermeant buffer)

\[
\sum_i z_i s_a(i) = 0
\]

where \( z_i \) is the valence of species \( i \). In this model, conservation of charge for the buffer reactions (Eq. 11) takes the form

\[
s_a(H^+) + s_a(NH_4^+) - s_a(HCO_3^-) - s_a(HPO_4^{2-}) - s_a(Buf^-) = 0
\]

The solute equations are completed with the chemical equilibria of the buffer pairs: HPO\(_4^{2-}\):H\(_2\)PO\(_4^-\), NH\(_3\):NH\(_4^+\), and HCO\(_3^-\):H\(_2\)CO\(_3\), and within the cell, Buf\(^-\):HBuf. Corresponding to the electrical potentials, \( \psi_a \), for \( \alpha = E, I, \) or M, is the equation for electroneutrality

\[
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Fig. 1. Schematic representation of ascending Henle limb (AHL) epithelium, consisting of AHL cell and lateral intercellular space (LIS), and AHL tubule model, whose lumen is lined by this epithelium. Intraepithelial fluxes are designated \( J_{ab}(i) \), where the subscripts \( a,b \) refer to luminal cell membrane (MI), tight junction (ME), lateral cell membrane (IE), or interspace basement membrane (ES). Along the tubule lumen, axial flows are designated \( F_M(i) \).
\[ \sum_i z_i C_\alpha(i) = 0 \] (13)

where for the cellular compartment (\( \alpha = 1 \)), the sum includes the contribution of the impermeant anion plus the unprotonated impermeant buffer.

With respect to water flows, volume conservation equations for lumen, interspace, and cell can be used to compute the three unknowns: luminal volume flow, lateral interspace hydrostatic pressure, and cell volume. Cell hydrostatic pressure is set equal to luminal pressure; total cell-impermeant content is assumed fixed. Alternatively, cell-impermeant concentration can be considered a model variable, which is adjusted to maintain a fixed reference volume. This formulation had been used in steady-state medullary models, in which external osmolality varied widely (55, 56). For the calculations in this paper, cell volume will be allowed to vary, but for the tubule model, cells will be assumed to be of uniform size. Across each cell membrane, the volume fluxes are proportional to the hydromic driving forces. With respect to the lateral interspace, its volume, \( V_E \), and its basement membrane area, \( A_{ES} \), are functions of interspace hydrostatic pressure, \( p_E \):

\[
\frac{V_E}{V_{EO}} = \frac{A_{ES}}{A_{ES0}} = 1.0 + \nu_E(p_E - p_t) \quad (14)
\]

where \( V_{EO} \) and \( A_{ES0} \) are reference values for volume and outlet area, and \( p_t \) is a compliance.

Solute transport may be convective, electrodiffusive (e.g., via a channel), coupled to the electrochemical potential gradients of other solutes (e.g., via a cotransporter or an antipporter), or coupled to metabolic energy (via an ATPase). This is expressed in the model by the flux equation:

\[
J_{\alpha\beta}(i) = J_{\alpha\beta} \cdot (1.0 - \sigma_{\alpha\beta}(i)) \cdot \xi_{\alpha\beta}(i) + h_{\alpha\beta}(i) \cdot \xi_{\alpha\beta}(i) \left[ \frac{C_\alpha(i) - C_\beta(j)e^{-\xi_{\alpha\beta}(i)}}{1 - e^{-\xi_{\alpha\beta}(i)}} \right] + \sum_j L_{\alpha\beta}(i, j)[\bar{\mu}_\alpha(j) - \bar{\mu}_\beta(j)] + J_{\alpha\beta}^{act}(i) \quad (15)
\]

In this equation, the first term indicates the role of the solute reflection coefficient in determining convective transport, and \( \xi \) is a (logarithmic) mean membrane solute concentration. In this model, all reflection coefficients are equal to 1.0, except for the lateral interspace basement membrane, where all reflection coefficients are equal to 0.0. The second term of Eq. 15 is the Goldman relation for ionic fluxes, where \( h_{\alpha\beta}(i) \) is a solute permeability, and \( C_\alpha(i) \) and \( C_\beta(j) \) are the concentrations of \( i \) in compartments \( \alpha \) and \( \beta \). Here,

\[
\xi_{\alpha\beta}(i) = \frac{z_i F}{RT}(\psi_\alpha - \psi_\beta) \quad (16)
\]

is a normalized electrical potential difference, where \( z_i \) is the valence of \( i \), and \( \psi_\alpha - \psi_\beta \) is the potential difference between compartments \( \alpha \) and \( \beta \). The third term of the solute flux equation specifies the coupled transport of species \( i \) and \( j \) according to linear nonequilibrium thermodynamics, where the electrochemical potential of \( j \) in compartment \( \alpha \) is

\[
\bar{\mu}_\alpha(j) = RT \ln[C_\alpha(j)] + z_i F \psi_\alpha. \quad (17)
\]

For each of these transporters, the assumption of fixed stoichiometry for the coupled fluxes allows the activity of each transporter to be specified by a single coefficient. The exceptions to this representation of coupled fluxes are the transporters for which kinetic models are available, namely NKCC2 (Eqs. 8 and 9, with parameters in Table 2a of Ref. 58), KCC4 (Eq. 22, with parameters in Table 3a of Ref. 58), and the luminal membrane \( \text{Na}^+ / \text{H}^+ \) (or \( \text{Na}^+ / \text{NH}_4^+ \)) exchanger, NHE3 (Eqs. 7–9, with parameters in Table 1 of Ref. 54). For each of these transporters, a single density parameter suffices to represent its activity.

The final term in Eq. 15 represents active transport, and in this model there is a single transport ATPase, the peritubular Na-K-ATPase represented by

\[
J_{\alpha\beta}^{act}(\text{Na}^+) = \frac{[J_{\alpha\beta}^{act}(\text{Na}^+)]_{max}}{[C_\alpha(i) + K_{Na}]^3 + \frac{C_{E}(\text{Na}^+)}{C_{E}(\text{K}^+) + K_{K}}} \quad (18)
\]

in which the half-maximal \( \text{Na}^+ \) concentration, \( K_{Na} \), increases linearly with internal \( \text{K}^+ \), and the half-maximal \( \text{K}^+ \) concentration, \( K_{K} \), increases linearly with external \( \text{Na}^+ \) (16a). The pump flux of \( \text{K}^+ \) plus \( \text{NH}_4^+ \) reflects the 3:2 stoichiometry,

\[
J_{\alpha\beta}^{act}(\text{K}^+) + J_{\alpha\beta}^{act}(\text{NH}_4^+)^3 = - (2/3)J_{\alpha\beta}^{act}(\text{Na}^+) \quad (19)
\]

with the transport of either \( \text{K}^+ \) or \( \text{NH}_4^+ \) determined by their relative affinities, \( K_{K} \) and \( K_{NH4} \),

\[
\frac{J_{\alpha\beta}^{act}(\text{NH}_4^+)}{J_{\alpha\beta}^{act}(\text{K}^+)} = \frac{C_{E}(\text{NH}_4^+)}{C_{E}(\text{K}^+)} \cdot \frac{K_{K}}{K_{NH4}} \quad (20)
\]

Of note, the model distinguishes two populations of peritubular Na-K-ATPase, that of the cell base, with fluxes designated \( J_{IS}(i) \) [dependent on peritubular \( \text{K}^+ \), \( C_5(K^+) \)] and that of the lateral cell membrane, with fluxes designated \( J_{IL}(i) \) [dependent on interspace \( \text{K}^+ \), \( C_6(K^+) \)]. With the overall electrical resistance of the interspace basement membrane \( \sim 1 \ \Omega \cdot \text{cm}^2 \), and that of the lateral cell membrane \( \sim 21 \ \Omega \cdot \text{cm}^2 \), interspace \( \text{K}^+ \) concentration is a weighted average of cytosolic and peritubular \( \text{K}^+ \) concentrations. In the model calculations, the presence of the interspace blunts the impact of peritubular solute changes on the Na-K-ATPase of the lateral cell membrane.

**Model Parameters**

Parameters were selected so that when the model epithelium is configured as a tubule, the model tubule will resemble rat
AHL, a 4-mm structure, divided approximately into medullary and cortical halves (21). In what follows, the distinctions between cortical and medullary segments will be minimal, specifically only a different NKCC2 isofrom in medulla (F) and cortex (B) (37). Tubule diameter is a uniform 20 μm along its length (18, 32), and there is amplification of luminal membrane area to 2 cm²/cm² and of peritubular membrane area to 12 cm²/cm² (33). The data that inform the selection of secretion has been in the range of 10 – 20% of the rate of Na⁺ reabsorption measured in vivo (17, 21). For parameter selection for this model, Na⁺ reabsorption measured in vivo (17, 21), and identified as NHE3 in the medullary thick ascending limb (2, 4). In the cortical segment, the Na⁺/H⁺ exchanger has been found to be NHE2 (12). Consistent with established NHE3 physiology, the luminal membrane antiporter mediates Na⁺/NH₄⁺ exchange (6). With respect to the model AHL, the kinetic model of NHE3, developed for rat proximal tubule and incorporating NH₄⁺ transport (54), is used in both medullary and cortical segments, since there is no kinetic model for NHE2. The electrical properties of the luminal membrane were first examined in the rabbit, in which the overall conductance was ~12 mS/cm², and almost exclusively a K⁺ permeability (24, 25). In the mouse, the conductance of the transcellular pathway was only slightly higher, 17 mS/cm², but was found to double (36 mS/cm²) with application of ADH (28). The predominant K⁺ channel in rat AHL luminal membrane has a 70-pS conductance and a high open probability that decreases sharply with cytosolic acidification; its K⁺ permeation is fit nicely by a Goldman equation (7). Subsequently, a 30-pS K⁺ channel was identified in rat AHL, also with high open probability, but with relatively rapid rundown in an excised patch (48); its functional properties are akin to those of ROMK (49). Within rat AHL luminal membrane, the relative importance of the 70- and 30-pS channels to the overall K⁺ conductance was found to be 80 and 20%, respectively (50). Of note, no NH₄⁺ permeation of the 70-pS channel was detected (7), while for ROMK, NH₄⁺ conductance may be greater than that for K⁺ (13). In the model developed here, the luminal membrane K⁺ pathway has been approximated as a single Goldman channel, with NH₄⁺ permeability 20% of that for K⁺. Of necessity, the K⁺ permeability is taken to be high (yielding a luminal membrane conductance of 56 mS/cm²) to avoid luminal K⁺ depletion with axial flow.

Within the peritubular cell membrane, the Na⁺-K-ATPase mediates exit of Na⁺ in exchange for K⁺ plus NH₄⁺, with

![Transporter Diagram](https://example.com/transporter_diagram.png)

**Fig. 2.** Transporters of the AHL cell under baseline, low-ammonia conditions. Bath conditions, electrical potentials, and solute concentrations are those for Table 2. Solute fluxes are also those for Table 2 but are shown here in pmol·mm⁻¹·min⁻¹, assuming a tubule diameter of 20 μm. Where solutes share a transporter (e.g., K⁺ and NH₄⁺), both fluxes are indicated. PD, potential difference.

The Na⁺/H⁺ antiporter has been demonstrated functionally (17, 21), and identified as NHE3 in the medullary thick ascending limb (2, 4). In the cortical segment, the Na⁺/H⁺ exchanger has been found to be NHE2 (12). Consistent with established NHE3 physiology, the luminal membrane antiporter mediates Na⁺/NH₄⁺ exchange (6). Within the peritubular cell membrane, the Na⁺-K-ATPase mediates exit of Na⁺ in exchange for K⁺ plus NH₄⁺, with

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stoichiometry 3-Na\(^+\):2-(K\(^+\) plus NH\(_4\)^+). The relative affinities of K\(^+\) and NH\(_4\)^+ for the Na-K-ATPase are comparable to proximal tubule (35), but in inner medullary collecting duct the K\(^+\) affinity is greater by a factor of 4 (46). This ratio is not known for AHL, so in view of its proximity to proximal tubule, and to avoid luminal NH\(_4\)^+ depletion along the model tubule, equal affinities are assumed. Along the entire AHL, peritubular K\(^+\)-Cl\(^-\) (or NH\(_4\)^+ - Cl\(^-\)) cotransport is specified by the kinetic model of KCC4 (58). The overall electrical conductance of the peritubular membrane in the rabbit has been found to be two or more times greater than that of the luminal membrane, i.e., 20–50 mS/cm\(^2\) (24, 26). In the mouse, however, this twofold higher conductance of peritubular membrane has been found to reverse (to 2-fold higher luminal conductance) with application of ADH (29). The predominant conductance remains that for K\(^+\), although a Cl\(^-\) transference number as high as 0.2 has been reported for the rabbit (24). An inwardly rectifying K\(^+\) channel has been identified in the peritubular membrane of rabbit AHL (31). In the model tubule, a K\(^+\) permeability was selected, which gives a partial K\(^+\) conductance of 36 mS/cm\(^2\), and NH\(_4\)^+ permeability is taken to be 20% of the K\(^+\) permeability. Peritubular Cl\(^-\) permeability is set to 25% of K\(^+\) permeability, and to allow for HCO\(_3\)^- permeation of Cl\(^-\) channels, HCO\(_3\)^- permeability is set to 20% of the Cl\(^-\) permea-
ability. The parallel arrangement of $K^+$ and $Cl^-$ channels can have a comparable functional effect as the $K^+/Cl^-$ cotransporter, and this has been examined in models of other tubule segments (55, 57). Thus, with the experimental constraint on peritubular membrane electrical conductances, the KCC4 density was selected to accommodate the necessary fluxes of $K^+$ and $Cl^-$; ultimately, both channel fluxes and cotransporter fluxes prove to be substantial. There are other coupled transport pathways in the peritubular membrane, including a Na+/H+ exchanger (34), which has been identified as NHE4 (11), a Cl-/HCO$_3^-$ exchanger, which has been identified as AE2 (1, 15), and a Na$^+-$(n)HCO$_3^-$ cotransporter (8, 22, 34), but whose presence there has been questioned (40). These have been included in the model (using single-parameter nonequilibrium thermodynamic formalism), but do not feature prominently in the flux accounting.

The study by Rivers et al. (38) in vesicles prepared from rat medullary thick AHL supplies important information on the permeability of both luminal and peritubular membranes to water, urea, $NH_3$, and protons. They found that the water permeability of luminal and peritubular membranes was comparable, with $P_W = 4 \times 10^{-2} \text{ cm/s}$. In this model, that membrane $P_W$ yielded relatively large volume flows; the $P_t$ chosen for model calculations was half the reported value, so that overall volume reabsorption in AHL remained under 10% of entering flow. Of note, using the reported luminal membrane $P_t$ and amplifying by membrane area of 2 cm$^2$/cm$^2$, one would predict an epithelial $P_t$ of $8 \times 10^{-3} \text{ cm/s}$ (because peritubular membrane has a several-fold greater area); this is substantially higher than the measured epithelial $P_t$ in rabbit AHL, $1 \times 10^{-3} \text{ cm/s}$ (10). The urea permeabilities measured by Rivers et al. (38) were also comparable for the two cell membranes, and those were incorporated into this model. $NH_3$ permeability was slightly different for luminal and peritubular membranes, 0.015 and 0.001 cm/s, and those were used as reported. It will be seen that, relative to the rates of luminal acidification and cellular uptake of $NH_4^+$, these $NH_3$ permeabilities are more than adequate to sustain the necessary base exit. Rivers et al. reported comparable membrane proton permeability measured at 20°C; for the present model at 37°C, the proton permeability was increased by the factor of three that they observed for temperature differences in $P_t$ and $NH_3$ permeabilities. There are no data available for CO$_2$ and H$_2$CO$_3$ permeabilities for AHL, so unit membrane permeabilities were taken from prior renal epithelial models: compared with the membrane $NH_3$ permeability, membrane CO$_2$ permeability was ~10-fold greater, and H$_2$CO$_3$ was ~10%.

The first measurements of tight junctional parameters of AHL came from experiments in isolated perfused tubules from rabbits. Overall epithelial conductance was 46.5 mS/cm$^2$, with a ratio of partial conductances of Cl$^-$-to-$Na^+$ of 0.45 (10). Subsequently, determinations of the Na$^+$ and Cl$^-$ permeabilities were $2.8 \times 10^{-5}$ and $1.4 \times 10^{-5} \text{ cm/s}$, respectively (9). The values for mouse AHL are comparable: an overall conductance of 90 mS/cm$^2$, with Na$^+$ and Cl$^-$ permeabilities $2.5 \times 10^{-5}$ and $1.2 \times 10^{-5} \text{ cm/s}$ (27). These permeabilities are incorporated directly as tight junction permeabilities in this model. The ratio of HCO$_3^-$ to Cl$^-$ tight junction permeability of rabbit AHL has been estimated at 0.4 (22), and this is used to assign the HCO$_3^-$ permeability. In rat AHL, epithelial $NH_4^+$ permeability has been measured at $6.0 \times 10^{-5} \text{ cm/s}$ (18). In the model, the transcellular $NH_4^+$ permeability, based on the unit membrane permeabilities in series, is $3 \times 10^{-5} \text{ cm/s}$, so that tight junction $NH_4^+$ permeability is set at $3 \times 10^{-5} \text{ cm/s}$. Tight junction $K^+$ permeability is taken to equal this $NH_4^+$ permeability, so that both are just slightly larger than the Na$^+$ permeability. Compared with the HCO$_3^-$ permeability, tight junction permeabilities of CO$_2$ and H$_2$CO$_3$ are fourfold greater, urea permeability is 20% less, and for the phosphate species, permeability is 40% of HCO$_3^-$ permeability. With these assigned permeabilities, the overall tight junction electrical conductance for this model AHL is calculated to be 22 mS/cm$^2$, and nearly identical to the conductance measured in rabbit AHL, 21 mS/cm$^2$ (26). Tight junction water permeability is taken to be small (~2% of the cell), and the solute reflection coefficients were 1.0.

The interspace basement membrane conductance was assumed to be ~40-fold greater than that of the tight junction, and solute permeabilities were proportional to diffusivity in free solution. The values shown are those used previously for the interspace basement membrane of distal convoluted tubule (57). All interspace basement membrane reflection coefficients are 0.0, except for the peritubular oncotic force (for which the reflection coefficient is 1.0). As in previous models, the LIS is a compliant structure, which distends in response to increases in interspace hydrostatic pressure. There is a minimum LIS volume, ~7% of cell volume, and a minimum basement membrane LIS outlet area, ~2% of epithelial area. Under most conditions, the peritubular oncotic force keeps the LIS collapsed to these minimal values. Staining for carbonic anhydrase is positive for luminal and peritubular membranes of rat AHL (36a), and this marks a difference from the rabbit AHL (14). Accordingly, rate constants for hydration of CO$_2$ are assumed to be 10,000-fold greater than those for the uncatalyzed reaction in lumen, cytosol, and lateral intercellular space.

**MODEL CALCULATIONS**

**Low Ammonia**

For the epithelial simulations, the 29 model equations were solved iteratively to double precision machine accuracy using Newton’s method. Table 2 contains the open-circuit solution of the epithelial model in which NKCC2 is the F-isoform. Luminal and peritubular conditions are equal, and suggestive of a Ringer-like solution, with the exception of a small ammonia concentration of 0.2 mM; PCO$_2$ is 50 mmHg. Solute fluxes in Table 2 are in nanomoles per second per centimeter squared, and these same fluxes appear in Fig. 2 with the dimension picomoles per millimeter per minute, assuming a tubule diameter of 20 $\mu$m. This AHL cell displays a relatively low cell Na$^+$ and high cell Cl$^-$, consistent with measurements of cell composition (5). Cytosolic pH of the model cell is 7.29, but will decrease with increments in ambient ammonia, consistent with observations (52). With dominant $K^+$ permeabilities of both peritubular and luminal cell membranes, the peritubular and transepithelial electrical potentials are comparable to measured values. [Greger and Schlatter (25) reported peritubular potential difference (PD) of −69 mV in rabbit cortical AHL; Good et al. (21) found transepithelial PD +6.2 and +13.4 mV in different groups of rat medullary AHL; Tsuruoka et al. (45) determined peritubular PD in rat medullary AHL −75 mV.] The total Na$^+$ flux by the model AHL is 251

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### Table 2. Model solution for AHL (NKCC F-isoform)

<table>
<thead>
<tr>
<th>Intensive Variables</th>
<th>Lumen</th>
<th>Interspace</th>
<th>Cell</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume, cm³</td>
<td>0.48 × 10⁻⁴</td>
<td>7.47 × 10⁻⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrical potential, mV</td>
<td>10.0</td>
<td>0.16</td>
<td>−72.2</td>
<td>0.00</td>
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<tr>
<td>Pressure, mmHg</td>
<td>0.00</td>
<td>2.02</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Concentration, mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺</td>
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</table>

### Membrane Flux Components

| Coupled fluxes, pmol·s⁻¹·cm⁻² |       |      |      |      |      |
| Na⁺-K⁺(NH₄⁺)-2Cl⁻ (Na⁺)       | 4770. |       |      |      |      |
| Na⁺-K⁺(NH₄⁺)-2Cl⁻ (K⁺)        | 4661. |       |      |      |      |
| Na⁺-K⁺(NH₄⁺)-2Cl⁻ (Cl⁻)       | 9540. |       |      |      |      |
| Na⁺-K⁺(NH₄⁺)-2Cl⁻ (NH₄⁺)      | 110.  |       |      |      |      |
| Na⁺/H⁺ (Na⁺)                  | 594.  | −619. | −120. |       |      |
| Na⁺/H⁺ (H⁺)                   | −554. | 619.  | 120.  |       |      |
| Na⁺/H⁺ (NH₄⁺)                 | −40.  |       |      |       |      |
| K⁺-Cl⁻ (K⁺)                   | 3550. |       |      |      |      |
| K⁺-Cl⁻ (Cl⁻)                  | 3530. |       |      |      |      |
| K⁺-Cl⁻ (NH₄⁺)                 | −20.4 |       |      |      |      |
| Cl⁻/HCO₃⁻ (Cl⁻)               | −80.  |       |      |      |      |
| Cl⁻/HCO₃⁻ (HCO₃⁻)             | 80.   |       |      |      |      |
| Na⁺-3HCO₃⁻ (Na⁺)              | 35.   |       |      |      |      |
| Na⁺-3HCO₃⁻ (HCO₃⁻)            | 106.  |       |      |      |      |
| Na₂HPO₄⁻ (Na⁺)                 | −82.  |       |      |      |      |
| Na₂HPO₄⁻ (HPO₄²⁻)             | −41.  |       |      |      |      |

### Pump fluxes, pmol·s⁻¹·cm⁻²

<table>
<thead>
<tr>
<th>Na-K-ATPase (Na⁺)</th>
<th>Na-K-ATPase (K⁺)</th>
<th>Na-K-ATPase (NH₄⁺)</th>
</tr>
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<tbody>
<tr>
<td>5199.</td>
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<td>−112.</td>
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</table>

Buf and HBuf, unprotonated and protonated cystolic impermeant buffer, respectively.
strating balanced forces for solute entry and exit. At the highest luminal Na⁺ concentrations, there is cell shrinkage; at very low lumen Na⁺, there is near zero lumen Cl⁻, cytosolic Cl⁻ depletion, and reversal of solute flux through KCC, so that the cell swells.

AHL luminal transporters are also likely to encounter a range of luminal K⁺ concentration, due in part to water abstraction from the descending Henle limb, and perhaps to diffusive K⁺ entry. Figure 4, A–C, displays transport by the model AHL (F-isoform only) over luminal K⁺ concentration from 0.5 to 15 mM; peritubular K⁺ is fixed at 5 mM. Other than K⁺, the solutions are symmetric, Ringer-like, with 0.2 mM ammonia. Luminal fluxes of Na⁺, K⁺, and the ammonia species are shown in the three panes of Fig. 4A. The middle pane contains total epithelial K⁺ flux, along with the portions through NKCC2 and through the luminal membrane K⁺ channel (labeled GK). As lumen K⁺ increases, there is increased K⁺ reabsorption, early on due to a sharp increase in NKCC transport, and then to progressive decline in secretory channel flux. Overall epithelial K⁺ transport is zero when lumen K⁺ is

![Figure 4](image-url)

**Fig. 4.** AHL fluxes with variation in luminal KCl (F-isoform). Bath and lumen conditions are those for Table 2, with the exception of luminal K⁺ concentration, which is varied from 0.5 to 15 mM (as KCl addition or subtraction). A: luminal fluxes of Na⁺, K⁺, and the ammonia species (pmol·mm⁻¹·min⁻¹). The middle pane contains total epithelial K⁺ flux, along with the portions through NKCC2 and through the luminal membrane K⁺ channel (labeled GK). B: selected luminal and peritubular transporter fluxes of Na⁺, K⁺, H⁺, and NH₄⁺ as a function of luminal K⁺ concentration. The sum of NKCC and NHE3 Na⁺ fluxes is the transcellular Na⁺ flux in the top pane in A. C: cell volume and the important cytosolic solute concentrations during variation in luminal K⁺.
0.7 mM. In Fig. 4B, coupled transporters are displayed, with NKCC fluxes in the top left corner indicating that displacement of NH$_4^+$ by K$^+$ accounts for much of the early increase in K$^+$ uptake by NKCC. With this decrease in NH$_4^+$ uptake, cell NH$_4^+$ drops, and the cell alkalinizes (increase in cell HCO$_3^-$, Fig. 4C). As a consequence of the alkalinization, there is a decrease in luminal NHE3 activity (Fig. 4B), but this is a small flux, and dwarfed by the increase in Na$^+$ absorption via NKCC. Over the full range of luminal K$^+$ concentrations, there is an increase in cytosolic Cl$^-$ and HCO$_3^-$, so that cell volume increases from 5 to $10 \times 10^{-4}$ cm$^3$/cm$^2$, in the absence of any volume-regulated transporters. In short, low luminal K$^+$ is predicted to lead to cytosolic acidosis that activates NHE3, which maintains AHL Na$^+$ reabsorption, despite the decline in NKCC uptake.

Variation in peritubular K$^+$ concentration has received attention as a modulator of AHL Na$^+$ reabsorption. Specifically, if medullary interstitial K$^+$ acts to blunt AHL Na$^+$ flux, then it could act to augment cortical K$^+$ secretion through enhanced Na$^+$ delivery (42). In the calculations of Fig. 5, A–C, the AHL model with the F-isoform NKCC is examined as peritubular baseline value (at 5 mM peritubular K$^+$). The impact of peritubular K$^+$ is to diminish KCC transport. The luminal concentration of NH$_4^+$ is varied from 2 to 50 mM, by addition of KCl. Otherwise, luminal and peritubular solutions are symmetric, Ringer-like, with 2.0 mM ammonia. With reference to Fig. 5B, the primary impact of peritubular KCl is to diminish KCC transport. The effect on cytosolic K$^+$ and Cl$^-$ (Fig. 5C) is straightforward, and most pronounced in the hypokalemic range (2–5 mM). At low peritubular K$^+$, there is a sharp increase in cytosolic Na$^+$, and this reflects the relative insensitivity of the Na-K-ATPase to variation in cell Na$^+$ at the higher concentrations, which come with increased NKCC flux (Eq. 18). At the highest peritubular K$^+$ concentration (50 mM), the increase in cytosolic K$^+$ and Cl$^-$ reduces NKCC Na$^+$ flux to ~40% of its baseline value (at 5 mM peritubular K$^+$), and about half of this reduction occurs in the first 15 mM increase in peritubular KCl. The increase in peritubular K$^+$ also inhibits Na-K-ATPase NH$_4^+$ entry, and the secondary decrease in NKCC flux blunts luminal NH$_4^+$ entry. In consequence, cytosolic NH$_4^+$ declines, the cell alkalinizes, and this blunts NHE3 activity, so that as a result of increasing peritubular K$^+$, the model predicts decreased Na$^+$ reabsorption via both NKCC and NHE3. Although Fig. 5A shows a decline in transcellular Na$^+$ entry across the range of peritubular K$^+$ concentrations, the model also predicts that over this range there is enhanced electrodiffusive flux of K$^+$ across the luminal membrane (curve $G_K$ in the middle pane of Fig. 5A), and over this range, luminal hyperpolarization from +6.4 to +32 mV (not shown). With a tight junction Na$^+$ conductance of 14.5 mS/cm$^2$, this change in PD translates into a positive Na$^+$ current of 0.4 mA/cm$^2$, or a Na$^+$ flux of 4 nmol·s$^{-1}$·cm$^{-2}$. With the 20-μm diameter tubule, this is 160 pmol-mm$^{-1}$·min$^{-1}$, and that is what appears as the junctional Na$^+$ flux in Fig. 5A. In short, with variation in peritubular K$^+$ concentration, luminal K$^+$ conductance and tight junctional Na$^+$ conductance act in parallel to maintain AHL Na$^+$ reabsorption.

The luminal concentration of NH$_4^+$ in end-proximal tubule is ~1–2 mM, and this can be expected to increase in descending Henle limb, in part due to water abstraction or with diffusive NH$_4^+$ entry from the medullary interstitium. The model allows prediction of the impact of luminal NH$_4^+$ on AHL solute transport, and this appears in Fig. 6, A–C. The calculations are done with the epithelial model incorporating the NKCC F-isoform, and using symmetric, Ringer-like solutions, except for variation of luminal NH$_4^+$ from 0.2 to 10 mM (NH$_4$Cl addition). The primary effect of increasing luminal NH$_4^+$ is the competition for K$^+$ entry on NKCC (Fig. 6B), so that by the time luminal NH$_4^+$ concentration has reached 10 mM, all of the Na$^+$ entry proceeds as Na$^+$-NH$_4^+$-2Cl$^-$ (Fig. 6B); overall, there is little change in NKCC Na$^+$ uptake. As a consequence of increased luminal NH$_4^+$ entry, there is an increase in cytosolic NH$_4^+$ and acidification of the cell (Fig. 6C). In turn, this increases NHE3 activity, so that beyond luminal NH$_4^+$ of 4.5 mM, the NHE3 Na$^+$ entry exceeds Na$^+$ flux across NKCC; ultimately, about half of NHE3 Na$^+$ entry participates in exchange for NH$_4^+$. Overall, with increases in luminal NH$_4^+$ there is predicted to be a substantial increase in AHL Na$^+$ flux (Fig. 6A), due to the increase in NHE3 Na$^+$ flux. By virtue of the kinetics of the Na-K-ATPase (Eq. 18), this increase in Na$^+$ transport requires a substantial increase in cytosolic Na$^+$ (Fig. 6C). Figure 6A also shows the prediction of net ammonia uptake from 100 pmol-mm$^{-1}$·min$^{-1}$ and above, with luminal NH$_4^+$ >6 mM. Reabsorption of this magnitude occurs only because peritubular NH$_4^+$ is set at 0.2 mM; more realistic fluxes are obtained when peritubular NH$_4^+$ is taken to be higher.

**Physiological Ammonia**

The impact of ammonia was relatively small in the calculations of Fig. 2, but is brought to the fore in Fig. 7, in which the ambient total ammonia is increased from 0.2 to 2.0 mM (addition of 1.8 mM NH$_4$Cl to both luminal and peritubular solutions used in Fig. 2). With high ambient ammonia there is acidification of the cell to pH 6.89, activation of NHE3, an increase in cytosolic Na$^+$, and a decrease in cytosolic K$^+$. Cytosolic NH$_4^+$ has increased from 0.4 to 8.4 mM. The total AHL Na$^+$ flux has increased to 301 pmol-mm$^{-1}$·min$^{-1}$, of which 272 is transcellular, with 170 via NKCC2 and 102 via NHE3. Of the NKCC2 flux, 126 pmol·mm$^{-1}$·min$^{-1}$ is transcellular, with 170 via NKCC2 and 102 via NHE3. The model predicts a substantial increase in AHL Na$^+$ flux (Fig. 6A). With the 20-μm diameter tubule, there is a major increase in luminal membrane proton secretion to 64 pmol-mm$^{-1}$·min$^{-1}$, respectively. Luminal membrane uptake of K$^+$ (126 pmol-mm$^{-1}$·min$^{-1}$) is still greater than conductive luminal K$^+$ exit (66 pmol-mm$^{-1}$·min$^{-1}$); the peritubular K$^+$ uptake via the Na-K-ATPase (154 pmol-mm$^{-1}$·min$^{-1}$) is balanced by KCC exit (153 pmol-mm$^{-1}$·min$^{-1}$), while conductive flux is 61 pmol-mm$^{-1}$·min$^{-1}$. With respect to acid/base transport, there is a major increase in luminal membrane proton secretion to 64 pmol-mm$^{-1}$·min$^{-1}$ and only a small increase in peritubular Na$^+/H^+$ exchange (33 pmol-mm$^{-1}$·min$^{-1}$); in this model, only the luminal NHE has the cytosolic proton modifier site. Base exit under these circumstances is nearly exclusively as NH$_3$, with only minor HCO$_3^-$ fluxes. With 2 mM ambient ammonia, there is now 85 pmol-mm$^{-1}$·min$^{-1}$ of net cellular NH$_4^+$ uptake, and an equal diffusive exit of NH$_3$. The cellular uptake of NH$_4^+$ is divided among NKCC, the Na-K-ATPase, and luminal and peritubular K$^+$ channels, and KCC: 44, 52, 22, and 6 pmol-mm$^{-1}$·min$^{-1}$; NH$_3$ extrusion by the NHE3 is ~39

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*Note: The above text is a Natural Language Representation of the given document. It has been formatted for clarity and readability.*
pmol·mm⁻¹·min⁻¹. With respect to total ammonia, there is negligible net uptake across the luminal membrane, only 1 pmol·mm⁻¹·min⁻¹.

In the case of higher ammonia concentrations, with the attendant reconfiguration of AHL fluxes, one may reexamine the impact of peritubular K⁺ concentration on AHL Na⁺ transport. This is addressed in Fig. 8, A–C, which repeats the calculations of Fig. 5, A–C, with ambient ammonia set to 2 mM. The salient finding in Fig. 8A is that as in the low-ammonia case, transepithelial Na⁺ uptake is decreased by two-thirds with increasing peritubular KCl. As was the case in Fig. 5A, with increasing peritubular K⁺, there is progressive luminal hyperpolarization, increased Na⁺ reabsorption across the tight junction, and stabilization of overall Na⁺ transport.

Fig. 5. AHL fluxes with variation in peritubular KCl (F-isoform). Bath and lumen conditions are those for Table 2, with the exception of peritubular KCl concentration, which is varied from 2 to 50 mM (as KCl addition or subtraction). A: luminal fluxes of Na⁺, K⁺, and the ammonia species (pmol·mm⁻¹·min⁻¹). The middle pane contains total epithelial K⁺ flux, along with the portions through NKCC2 and through the luminal membrane K⁺ channel (labeled GK). B: selected luminal and peritubular transporter fluxes of Na⁺, K⁺, H⁺, and NH₄⁺ as a function of peritubular K⁺ concentration. C: cell volume and cytosolic solute concentrations during variation in peritubular K⁺.
The major difference from the case with low ammonia is the augmented activity of NHE3 (Fig. 8B). This increase in NHE3 activity follows from the cytosolic acidification produced by NH4+ entry (Fig. 8C), and NHE3 accounts for ~40% of transcellular Na+ flux across the whole range of peritubular K+ concentrations. Although there are now two important pathways for luminal Na+ reabsorption, the fluxes through both are blunted by peritubular K+ uptake. The principal pathways for cellular NH4+ uptake are the luminal NKCC and the peritubular Na-K-ATPase. Peritubular K+ acts directly to blunt peritubular uptake of NH4+ (competitively), and indirectly to decrease luminal NH4+ uptake via inhibition of NKCC activity. Together, the decrease in NH4+ entry alkalinizes the cell (Fig. 8C), and due to sensitivity of NHE3 flux to cytosolic pH, Na+ entry via this transporter drops.

Fig. 6. AHL fluxes with variation in luminal NH4Cl (F-isoform). Bath and lumen conditions are those for Table 2, with the exception of luminal NH4+ concentration, which is varied from 0.2 to 10 mM (as NH4Cl addition). A: luminal fluxes of Na+, K+, and the ammonia species (pmol-mm⁻¹-min⁻¹). The middle pane contains total epithelial K+ flux, along with the portions through NKCC2 and through the luminal membrane K+ channel (labeled Gk). B: selected luminal and peritubular transporter fluxes of Na+, K+, H+, and NH4+ as a function of luminal NH4+ concentration. C: cell volume and cytosolic solute concentrations during variation in luminal NH4+.
One critical aspect of the model's representation of ammonia transport is the brisk uptake via NKCC2. Specifically, this provides an acid load, and obligates substantial Na\(^+\)/H\(^+\) transport of NHE3. For the calculation of Fig. 9A, the time-dependent AHL model (F-isoform) is used to simulate an experiment in which rat AHL epithelium is subjected to a luminal NH\(_4\)Cl pulse. For these calculations, the model equations are represented as a first-order backward finite difference scheme. From \(t = -100\) s to \(t = 0\), the epithelium is at the low-ammonia steady state of Table 2. Then at \(t = 0\), 20 mM NH\(_4\)Cl is added to the luminal solution and maintained until \(t = 200\) s, when the initial NH\(_3\) concentration (0.2 mM) is restored. The full curve of cytosolic pH as a function of time is shown in the top pane, and expanded scale views of pulse and recovery are shown in the two bottom panes. It is clear that the pulse and recovery are asymmetric, notably that cellular acidification is faster than recovery. This derives from the differences in luminal and cytosolic K\(^+\) concentrations, and their impact on NH\(_4\) binding to NKCC2. This figure may be compared with Fig. 3A of Watts and Good (52), who found that with the luminal NH\(_4\)Cl pulse, there was cytosolic acidification from pH 7.1 to pH 6.5 at the rate of 11.1 pH units/min. In the model AHL, linear regression of the linear portion of the pulse yields a slope of 7.8 pH units/min. In the preparation of Watts and Good, cytosolic buffering was determined to be 50 mM, and in this model, total impermeant cellular buffer is 37 mM (Table 2), so that the data are insufficient to determine a unique set of coefficients, and it was emphasized that we have almost no information from internal to external flux experiments. The significance of this limitation to the present work is unknown. The other important coupled transporter of the luminal membrane is NHE3, and in this case a kinetic model was available (54). It is important to note that with respect to the data base informing the NHE3 model, there were transport results from bidirectional studies.

DISCUSSION

This mathematical model of the AHL epithelium has followed the pattern that has evolved through a succession of renal epithelial models. Nevertheless, it has proven to be a more complex construction in two important ways: The first is that the AHL epithelium experiences large variation in solute concentrations on both luminal and peritubular surfaces. This means that the biophysical representation of the important solute transporters needed to be more robust than the convenient, single-coefficient, linear nonequilibrium thermodynamic transporters that served well in the proximal tubule. As prerequisites to the AHL model, kinetic representations of luminal NKCC2 and peritubular KCC4 were constructed (58). With respect to those transporters, it was acknowledged that the data are insufficient to determine a unique set of coefficients, and it was emphasized that we have almost no information from internal to external flux experiments. The significance of this limitation to the present work is unknown. The other important coupled transporter of the luminal membrane is NHE3, and in this case a kinetic model was available (54). It is important to note that with respect to the data base informing the NHE3 model, there were transport results from bidirectional studies. The second complexity of the model AHL is that ammonia transport is not just an add-on for more complete acid/base bookkeeping, but appears to be integral to any realistic representation of Na\(^+\) transport. This refers to the substantial rate of

![Diagram of transporters in the AHL cell](http://ajprenal.physiology.org/)
NH$_4^+$ cotransport with Na$^+$ on NKCC2 and in exchange for Na$^+$ on NHE3. It also refers to the effect of ambient NH$_4^+$ to acidify the cell, and the powerful modulation of NHE3 activity by cytosolic acidosis. In the presentation of model calculations, it has been necessary to display a relatively comprehensive tableau of transporters and cytosolic conditions, to make sense of overall epithelial transport.

Perhaps the most important feature of the AHL model is the series configuration of NKCC2 and KCC4, and one natural question is to what extent the kinetics of the two transporters coordinate transport and accommodate changes in throughput, whether triggered from luminal or peritubular conditions. This was most easily examined in systems with low ammonia, suggestive of in vitro perfusion studies, but it must be acknowledged that an obvious difference between the model and AHL perfusions in vitro are the magnitudes of the fluxes. Even in the absence of ammonia, model Na$^+$ fluxes are about twofold higher than reported values, and with ammonia they increase.
by a third (Figs. 2 and 7). It is not clear how to rationalize these differences, but the Na⁺ fluxes of the model are in the range of what will be necessary to simulate AHL transport in vivo. In simulations using B- and F-isoforms of NKCC, the differences in epithelial performance were not striking, and most of the simulations were carried out using the F-isoform. With either isoform, the sensitivity of overall epithelial Na⁺ transport to luminal Na⁺ concentration was apparent at luminal concentrations <130 mM (Fig. 3). The limiting concentrations of luminal Na⁺ (for either F- or B-isoforms) were in the range of 30–35 mM (Fig. 3), and the limiting concentration for luminal K⁺ was ~0.7 mM (Fig. 4). These are limiting values, and necessarily lower than concentrations observed with micropuncture of early distal tubule of hydropenic rats (e.g., 42 and 1.5 mM for Na⁺ and K⁺) (30).

The central experiment considered in this work is the impact of peritubular K⁺ concentration on AHL function. Stokes (42) undertook such experiments using rabbit medullary AHL in vitro (in the absence of ammonia), and examined increases in either peritubular or luminal K⁺ from 5 to 25 mM. The variations in K⁺ concentration were done as K⁺-for-Na⁺ substitution, and measurements were made of fluxes of Na⁺, K⁺, and Cl⁻, along with transepithelial voltage. Stokes’ data and results of model simulations are displayed in Table 3. With peritubular K⁺ replacement, the most dramatic flux change is that for Cl⁻, nearly 50% in the model, and close to 100% in the experiment.

For both model and experiment, the reabsorptive K⁺ flux becomes secretory, and the decrease in reabsorptive Na⁺ flux is less than the decrease in Cl⁻ flux, consistent with a formation of NaCl reabsorption to Na⁺-for-K⁺ exchange. In the model, transepithelial Na⁺ flux actually increases, due to reabsorptive tight-junctional Na⁺ flux, produced by an increase in the positive lumen PD (as in Fig. 5B), plus a favorable Na⁺ diffusion gradient from lumen to blood; in the experiments, the PD change was small and in the opposite direction. When the model epithelium is short-circuited, eliminating the diffusion potential, peritubular K⁺ does blunt AHL Na⁺ reabsorption. Nevertheless, even in the short-circuited model epithelium, the transepithelial Na⁺ gradient (lumen-to-blood) still produces substantial paracellular Na⁺ reabsorption (Table 3). Of note in the AHL model, the decrease in reabsorptive Cl⁻ flux increases to 75% when the peritubular K⁺ concentration is increased to 50 mM. With luminal K⁺ substitution, the most dramatic changes in both experiment and model are the decrease in reabsorptive Na⁺ flux. In the model, this is due to depolarization of the tubule lumen along with the Na⁺ concentration gradient favoring secretion; in the experiment, the change in epithelial PD again appears inadequate to rationalize the Na⁺ flux. In both model and experiment, the increase in luminal K⁺ produces substantial K⁺ reabsorption, and relatively little change in Cl⁻ flux.
Table 3. Effect of peritubular and luminal K⁺ on medullary AHL PD and solute fluxes: comparison with data from rabbit tubules (Stokes, 1982)

<table>
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<th>Lumen</th>
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</tr>
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<tbody>
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<td>K⁺</td>
<td>Cl⁻</td>
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<th>Lumen</th>
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For the model AHL, the calculations of Figs. 5 and 8 provide mechanistic rationalization of the overall epithelial impact of peritubular K⁺ addition. When ambient ammonia was low, increasing KCl from 5 to 35 mM produced an 82% reduction in KCC transport and a 43% reduction in transcellular luminal Na⁺ entry; with this, there was a 30% increase in cell volume (Fig. 5, A–C). When ambient ammonia was higher, this same increase in KCl reduced ammonia to AHL acidifies the cell (52); there is proton secretion by NKCC, compared with KCC, may reflect the fact that KCC is balanced by NH₃ exit, and this leaves a substantial requirement for proton extrusion, namely 97 pmol·mm⁻¹·min⁻¹ (20-μm tubule diameter). This rate of NH₃ uptake can be compared with the steady-state entry of NH₄⁺ of 151 pmol·mm⁻¹·min⁻¹ via NKCC in the model epithelium with 10 mM luminal ammonia and 0.2 mM peritubular ammonia (Fig. 6B). Furthermore, the model prediction of steady-state cytosolic acidification with addition of ambient ammonia (pH = 7.29 to pH = 6.89, Figs. 2 and 7) is comparable to the observation of Good and Watts (23), pH = 7.10 to 6.50 in going from 0 to 4 mM ammonia. In the model, much of the NH₃ entry is balanced by NH₄⁺ exit, and this leaves a substantial requirement for proton extrusion, namely 97 pmol·mm⁻¹·min⁻¹, split between luminal (64 pmol·mm⁻¹·min⁻¹) and peritubular (33 pmol·mm⁻¹·min⁻¹) Na⁺/H⁺ exchangers (Fig. 7). Luminal NHE3 is an important defender of AHL pH, and inhibition of NHE3 renders the cell acidic (23). NHE3, at least as it functions in proximal tubule is activated by cytosolic acidification, and this activation is progressively amplified down through pH = 6.0 (3). NHE3 activation by cellular protons has also been documented for AHL, but the shape of the curve is different, showing a leveling off of proton extrusion in the range of cell pH of 7.0–6.5 (51). Nevertheless, the absolute magnitude of NHE3 proton flux is susceptible to other influences, and approximately doubled from 68 to 144 pmol·mm⁻¹·min⁻¹ in response to medium hypocotylicity of
50 mosmol/kgH$_2$O (53). This latter flux is of the magnitude that the model suggests is required to defend against the acid challenge of physiological levels of medullary ammonia. What is difficult to understand is why such proton extrusion rates are never seen in physiological reabsorption experiments, for which net HCO$_3^-$ flux in vitro is typically 10 pmol-mm$^{-2}$-min$^{-1}$.

Ammonia reabsorption is also low in the isolated perfused AHL, and uninfluenced by amiloride (23). This observation is surprising, especially in view of the brisk NH$_4^+$ backflux on NHE3. In the model, this is a consequence of substantial NHE3 sodium flux, appreciable cytosolic NH$_4^+$ concentration, and the assumption of competitive binding of NH$_4^+$ to the NHE3 proton binding site (with affinity similar to that in proximal tubule vesicles). In the model with ammonia, Na$^+/NH_4^+$ exchange is predicted to be about 90% of the NH$_4^+$ uptake via NKCC2 (Fig. 7). By itself, this would provide an important catalytic role for NH$_4^+$ in ABL Na$^+$ reabsorption. Yet it would appear that such a mechanism would predict that NHE3 inhibition with amiloride could break this cycle, but that did not happen (23). Alternatively, amiloride does acidify the ABL cell (23), and might well increase cytosolic NH$_4^+$, so that there could be a secondary impact of amiloride on NH$_4^+$ entry via NKCC2. A second rationalization of low net NH$_4^+$ reabsorption is the presence of substantial rates of NH$_3$ backflux across the luminal membrane, and in the model shown in Fig. 7, this is ~95% of net luminal NH$_3^+$ uptake. In order that NH$_3$ backflux provide significant amounts of luminal NH$_3^+$ to drive reabsorptive Na$^+$ flux across NKCC2, there still need to be equimolar amounts of NHE3 proton secretion for luminal NH$_3$ titration.

Ultimately, the configuration of the ABL transporters needs to yield an epithelial cell that can reabsorb three quarters of delivered Na$^+$ in association with a cation, K$^+$ or NH$_4^+$, whose concentration is at most a few percent of the Na$^+$ concentration. In the case of K$^+$, this is achieved with a luminal cell membrane that is highly K$^+$ permeable plus an interstitium-to-lumen K$^+$ concentration gradient favorable to secretion. In the case of NH$_4^+$, there is certainly the capability to engender reabsorptive Na$^+$ flux on NKCC2, and given the luminal concentration of NH$_4^+$, it is likely that this flux is substantial. For this mechanism to be significant, it remains to identify the transporters responsible for luminal ammonium recycling. Although data are not available to support substantial Na$^+/NH_4^+$ flux on NHE3, diffusive NH$_3$ exit in parallel with proton secretion would be equivalent. This still posits a key role for luminal NHE3, with what would appear to be luminal proton secretion well in excess of measured HCO$_3^-$ reabsorption. For either K$^+$ or NH$_4^+$, balancing luminal membrane fluxes through uptake and secretory pathways can best be assessed in a model of the ABL as a tubule, in which he luminal concentrations of K$^+$ and NH$_4^+$ adjust to their respective equilibrium levels.

GRANTS

This investigation was supported by Public Health Service Grants R01-DK-29857 from the National Institute of Arthritis, Diabetes, and Digestive and Kidney Disease.

DISCLOSURES

No conflicts of interest are declared by the author.

REFERENCES

Intracellular distribution of carbonic anhydrase in the rat kidney.


