Local pH domains regulate NHE3 mediated Na\(^+\) reabsorption in the renal proximal tubule

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Abstract

The proximal tubule Na\(^+\)/H\(^+\) exchanger 3 (NHE3), located in the apical dense microvilli (brush border), plays a major role in the reabsorption of NaCl and water in the renal proximal tubule. In response to a rise in blood pressure NHE3 redistributes in the plane of the plasma membrane to the base of the brush border where NHE3 activity is reduced. This NHE3 redistribution is assumed to provoke pressure natriuresis, however, it is unclear how NHE3 redistribution per se reduces NHE3 activity. To investigate if the distribution of NHE3 in the brush border can change reabsorption rate, we constructed a spatiotemporal mathematical model of NHE3 mediated Na\(^+\) reabsorption across a proximal tubule cell and compared the model results with \textit{in vivo} experiments in rats. The model predicts that when NHE3 is localized exclusively at the base of the brush border it creates local pH microdomains that reduce NHE3 activity by >30%. We tested the model’s prediction experimentally: rat kidney cortex was loaded with the pH sensitive fluorescent dye BCECF and cells of the proximal tubule were imaged \textit{in vivo} using confocal fluorescence microscopy before and after increasing blood pressure by \textasciitilde 50 mmHg. The experimental results support the model by demonstrating that raising blood pressure induces the development of pH microdomains near the bottom of the brush border. These local changes in pH reduce NHE3 activity which may explain the pressure natriuresis response to NHE3 redistribution.
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Introduction

Renal sodium excretion and arterial blood pressure control are tightly connected. The arterial pressure provides the driving force for the glomerular ultrafiltration of plasma that takes place in the kidney. Most of the ultrafiltrate is reabsorbed as the fluid passes along the renal tubules, with the major part of the reabsorption taking place in the proximal tubule, and only a small fraction (~1%) reaches the end of the collecting duct where it emerges as the final urine. The balance between glomerular filtration and tubular reabsorption is controlled by a variety of neuroendocrine mechanisms and is central to the kidney’s ability to maintain sodium and water homeostasis. An acute increase in blood pressure of less than 50 mmHg triggers an increase in renal salt and water excretion, a response known as pressure natriuresis (9; 24; 35). Since the filtration rate stays constant due to the process of renal autoregulation (2), increased excretion must be due to decreased tubular reabsorption of salt and water (9; 10; 35). By acutely increasing the excretion of salt and water, pressure natriuresis causes a decrease in the extracellular fluid volume, which contributes to restoration of arterial blood pressure (9; 10; 35). Pressure natriuresis is currently accepted to be of central importance for the long term control of the arterial blood pressure (19).

Pressure natriuresis is partly due to reduced reabsorption of NaCl and water in the proximal tubule (9; 10; 35), but many questions remain as to the exact molecular mechanisms that underlie the reduction in water and salt reabsorption. Specific molecular transporters in the membranes of the cells lining the proximal tubule actively reabsorb Na⁺, Cl⁻, and HCO₃⁻ across the tubular epithelium. The reabsorption of the solutes establishes a small but effective osmotic gradient across the epithelium driving secondary reabsorption of water (43). The proximal tubule has a very high transport capacity, facilitated by a characteristic apical brush-border. The brush border is a compact
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microvillar structure that increases the surface area of the cells, thereby contributing to their large reabsorptive capacity. One of the major membrane proteins that transports Na\(^+\) across the apical membrane in the proximal tubule is the Na\(^+\)/H\(^+\) exchanger isoform 3 (NHE3), which exchanges one luminal Na\(^+\) for one cytosolic H\(^+\). Under normal resting conditions, NHE3 is uniformly distributed in the brush border membrane, but during pressure natriuresis, NHE3 is translocated to the base of the brush border. This translocation is believed to be central for the reduced tubular salt and water reabsorption (42; 55) and is associated with decreased proximal tubule Na\(^+\) reabsorption (57). However, NHE3 transport activity measured in brush border membrane vesicles isolated after an acute increase in blood pressure was unchanged (42; 55). Immuno-electron microscopy and biochemical studies provide convincing evidence that NHE3 is not internalized during acute hypertension \textit{in vivo}. Rather, NHE3 remains at the base of the microvilli, in lipid raft domains of the plasma membrane that do not enter the intermicrovillar cleft region (41; 54). Hence, it is unclear if and how translocation of NHE3 to the base of the brush border is associated with the reduced tubular salt and water reabsorption.

Proximal tubule reabsorption has been modeled exhaustively, and the models include detailed kinetic expressions for NHE3 activity (51; 52). One of the central parameters regulating NHE3 activity is intra- and extracellular pH, as H\(^+\) is a substrate for the transporter and NHE3 activity is pH sensitive (51). In the lumen of the proximal tubule, the main pH buffer is the bicarbonate system. In the cytosol of the proximal tubule cells, the bicarbonate system is also an important pH buffer together with various other buffers, especially intracellular proteins (15; 21). Models of the proximal tubule (51; 52) have focused on the uptake of different ions, and the brush border has been considered with respect to mechanical stress and flow near and in the brush border. An important conclusion from model simulations is that there is almost no flow in and near the brush border,
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which has also been demonstrated experimentally (4; 18). However, the effect of redistributing NHE3 within the brush border on Na⁺ reabsorption has not yet been addressed.

The main hypothesis of the present study is that local pH micro-domains that develop following redistribution of NHE3 during acute hypertension can explain, at least in part, the reduction in tubular salt and water reabsorption during pressure natriuresis. To test this, we have made a spatiotemporal model of NHE3 mediated Na⁺ reabsorption in the proximal tubule that includes the brush border microvilli, and used this model to simulate the effects of redistribution of NHE3 within the brush border. The model has been constructed to address this specific problem. Consequently, aspects of tubular reabsorption that are not relevant for assessing the effect of NHE3 redistribution have not been included. Specifically, we have not included mechanisms that have not been described in the context of pressure natriuresis. The model predicts that when NHE3 is redistributed to the lower part of the brush border it creates local changes in pH, both in the lumen and in the cytosol, that reduce NHE3 activity. To test the model experimentally, we determined the changes in cytosolic pH in proximal tubule cells in rats in vivo before and after an acute increase in blood pressure. The results support the findings of the model, namely, that an acute increase in blood pressure gives rise to the development of a pH microdomain at the base of the brush border.

Materials and methods

The model

The proximal tubule (PT) cell is polarized with a basolateral membrane facing the interstitial side, and an apical microvillar membrane facing the tubular lumen. The present model focuses specifically on the NHE3 mediated transepithelial Na⁺ transport in the early part (S1/S2) of the proximal tubule, and on the possible effects on this transport of the redistribution of NHE3 within the membrane of the microvillus. For simplicity we assumed that the microvilli are uniform in size
Local pH domains in the renal proximal tubule and distribution. To further simplify the model we considered only a single microvillus together with the underlying part of the cell. This is, obviously, a crude approximation, but it is useful for this particular problem we address herein. Consequently, the domain of the model consists of a cylinder (see Fig. 1), where the top part represents the microvillus together with the surrounding tubular fluid, and the lower part represents the cell cytoplasm beneath the microvillus, bounded at the bottom by the basolateral cell membrane. It is further assumed that the cytosol below the microvilli in the cell body is homogeneous throughout the cell. We have neglected the influence of the cell edges, cell nucleus and other non-uniformly distributed organelles.

Because of the axiosymmetry of the cylinder, the 3D problem can be reduced to a 2D problem. The axiosymmetry implies that the full 3D model can be obtained by rotating the corresponding 2D model about its z-axis. Here the z-axis is orientated in the centre of a microvillus, and the model is a cross section of one microvillus and the cell body below. The limitation of the approach is that it is impossible to model the whole cell. However, it is an efficient method to model objects that have axial symmetry, because the computations are done in a 2D geometry instead of in a 3D geometry. That is computationally more efficient.

The NHE3 is a non-electrogenic transporter. As one Na\(^+\) is exchanged for one H\(^+\), the net charge transfer is zero. Accordingly, transepithelial and membrane potentials in the PT are insensitive to amiloride (16; 27). We are unaware of studies showing that pressure natriuresis is accompanied by changes in transepithelial or membrane potentials in the PT. We have, therefore, assumed that the plasma membrane potential and the transepithelial potential are constant. Finally, we assume that the concentrations of ions and other constituents in the extracellular compartment at the basolateral side and in the bulk lumen (>0.5 \(\mu\)m from the tip of the microvilli) remain constant.
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The structure of the cylinder is illustrated in Fig. 1. The cytosol compartment is colored blue (Fig. 1c) and the lumen is red (Fig. 1c). The model includes the tubular lumen between the microvilli and a thin layer 0.5 μm of tubular fluid off the tip of the microvilli. At the upper edge, we have assumed constant concentrations of the various ions, which reflects physiological values in the early part of the tubular lumen. At the bottom the cylinder is bounded by the basolateral membrane, and again we have assumed constant concentrations of ions in the extracellular fluid facing this edge. The model includes transport and diffusion of the relevant ions and molecules across both the apical and the basolateral membranes, and within the cytosolic compartment. In the apical membrane we have implemented a model of NHE3 and diffusion of CO₂ and NH₃ over the membrane. In the basolateral membrane we have included a model of the Na⁺/K⁺ pump and the Na⁺-HCO₃⁻ co-transporter. The model was implemented in COMSOL 4.1 (COMSOL A/B (1)) and solved using the finite element method.

Transport of molecules and ions were modeled in the cytosol and/or the lumen. Since fluid flow near the brush border is very low (about 0.25% of the average tubular flow), and further reduced inside the brush border itself (4; 18), convective transport can be ignored. Consequently, diffusion is assumed to be the only mechanism for transport of ions and molecules within the luminal and cytosolic compartments. Ions can only move between the lumen and the cytosol by the defined transmembrane fluxes (see below). Finally, a given ion or molecule may be added to or removed from the luminal or cytosolic compartment by a chemical reaction, e.g. by being bound to a buffer.

Diffusion and chemical reactions were modeled using the diffusion-reaction equation,

\[ \frac{\partial c}{\partial t} = \nabla \cdot (D \nabla c) - R \]
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where \( c \) is the concentration of the diffusible ions or molecules, \( D_c \) is a diagonal matrix where the elements in the diagonal are the diffusion coefficients for \( c \) in the \( x \) and \( z \) directions, and \( R \) expresses chemical reactions including buffer reactions. Below, when presenting the individual reactions, “\( D \)” will refer to the diffusion coefficient of the ion or molecule in that equation.

Geometry of the model

The microvilli in the S1 to S2 segments of the proximal tubule are ~3 \( \mu \)m long, have a radius of ~45 nm at the base, and are localized on the apical side of the cell, ~7 \( \mu \)m away from the basolateral membrane (12; 33; 53; 54). We have implemented those geometries in the model (Fig. 1).

In the model, the microvillus has a radius of 45 nm at the base, while the total model (microvillus plus surrounding fluid) has a radius of 82 nm. That corresponds to a distance of 74 nm between neighboring microvilli. Depending on the tubular flow, the distance between neighboring microvilli in the S2 segment has been reported to be between 62 and 90 nm (33). The cytosol volume is 2.6 times the volume of the lumen in this model. The microvillus curves, so it ends in a rounded point.

In this model, the microvilli increases the apical surface area with a factor of 26, and the microvilli density is 37 per \( \mu \)m\(^2\), which is in close agreement with previous models of the proximal tubule brush border (25).

The mesh, which is used by the finite element method, was generated using triangles together with a boundary layer at the surface of the brush border. The size of the mesh was maximum 5 nm, minimum 2 pm and maximum element growth rate was 1.1.
Fluxes across membranes

The purpose of the model is to examine the effects of NHE3 redistribution in the microvilli on NHE3 mediated tubular Na\(^+\) reabsorption. We have, therefore, focused on the transport mechanisms that are relevant in this setting. The model includes fluxes of Na\(^+\), CO\(_2\), NH\(_3\), NH\(_4\)\(^+\) and H\(^+\) across the apical membrane. All fluxes are listed in Table 1, the rates in Table 2, and the parameters are given in Table 3. At the apical side, NHE3 transports Na\(^+\) into the cell in exchange for H\(^+\), and, to a minor degree, in exchange for NH\(_4\)\(^+\). The kinetics of NHE3, including parametric values, are based on previous published models by Weinstein et al (51; 52). CO\(_2\) is assumed to diffuse freely across plasma membranes as in other models of Na\(^+\) reabsorption in the proximal tubule (52). NH\(_3\) is also allowed to diffuse freely across the apical cell membrane (52). Because we focus the model on the effect of NHE3 redistribution, we have not included K\(^+\) and Cl\(^-\) in the model. If Na\(^+\) forms large concentration gradients inside the same compartment, it would be necessary to model the other major ions. We checked the assumption of equal Na\(^+\) distribution, and indeed the model predicted that variations in Na\(^+\) would be around one in one thousands.

At the basolateral side, Na\(^+\) is transported out of the cell by either the Na\(^+\)/K\(^+\) pump or the Na\(^+\)-HCO\(_3^-\) co-transporter. The Na\(^+\)/K\(^+\) pump model is from Weinstein et al (52), and implemented assuming a constant intracellular concentration of K\(^+\) and constant extracellular concentrations of Na\(^+\) and K\(^+\). The model of the Na\(^+\)-HCO\(_3^-\) co-transporter is adapted from Gross et al (17), and it does not take external Na\(^+\) and HCO\(_3^-\) into account. We have adjusted the maximum fluxes of the basolateral Na\(^+\)-HCO\(_3^-\) co-transporter and Na\(^+\)/K\(^+\) pump to set the initial cytosolic Na\(^+\) and HCO\(_3^-\) concentrations at physiological levels. We have assumed that external Na\(^+\), K\(^+\) and HCO\(_3^-\) concentrations are constant at the basolateral side. We also tested an alternative model of the Na\(^+\)-HCO\(_3^-\) co-transporter in which we assumed linear kinetics, and the model gave the same results.
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Reactions

The reactions between the protons and the buffer systems were implemented explicitly. Thus, it is possible to change the diffusion coefficient of each solute directly. All the details of the reactions are provided in Table 4. Below, the subscripts indicate if the reaction takes place in the cytosol (cyt) or lumen (lum).

Cytosolic pH is buffered by various molecules (B), and we have lumped all the reactions into a general buffer reaction:

\[ H_{\text{cyt}}^+ + B_{\text{cyt}} \xrightarrow{k_1} B_{\text{cyt}}^+ \]

which we have modeled with the rate equation:

\[ R_{\text{buffer}} = k_{\text{buffer1}} [H_{\text{cyt}}^+] [B_{\text{cyt}}] - k_{\text{buffer2}} [B_{\text{cyt}}^+] \]

The description of the diffusion and reactions of the cytosolic buffer becomes:

\[
\frac{\partial [B_{\text{cyt}}]}{\partial t} = \nabla \cdot (D V [B_{\text{cyt}}]) - (k_{\text{buffer1}} [H_{\text{cyt}}^+] [B_{\text{cyt}}] - k_{\text{buffer2}} [B_{\text{cyt}}^+])
\]

\[
\frac{\partial [B_{\text{cyt}}^+]}{\partial t} = \nabla \cdot (D V [B_{\text{cyt}}^+]) + (k_{\text{buffer1}} [H_{\text{cyt}}^+] [B_{\text{cyt}}] - k_{\text{buffer2}} [B_{\text{cyt}}^+])
\]

Above “D” is the diffusion coefficient for the endogenous pH buffer in the cytosol.

We also included the buffer reaction between NH\(_3\) and NH\(_4^+\) (44; 45; 52), with the following rate equation.

\[ R_{\text{Amm}} = k_{\text{Amm1}} [H^+] [NH_3] - k_{\text{Amm2}} [NH_4^+] \]

The corresponding equations for reaction and diffusion in the cytosol become:
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\[
\frac{\partial \left[ NH_3_{cyt} \right]}{\partial t} = \nabla \cdot (D \nabla \left[ NH_3_{cyt} \right]) - \left( k_{kAmm1} \left[ H^+ \right] \left[ NH_3_{cyt} \right] - k_{kAmm2} \left[ NH^+_4_{cyt} \right] \right)
\]

\[
\frac{\partial \left[ NH^+_4_{cyt} \right]}{\partial t} = \nabla \cdot (D \nabla \left[ NH^+_4_{cyt} \right]) + \left( k_{kAmm1} \left[ H^+ \right] \left[ NH_3_{cyt} \right] - k_{kAmm2} \left[ NH^+_4_{cyt} \right] \right)
\]

With the equations for the lumen:

\[
\frac{\partial \left[ NH_3_{lum} \right]}{\partial t} = \nabla \cdot (D \nabla \left[ NH_3_{lum} \right]) - \left( k_{kAmm1} \left[ H^+ \right] \left[ NH_3_{lum} \right] - k_{kAmm2} \left[ NH^+_4_{lum} \right] \right)
\]

\[
\frac{\partial \left[ NH^+_4_{lum} \right]}{\partial t} = \nabla \cdot (D \nabla \left[ NH^+_4_{lum} \right]) + \left( k_{kAmm1} \left[ H^+ \right] \left[ NH_3_{lum} \right] - k_{kAmm2} \left[ NH^+_4_{lum} \right] \right)
\]

In both the lumen and the cytosol, we have explicitly modeled the bicarbonate buffer system.

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

where the first reaction is catalyzed by carbonic anhydrase (CA). Because of the efficient catalysis by CA, and high rate of the second reaction it is, under most conditions, sufficient to consider only the net reaction (6; 49):

\[ CO_2 + H_2O \xrightarrow{k_{CA1}} \xrightarrow{k_{CA2}} H^+ + HCO_3^- \]

We have assumed that the activity of water is constant, and then the corresponding rate equation becomes:

\[
R_{Bicar} = k_{kCat} \left[ H^+ \right] \left[ HCO_3^- \right] - k_{kCat1} \left[ CO_2 \right]
\]

The corresponding equations for reaction and diffusion in the cytosol become:
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\[
\frac{\partial [\text{HCO}_3\text{, cyt}]}{\partial t} = \nabla \cdot (D \nabla [\text{HCO}_3\text{, cyt}]) - (k_{\text{Ca}2} [H^+\text{, cyt}][\text{HCO}_3\text{, cyt}]) - k_{\text{Ca}1} [\text{CO}_2\text{, cyt}]
\]

\[
\frac{\partial [\text{CO}_2\text{, cyt}]}{\partial t} = \nabla \cdot (D \nabla [\text{CO}_2\text{, cyt}]) + (k_{\text{Ca}2} [H^+\text{, cyt}][\text{HCO}_3\text{, cyt}]) - k_{\text{Ca}1} [\text{CO}_2\text{, cyt}]
\]

With the equations for the lumen:

\[
\frac{\partial [\text{HCO}_3\text{, lum}]}{\partial t} = \nabla \cdot (D \nabla [\text{HCO}_3\text{, lum}]) - (k_{\text{Ca}2} [H^+\text{, lum}][\text{HCO}_3\text{, lum}]) - k_{\text{Ca}1} [\text{CO}_2\text{, lum}]
\]

\[
\frac{\partial [\text{CO}_2\text{, lum}]}{\partial t} = \nabla \cdot (D \nabla [\text{CO}_2\text{, lum}]) + (k_{\text{Ca}2} [H^+\text{, lum}][\text{HCO}_3\text{, lum}]) - k_{\text{Ca}1} [\text{CO}_2\text{, lum}]
\]

Carbonic anhydrase isoform II (CAII) is present in the cytosol of the proximal tubule cells, whereas the membrane associated isoforms CAIV and CAXII are exposed to the lumen. In addition, CAXIV is also present in the lumen in rodents (41). CA is one of the enzymes with the highest turnover number, but the maximum turnover is difficult to estimate, especially in the lumen. We have assumed that CA is uniformly distributed both in the lumen and in the cytosol. In a non-catalyzed system \(k_{\text{CAT}} = 0.037 \text{ s}^{-1}\) (23), and in the kidney the rate is assumed to be 10,000 times higher due to CA (32). 95% of total CA in the proximal tubule is the cytosolic isoform CAII, and the remaining 5% is the membrane attached isoforms (41). CAII has a turnover number that is 30-230% higher than the isoforms exposed to the lumen, and the efficiency coefficient \((k_{\text{cat}}/K_m)\) is 200-300% higher for CAII (20; 39). Hence, the major part of CA is present in the cytosol, only a minor part (5%) is exposed to the lumen and, with respect to the absolute activity, that number is even smaller. Because of the uncertainty with regard to the size of the luminal CA activity, simulations were performed for a range of luminal CA activities relative to the cytosolic CA activity.

We have assumed that proton mobility can be described as simple diffusion, as is also the case with the other ions and molecules in the model. The diffusion coefficients are obtained from \textit{in vitro}...
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studies in cardiomyocytes (44; 45). The corresponding equations for reaction and diffusion of protons in the cytosol and lumen become:

\[
\frac{\partial [H_{\text{cyt}}^+]}{\partial t} = \nabla \cdot (D \nabla [H_{\text{cyt}}^+]) - \left( k_{\text{Ca}^2} [H_{\text{cyt}}^+] [HCO_3_{\text{cyt}}] - k_{\text{Ca}^1} [CO_2_{\text{cyt}}] + \right)
\]

\[
+ \left( k_{\text{Ammonium}} [H_{\text{cyt}}^+] [NH_3_{\text{cyt}}] - k_{\text{Ammonium}} [NH_4^+] + \right)
\]

\[
- k_{\text{Buffer}} [H_{\text{cyt}}^+] [B_{\text{cyt}}] - k_{\text{Buffer}} [B_{\text{cyt}}^+] \]

\[
\frac{\partial [H_{\text{lum}}^+]}{\partial t} = \nabla \cdot (D \nabla [H_{\text{cyt}}^+]) - \left( k_{\text{Ca}^2} [H_{\text{lum}}^+] [HCO_3_{\text{lum}}] - k_{\text{Ca}^1} [CO_2_{\text{lum}}] + \right)
\]

\[
+ \left( k_{\text{Ammonium}} [H_{\text{cyt}}^+] [NH_3_{\text{lum}}] - k_{\text{Ammonium}} [NH_4^+] + \right)
\]

\[
- k_{\text{Buffer}} [H_{\text{cyt}}^+] [B_{\text{cyt}}] - k_{\text{Buffer}} [B_{\text{cyt}}^+] \]

The diffusion equation for Na\(^+\) is

\[
\frac{\partial [Na_{\text{cyt}}^+]}{\partial t} = \nabla \cdot (D \nabla [Na_{\text{cyt}}^+])
\]

\[
\frac{\partial [Na_{\text{lum}}^+]}{\partial t} = \nabla \cdot (D \nabla [Na_{\text{lum}}^+])
\]

**Boundary and initial conditions**

The initial conditions are given in Table 5. Initially all ions and molecules were distributed homogeneously in the cytosol and/or lumen. We assumed that 0.5 µm from the tip of the microvilli the luminal concentrations of CO\(_2\), NH\(_4^+\), H\(^+\) and Na\(^+\) are constant, and that the concentrations on the interstitial side of the basolateral membrane remain constant.

Axial symmetry is applied along the z-axis. On the other vertical boundary of the lumen and the cell cytosol, we have applied no flux conditions, that is, no transport across these boundaries. On the boundaries that correspond to the cell membrane, the boundary conditions are given by the fluxes across the membrane (Table 1).
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**Activation**

In the model, the microvillus is 3 µm long, and it is assumed that NHE3 is initially uniformly distributed in the membrane, except for the curved inner part of the wrinkle (Fig. 1B). To model redistribution of NHE3, the flux carried by NHE3 is compressed to a 40 nm band at the base (Fig. 1C) (55). During control conditions, the NHE3 mediated flux is distributed evenly across the plasma membrane of the microvillus (Fig 1B, green bar). To simulate pressure natriuresis where NHE3 is redistributed to the base of the microvillus, we rescaled the NHE3 activity (Table 3, $A_{surface}$), and localized it to a thin band near the base of the microvillus (Fig. 1C, green bar). Total maximum NHE3 activity is, therefore, the same before and after activation.

**Experimental description**

All experiments were performed on male Sprague-Dawley rats (200-250 g body wt) that were kept under diurnal light conditions and had free access to food and water. The rats were obtained from Charles River Breeding Lab (Wilmington, MA), and were kept under specific pathogen-free conditions. All experimental protocols were approved by the University of Southern California Animal Care and Use Committee. Rats were anesthetized with Inactin (Sigma, 100 mg/kg, intraperitoneally) and non-surviving surgery was carried out on a temperature controlled rat operating pad (Vestavia Scientific, Birmingham, AL). The trachea was cannulated to facilitate breathing, and a catheter was inserted into the carotid artery for continuous blood pressure measurements by a pressure transducer using a BP-1 Blood Pressure Monitor (World Precision Instruments Inc, Sarasota, FL). Albumin (1% BSA)-containing saline and EIPA (5-(N-Ethyl-N-isopropyl) amiloride, 1.5 mg/kg, Sigma) injections were administered via a catheter inserted into the right jugular vein. Blood pressure was increased acutely by constriction of the superior mesenteric and celiac arteries, and the abdominal aorta below the renal arteries with silk ligatures as previously
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described (55-57). The rat was temporarily removed from the microscope stage when the arteries were constricted.

In vivo imaging of PT was achieved using modifications of previously described methods (22; 40). The left kidney was located by palpation and was exteriorized via a left flank incision. The pH sensitive dye BCECF-AM (2’ ,7-Bis-(2-Carboxyethyl)-5-(and-6)-Carboxyfluorescein, Acetoxymethyl Ester) (50 µg, Invitrogen) was dissolved in 2 µl DMSO, diluted with 0.9% saline to 50 µl, and injected into the subcapsular space. The rat was transferred to the microscope stage and maintained at 37 °C with a homeothermic blanket. Intravital fluorescence microscopy was performed using a Leica TCS SP5 confocal imaging system (Leica Microsystems, Heidelberg, Germany) with a HCx PL HPO CS 63x (1.3 NA) glycerol immersion objective. The proximal tubule was identified by the apical brush border structure and the basolateral nucleus BCECF was excited with a 496 and 458 nm argon laser and emission detected at 500-600 nm. BCECF (496/458 nm excitation) ratio images were generated using the Leica LAS imaging software.

Results

NHE3 redistribution decreases Na⁺ reabsorption

Under resting conditions, NHE3 is distributed uniformly along the microvilli of the brush border, but during pressure natriuresis NHE3 redistributes to the base of the brush border (42; 55). In the model, we implemented this redistribution with a step function that changes from 1 to 0 within 10 s (Fig. 2A), where 1 indicates uniform NHE3 distribution and 0 that NHE3 is at the base. Following redistribution, the model predicted a decrease in average cytosolic Na⁺ concentration (Fig. 2B) and a transient decrease in average cytosolic pH (Fig. 2C). Note that the transient decrease in average cytosolic pH coincides with the drop in Na⁺ concentration. In addition, the model predicted a reduction (32%) in NHE3 mediated Na⁺ reabsorption, which is quantified as the Na⁺ flux across the
basolateral side (Fig. 2 D). In these simulations, luminal and cytosolic CA activity were identical. The effect in the model was completely reversible and normal Na$^+$ reabsorption was restored when NHE3 was redistributed to the entire brush border surface (not shown).

**Reduced Na,K-ATPase activity**

The model predicts that there is a drop in cytosolic Na$^+$ concentration following redistribution of NHE3 (Fig. 2). The activity of the Na,K-ATPase is also reduced during pressure natriuresis by 25-50% (58; 59). We have implemented that in the model to address the decrease in cytosolic Na$^+$ concentration. Fig. 3 shows simulations where we have gradually decreased the activity of the Na$^+$ pump at the basolateral side by up to 50%. Otherwise the parameters were as in Table 3. As expected lowering the activity of the Na,K-ATPase increased the cytosolic Na$^+$ concentration (Fig. 3A) and lowered the reabsorption, secondary to a reduced, inward directed Na$^+$ gradient (Fig. 3C). The reduced reabsorption led to a slight cytosolic acidification (Fig. 3B), which is due to a reduced NHE3 activity and increase in Na$^+$-HCO$_3^-$ co-transporter activity.

When the activity of the Na,K-ATPase is kept constant, the change in Na$^+$ absorption due to redistribution of NHE3 results in decreased cytosolic Na$^+$ concentration (Fig. 3A). If we assume that cytosolic Na$^+$ should remain unchanged during the pressure natriuresis, the Na, K-ATPase activity should be decreased to ~63.5% of the initial activity following the redistribution of NHE3 (Fig. 3A), which is within the experimentally observed range. This decrease in Na,K-ATPase activity offsets the decrease in cytosolic Na$^+$ due to the reduced uptake of Na$^+$ via NHE3. The combined decrease in Na/K-ATPase and NHE3 activity results in a decrease in Na$^+$ reabsorption of 37% in contrast to the 32% decrease when only NHE3 activity decreased (see above).
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Carbonic anhydrase activity

NHE3 is sensitive to both cytosolic and luminal pH (51), thus, the activity of the carbonic anhydrase (CA) is important, since it determines the apparent availability of the bicarbonate buffer system. However, there is uncertainty regarding the level of luminal CA activity. The activity of the luminal CA was initially modeled as equivalent to the activity in the cytosol despite the possibility that the activity in the lumen could be 10-100 fold lower. We have explored how the activity of luminal CA affects reabsorption (Fig. 4). Reducing the activity of luminal CA below that of the cytosolic activity decreased the average cytosolic Na⁺ concentration and the reabsorption of Na⁺, but cytosolic pH remained almost unaffected. The reduction in cytosolic Na⁺ concentration, which the model predicted at luminal CA levels that was 10 fold lower, could be adjusted by decreasing Na,K-ATPase activity by 38.6%.

Figure 5 shows model predictions of pH in cytosol and lumen prior to, and 5000 s following, the redistribution of NHE3. The model has reached a steady-state in both of these situations. Before perturbation, there was no pH gradient in the cytosol or in the lumen, while at 5000 s, when NHE3 activity was localized to the base of the microvilli, pH gradients appeared. It is interesting to note that the cytosolic pH domain is smaller compared to the luminal pH domain (Fig. 5). The cytosolic pH domain is localized in the vicinity of the NHE3 cluster. In contrast, the luminal pH domain extends over a larger area, occupying the lower part of brush border structure. Notice that there are gradients both along and between the villi. The pH domain in the intervillous space is larger, because the buffer capacity is lower in the lumen compared to the cytosol.

In the cytosol, pH initially decreased transiently on a global scale (Fig. 2C), while pH gradually increased near the clustered NHE3 and a higher pH level was established (Fig. 5). During the same
time, pH decreased locally in the lumen. Thus, the redistribution of NHE3 provokes the emergence of local, transmembrane acidification/alkalinization because NHE3 exchanges Na⁺ for H⁺. Because NHE3 is inhibited by increasing cytosolic pH (i.e. by decreasing cytosolic [H⁺]) (51), the model predicts that total activity of NHE3 will decrease following redistribution to the base of the brush border. However, the model did not predict Na⁺ domains of any significance. The model did not suggest that there would be local variations in the concentration of Na⁺ of more than 0.05 mM in the lumen and 0.07 mM in the cytosol. In contrast to the situation for protons, the concentration of Na⁺ is relatively high in both the lumen and in the cytosol, the mobility is high, and there are no Na⁺ buffering effects. Together, these three factors act against the development of Na⁺ micro-domains in this model.

Diffusion and concentration of mobile buffer

The model contains a mobile pH buffer that models the effect of proteins and other molecules that buffer cytosolic pH. The diffusion coefficient of the buffer is estimated (33x10⁻⁷ cm²/s) from studies and models of cardiomyocytes (44). Hence, we have modeled the impact of changing the diffusion coefficient of the buffer (mobile) from 0.33 to 330 x10⁻⁷ cm²/s. However, changes in diffusion coefficient over this large range changed reabsorption by less than 5%.

The concentration of buffer was approximated from studies in proximal tubule cell lines where the total buffer capacity is reported to be between 20 and 45 mM per pH unit (15; 21). In the model, the concentration of the endogenous buffer was set at 26 mM (pKa 7.57) and the bicarbonate concentration at 14 mM (pKa 6.35). We have explored the effect of changing the buffer capacity, i.e., the concentration of the intracellular H⁺ buffer. We have simulated the model for concentrations of endogenous buffer that are a factor 10 higher or lower than in previous
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Simulations. Prior to redistribution of NHE3, changes in the H\(^+\) buffer concentration did not affect the Na\(^+\) concentration, pH or the reabsorption rate. However, following redistribution of NHE3 the changes in the endogenous H\(^+\) buffer concentration had a modest impact on average cytosolic Na\(^+\) concentration and reabsorption, but only little effect on average cytosolic pH, except for the transient drop. If the Na,K-ATPase activity was modified so cytosolic Na\(^+\) concentration was constant, the model predicted that, when the pH buffer was reduced with a factor of 10, the NHE3 redistribution would reduce Na\(^+\) reabsorption by 50%. When the pH buffer concentration was increased with a factor of 10, the NHE3 mediated Na\(^+\) reabsorption was reduced with 25%. That is in contrast to the 37% reduction in NHE3 mediated Na\(^+\) reabsorption the model predicted for a “normal” buffer level. These effects can be ascribed to a higher pH in the cytoplasmic microdomain for the low buffer concentrations, and less dominant pH domains in the case of the the higher buffer concentration (results not shown).

Location of NHE3

Both biochemical and image analysis indicate that NHE3 is translocated to the very base of the microvilli (42; 55), which we have implemented in the model. The results indicate that the reduction of Na\(^+\) reabsorption is based on local changes in pH inside the cell (i.e. cytosol), as well as outside the cell (i.e. in the lumen), when NHE3 is concentrated in local areas. To address how the area of NHE3 redistribution correlates with the reduction in reabsorption, we have simulated several scenarios where the area of the NHE3 redistribution site is changed (Fig. 6). In Figure 6 the dashed lines correspond to simulations with an unchanged Na,K-ATPase activity, whereas the solid lines correspond to a decrease in Na,K-ATPase activity to 63.5% of initial activity. The reabsorption of Na\(^+\) and the cytosolic Na\(^+\) concentration remains unchanged as long as the area to which NHE3 is
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redistributed is above 20-30% of the initial area, and the changes are pronounced only when the
area is below 5-10% of the initial area. In the previous simulations the ratio of the area before and
after redistribution was 2%.

Mixing of the fluid in the luminal part of the brush border

Experimental and theoretical studies suggest that there is little or no flow in the brush border region
(4; 18). If there is a flow in the brush border, it would mix the luminal fluids better and hence
reduce the extracellular luminal pH domain. To test the possible consequences of the no-flow
assumption, we simulated the situation where the extracellular region of the brush border is well-
mixed, i.e. where no local extracellular gradients can appear. We did this by allowing NHE3 to
remain evenly distributed in the microvilli on the luminal side, while the cytosolic NHE3 were still
redistributed to the base of the microvilli. In this case, the model suggests that the NHE3 mediated
Na⁺ reabsorption will be reduced by 31%, which is slightly lower than 37% that is predicted by the
model when the lumen is acidified locally.

Experimental support

The model predicted that translocation of NHE3 to the base of the brush border induces local pH
domains. To test this prediction experimentally, we measured cytosolic pH changes in optical
sections of the proximal tubule in vivo in rats using confocal fluorescence microscopy both before
and after increasing blood pressure (by approximately 50 mmHg). Following BCECF loading, the
tubules were open indicating that there was flow through the tubule and that the kidney was not
damaged. Initially, the ratio of BCECF fluorescence was uniform throughout the cells, except for a
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...slightly higher intensity in the nucleus (Fig. 7 A and C). This is in accordance with previous investigations, which has also found that BCECF accumulates in the nucleus (50).

Following elevation of the blood pressure (Fig. 7E), we observed an isolated increase in BCECF fluorescence ratio near the base of the apical brush border indicating alkalization of the cytosol in that microdomain (Fig. 7B and D, see arrows), as predicted by the models. Specifically, the band of red/yellow in panel D is more intense than the band of yellow in panel C. The rat was temporarily removed from the microscope stage during constriction of the arteries, so the tubule in Fig. 7A is not the same as in Fig. 7B. The band persisted for at least 10 min. After 10 min the experiment was terminated and the rat removed from the microscope.

The increase in BCECF fluorescence ratio near the base of the brush border was observed in several different proximal tubule segments and cells as shown in the low power image (Fig. 8A). To test the hypothesis that the local alkalization near the brush border in proximal tubule cells (Fig. 8) was due to the redistribution of NHE3, we injected EIPA (1.5 mg/kg) intravenously following the increase in blood pressure. EIPA is an inhibitor of Na⁺/H⁺ exchangers including NHE3 (46). EIPA caused a moderate decrease in the BCECF fluorescence ratio throughout the cells corresponding to a reduction in cytosolic pH (Fig. 8). Specifically, the band of yellow in panel E is slightly less intense than the band of yellow in panel C. Importantly, EIPA administration diminished the local pH gradient near the base of the brush border in response to the elevated blood pressure (Fig. 8C, E, see arrows).

**Discussion**

In this study, we used a multidisciplinary approach to investigate the effect of NHE3 redistribution within the brush border of the S1/S2 segments of the proximal tubule and tested whether redistribution could contribute to the reduced Na⁺ reabsorption following an acute increase in blood...
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pressure. We constructed a spatiotemporal model of NHE3 mediated proximal tubular Na\(^+\) reabsorption, which predicted that redistribution of NHE3 to the base of the brush border leads to the development of local pH domains near the base of the brush border on both the cytosolic and luminal sides. The local changes in pH reduced NHE3 activity because NHE3 is pH sensitive, and because H\(^+\) is a substrate for NHE3. To validate the predictions of the model, we measured the changes in cytosolic pH in the proximal tubule in vivo. We found that an acute increase in blood pressure, which is known to induce redistribution of NHE3 to the base of the brush border, resulted in the development of a microdomain of high pH in the area.

The model was constructed to address the specific question of whether redistribution of NHE3 in the brush-border by itself can reduce Na\(^+\) reabsorption. The model is based on conservative assumptions regarding parameter values of which we have only limited prior knowledge. We have tested the effect of varying the values of some of the central parameters in the model. We found that for a conservative set of parameters the model can explain a decrease in NHE3 mediated Na\(^+\) reabsorption of ~30% following the onset of the pressure natriuresis.

The model was sensitive to luminal CA activity (Fig. 4). We did not include other diffusible buffer systems in the tubular lumen, e.g., phosphate. Phosphate does not play a significant role as buffer at the higher levels of CA activity, but may become quantitatively important at the lowest levels due to the less efficient buffering by bicarbonate. Very low levels of luminal CA activity are most likely not physiologically relevant, but the omission of the luminal phosphate buffer could lead to an overestimation of the effects of NHE3 redistribution on Na\(^+\) reabsorption at the lowest levels of luminal CA activity. In this connection, it should be noticed that it is not known whether part of the luminal CA is also redistributed during pressure natriuresis.
The model predicted that redistribution of NHE3 to the base of the brush border, by itself, would reduce proximal tubular NHE3 Na\(^+\) reabsorption by at least 32%. NHE3 is the dominant Na\(^+\) transporter in the apical membrane in the proximal tubule, and studies have suggested that it may be responsible for up to 50% of proximal tubular Na\(^+\) reabsorption (2; 52). In accordance with this, a proximal tubular specific NHE3 knock out mouse model showed a 27% reduction in volume reabsorption compared to the wild type (28). Previous experimental studies in rats have found reductions of proximal tubular total Na\(^+\) reabsorption of up to 40% during pressure natriuresis (9; 10; 57). The predictions of the model are close to the experimental observations. But the gap between the predictions of the model suggests that factors other than the simple redistribution of NHE3 also contribute to the reduction in proximal reabsorption.

A reduction in NHE3 activity will automatically reduce bicarbonate reabsorption. In the first part of the proximal tubule, the concentration of Cl\(^-\) increases due to the preferential reabsorption of bicarbonate together with Na\(^+\). This differential reabsorption of \(HCO_3^-\) over Cl\(^-\) creates an electrochemical transepithelial gradient that mediates reabsorption of Cl\(^-\) in the latter part of the proximal tubule. The reabsorption of Cl\(^-\) will drive additional Na\(^+\) reabsorption. Hence, reduced NHE3 activity in the early part of the proximal tubule reduces Na\(^+\) reabsorption in both the early part and the late part of the proximal tubule. Hence, the reduction in Na\(^+\) reabsorption of 32-36%, due to the redistribution of NHE3 in the brush border, could be amplified by such secondary consequences.

We have not included the transepithelial potential or membrane potential in the model and the literature does not, to the best of our knowledge, support that the transepithelial or membrane potential should change in the proximal tubule during pressure natriuresis. Reabsorption of sodium by NHE3 is not electrogenic, and does not contribute to the membrane potential. Experiments have shown that neither the membrane potential of the epithelial cells in proximal tubules from rabbits...
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(27), nor the transepithelial potential in the proximal tubule of the rat (16) are affected by amiloride. When the flow in PT is increased from 5 to 19 nl/min the rate of PT reabsorption increases about 100%, but the transepithelial potential remains constant (13; 14). The flow induced reabsorption is mediated by NHE3 and proton pumps (13; 14). Therefore, we find it unlikely that a reduction in NHE3 activity will give rise to a significant change in transepithelial potential or membrane potentials, which otherwise could have secondary effects on SGLT2 activity and paracellular transport.

Experimentally, we measured cytosolic pH in the proximal tubule before and after acute blood pressure elevations by intravital confocal microscopy after subcapsular injection of a pH sensitive dye. The pH sensitive dyes are, in principle, pH buffers and the additional buffer capacity has the potential to obscure the results. Initially, 50 μg BCECF-AM was dissolved in 50 μl, which corresponds to a concentration of 1.7 mM. Following the subcapsular injection of with the dye, we observed that most of the dye was localized to a small area with a radius of 2-3 mm. Measurements were only collected in areas with low to moderate loading. Assuming a modest intracellular accumulation of the dye, we estimate an intracellular dye concentration of 1-5 mM in the areas where measurements were made. The cytosolic pH buffer capacity is between 20 and 45 mM per pH unit (15; 21), so an additional 1-5 mM will, at the most, increase the buffer capacity by 25%. In myocytes, 0.4 mM of the pH sensitive dye SNARF-1 has only a marginal effect on cytosolic pH and is sufficient to measure pH and gradient formation (48). In the renal PT, an increase in pH buffer concentration of 0.4 mM would correspond to a change in buffer capacity of only 1%. We have tested the effect of increasing intracellular buffer capacity with the model and found that increasing the endogenous buffer concentration by a factor of 10 had only a marginal effect on changes in reabsorption rate following redistribution of NHE3 (not shown).
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Reabsorption of Na\(^+\) and pH regulation has been modeled extensively in the proximal tubule (see (25; 26; 51; 52)). Those models paid careful attention to correct stoichiometric reabsorption between all the different ions and other molecules involved in these complex processes. In the current study, we focused on the effect of changing the position of NHE3 in the brush border. The model used in the present study is not a general model of Na\(^+\) reabsorption in the proximal tubule, but a specific model of the NHE3 mediated Na\(^+\) reabsorption across a proximal tubule cell. Because the activity of NHE3 is pH dependent, we included the essential reactions that determine local pH. We simplified the model by only including bicarbonate, ammonium and an unspecified mobile cytosolic buffer. Additional buffers in the proximal tubule include phosphate and formate (2; 25; 52). Phosphate is reabsorbed together with Na\(^+\) at the luminal membrane by the sodium phosphate co-transporter, which is endocytosed during pressure natriuresis (35). In this model, we focused only on the effect of NHE3 redistribution, while keeping all other possible variables constant. Adding the endocytosis of the sodium phosphate co-transporter would have amplified the effect of pressure natriuresis on tubular reabsorption, but would have resulted in a more complex model. The sodium phosphate co-transporter accounts for less than 2% of proximal tubular Na\(^+\) reabsorption (52), nonetheless, a change in phosphate metabolism could also alter Na\(^+\) reabsorption indirectly through effects on the pH. Phosphate (H\(_2\)PO\(_4^-\)) has a pKa of 7.21 (29), so if the concentration is reduced, pH would increase (cytosolic pH is about 7.3) and inhibit NHE3 activity further. Formate is secreted by the Cl\(^-\)/(HCO\(_3^-\), formate) exchanger (2) and the cytosolic concentration of formate is low (<1 mM) compared to bicarbonate (about 25 mM), while their pKa values are similar (25; 26; 52). Hence, including tubular formate in the model would not change the conclusion of the present study.

The present model focuses on the effect of NHE3 redistribution on tubular Na\(^+\) reabsorption.

Consequently we have not included other transport mechanisms that mediate apical Na\(^+\) transport,
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primarily the Na\(^+\) coupled co-transporters. It could be argued that up regulation of the activity of other transporters like the sodium-glucose (SGLT1 and 2) and the sodium-amino acid co-transporters could attenuate the effect of NHE3 redistribution. Although we cannot exclude this possibility, it has to be considered that, because of the relatively small concentrations of glucose and amino acids in the filtrate, these transporters are only responsible for a minor fraction of proximal tubular Na\(^+\) reabsorption. Thus, mice deficient in SGLT2, the isoform located in the proximal tubule, have an unimpaired tubular reabsorption of Na\(^+\), K\(^+\) and Cl\(^-\) (47). Furthermore, an increased proximal tubular Na\(^+\) reabsorption by these co-transporters would require additional amounts of the relevant solutes. Since there is no change in GFR during pressure natriuresis it is difficult to see how this could occur. We therefore find it unlikely that compensatory increases in the activity of these co-transporters lead to a major attenuation of the effect of NHE3 redistribution on tubular Na\(^+\) reabsorption.

Based on experimental and theoretical studies we assumed that there is no flow in the luminal part of the brush border (4; 18). Flow in the brush border, will mix the contents of the luminal fluids, and, hence, reduce local gradients in the extracellular luminal pH domain. To test the effect of a more “well-mixed” fluid in the luminal brush border region, we assumed that NHE3 remains evenly distributed in the microvilli on the luminal side, while the NHE3 on the cytosolic side is still redistributed to the base. This is clearly an artificial situation, but allows us, in a simple way, to model a situation where there is no local pH gradients on the luminal side. With this assumption the decrease in NHE3 mediated reabsorption was only 31%, which is slightly lower than the 37% that is predicted by the model when the lumen is allowed to acidify locally. The slightly reduced effect is not surprising since NHE3 activity increases when luminal pH increases from 6 to 8 (51). The effect of the mixing prevents the development of local acidification in the tubular lumen, and prevents part of drop in NHE3 activity. It is, however, also evident that the major effect of the
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Redistribution of NHE3 is due not to the localized extracellular acidification, but rather to the intracellular alkalinization.

In addition to NHE3, the apical membrane of the proximal tubule also contains Cl⁻/HCO₃⁻ and Cl⁻/HCO₂⁻ exchangers. The spatial distribution of the Cl⁻/HCO₂⁻ and the Cl⁻/HCO₃⁻ exchangers in the microvilli during pressure natriuresis is, to the best of our knowledge, not known. Both of the transporters exchange one cytosolic HCO₂⁻ or HCO₃⁻ for one luminal chloride, which counteracts the pH effect of NHE3. Hence, if the two exchangers were to be redistributed together with NHE3 to the base of the brush border, this would reduce the effect of the redistribution of NHE3. But since the total flux of these two exchangers is about 25% of the total NHE3 flux, the pH effect due to NHE3 redistribution would still be present.

Previous models of Na⁺ reabsorption in the proximal tubuli have modeled the entire proximal tubule including the brush border and, for computational reasons, the microvilli were described as one-dimensional, i.e., infinitely thin. In contrast, in this study we have modeled the full geometry of a single microvillus. On the other hand, our model lacks some of the detail of the previous models by Weinstein et al. In the present study, we focused on the NHE3 mediated Na⁺ reabsorption, and on the local effects when NHE3 is redistributed within the microvillar membrane during pressure natriuresis. In previous models that included formate metabolism and CA activity in the proximal tubule, the authors did not report local pH changes in the brush border; nor did we observe pH changes in the unperturbed state with evenly distributed NHE3 (Fig. 5). Following NHE3 redistribution our model predicts the development of pH domains that extend both longitudinally and perpendicular to the microvillar membrane.

Clearly, such an effect is only detectable in a 2D model. For computational reasons it would not be feasible to model the entire proximal tubule in this fashion. Thus, different problems require different models and assumptions.
Numerical analysis of the alterations in BCECF fluorescence ratio was not performed because intravital calibration of BCECF fluorescence to pH values was not feasible. Nevertheless, the acute hypertension-induced development of local pH gradients at the base of the apical brush border was directly visualized (Fig. 7 and 8) and provided experimental proof-of-concept for our main hypothesis. The pH domain that we observed in vivo appears spatially larger than those predicted by the mathematical model. We speculate that at least two factors could contribute to this difference. First, the intact convoluted proximal tubule has a complex 3D structure. Optical sectioning may not always cut cells perfectly along their apico-basilar axis. Depending on the position and angle of the confocal optical section (plane) through the cell body, more or less of the apical microdomains will be present in the image. This may lead to the overestimation of the thickness of the pH microdomain. A second possibility for the discrepancy between the mathematical model and the in vivo imaging could be that we have overestimated the pH buffer capacity in the model, that is, when we reduce the amount of pH buffer in the mathematical model the pH domains becomes wider.

Microdomains have been recognized for a long time. Ca²⁺ microdomains are known to play an important role in regulation of cell signaling: Ca²⁺ microdomains near the mouth of Ca²⁺ channels (36; 38), in synapses (30; 31), submembrane Ca²⁺ microdomains between ER/SR and the plasma membrane (34), or in wrinkled membrane spaces (7; 8; 11; 37). The regulation of Ca²⁺ microdomains appears to be closely connected to the tight buffering of free cytosolic Ca²⁺ (38). Ca²⁺ microdomains are usually only observed in the vicinity of Ca²⁺ channels or in larger volumes when that volume is partly isolated from the rest of the cell e.g. the synapses (5). Ca²⁺ microdomains occur when a large influx of Ca²⁺ exceeds the diffusion capacity of Ca²⁺ together with an exhaustion of the Ca²⁺ buffer capacity. In the present study, we did not observe a complete exhaustion of the H⁺ buffer capacity, but we did observe a high localized influx. However, the model did not take into account the fact that there are numerous vesicles at the base of the brush
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border structure, and that NHE3 is actually translocated to the vicinity of these vesicles following redistribution (12; 55). Hence, translocation is predicted to create a restricted space in which pH could increase even more than suggested by the model, analogous to the Ca\(^{2+}\) microdomains that exist between SR/ER and the plasma membrane (34).

The concept of inhomogeneous cellular pH has been described previously. It is widely accepted that different organelles, e.g. mitochondria, have pH values that are different from cytosolic pH. In proximal tubule cells, where the mitochondria are located close to the basolateral membrane (3; 12), it has been reported that average cytosolic pH is more alkaline than the regions close to mitochondria (3), and the gradient has been attributed to the H\(^+\) pumping by the mitochondria (i.e. F1F0-ATPase). However, the same study also showed that the gradient between the apical and basolateral side was, in part, due to NHE3 activity at the apical side and Na\(^+\)-HCO\(_3^-\) co-transporter activity at the basolateral side (3). Interestingly, the authors speculated that cytosolic pH gradients could have regulatory effects (3).

In summary, we established a mathematical model of the effects of NHE3 redistribution in the renal proximal tubule. The model predicts the development of a pH microdomain near the base of the apical brush border membrane when NHE3 redistributes to the base of the microvill. The model also suggests that this pH microdomain has regulatory effects on proximal tubular Na\(^+\) reabsorption. In addition, experimental support for the model was obtained by visualizing changes in cytosolic pH in response to elevated blood pressure in cells of the proximal tubule in vivo using confocal fluorescence microscopy. This local pH microdomain may, via a reduction in NHE3 activity, play a role in pressure natriuresis, an important physiological mechanism for blood pressure control and maintenance of body fluid and electrolyte homeostasis.
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Grants

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Figure Legends

Figure 1. Mathematical model of Na\(^+\)/H\(^+\) exchanger isoform 3 (NHE3) redistribution in the proximal tubule (PT) brush border in response to an acute increase in blood pressure.

A) The model included basolateral Na,K-ATPases and Na\(^+\)-HCO\(_3^−\) co-transporters and the apical NHE3. Following an acute increase in blood pressure NHE3 is redistributed to the base of the microvilli. In the model the height of the microvilli is 3 µm and the height of the cell body is 7 µm.

B) We assumed that the brush border structure is homogeneous and only modeled one of the microvilli and the corresponding cell body below. The structure of the model included the cell body and the microvilli (in blue) and part of the lumen (in red). NHE3 was assumed initially to be distributed uniformly in the microvilli of the brush border excluding the lowest (50 nm) curved part of microvilli as marked with the green bar. C) Following redistribution NHE3 was located to the lowest (50 nm) part just above the curved part as marked with the green bar.

Figure 2. Simulation of redistribution of NHE3.

A) The redistribution of NHE3 in the brush border was modeled using a step function where its location was changed from being uniformly distributed to being located only at the base of the microvilli at time 5000 s. B, C) The redistribution of NHE3 in the model was associated with a decrease in the average cytosolic Na\(^+\) concentration and a transient drop in cytosolic pH. D) Na\(^+\)
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reabsorption decreased rapidly and remained constantly low even after pH had partly normalized.

Luminal CA activity was the same as cytosolic CA activity.

**Figure 3: A decrease in Na,K-ATPase activity decreases Na\(^+\) reabsorption both before and after NHE3 redistribution.**

The mathematical model was simulated for various values of the basolateral Na,K-ATPase activity. The activity of Na,K-ATPase was reduced relative to the value previously used (see Table 3): from: 1 (cyan), 0.8 (pink), 0.7 (blue), 0.65 (yellow), 0.635 (green), 0.6 (red), 0.5 (black). A) Reducing the Na,K-ATPase activity increased the cytosolic bulk concentration of Na\(^+\) and B) the simulations showed that decreasing the Na,K-ATPase level decreased average cytosolic pH. C) A reduced Na,K-ATPase activity would also reduce the Na\(^+\) reabsorption. Luminal CA activity was 10% of the cytosolic in all the simulations.

**Figure 4. Luminal carbonic anhydrase (CA) controls Na\(^+\) reabsorption.**

The activity of luminal CA activity was changed relative to cytosolic CA activity from: 1 (blue), 0.3 (yellow), 0.1 (green), 0.05 (red) and 0.01 (black) and the impact was tested on Na\(^+\) reabsorption before and after NHE3 redistribution. A) Decreasing luminal CA activity decreased cytosolic bulk Na\(^+\) concentration slightly, B) but cytosolic pH was hardly affected. C) The reabsorption of Na\(^+\) was also decreased when luminal CA activity was decreased, and the relative difference in reabsorption following redistribution of NHE3 was increased when luminal CA activity was decreased.
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**Figure 5: Local pH domains.**

The illustration shows the pH in the cytosol (left column) and in the lumen (right column) before NHE3 is redistributed to the base of the microvilli (top panels). Note that each panel has its own pH scale. During control conditions there are no pH gradients in the cytosol nor in the lumen. Modeling acute hypertension by redistributing NHE3 to the base of the microvilli (lower panels) shows that redistribution of NHE3 can give rise to the development of pH microdomains near the base of the microvilli. Luminal CA activity was the same as the cytosolic activity.

**Figure 6: Relative distribution of NHE3 in the brush border structure and the reabsorption rate.**

NHE3 was redistributed in the microvilli and A) the average cytosolic Na\(^+\) concentration and B) reabsorption following redistribution are shown. All parameters were as listed in Table 3. Luminal CA activity was 10% of the cytosolic in all the simulations. The position of NHE3 was changed so it was moved away from the tip and concentrated towards the base. The x-axis shows to which degree it was concentrated. A value of 1 indicates that the area NHE3 is distributed in before and after redistribution is the same, i.e. no change. A value of 0.2 indicates that the area of distribution of NHE3 after translocation is 20% of the initial area. The dashed curves show the situations where basolateral Na,K-ATPase has an unchanged activity and the solid curves show when the activity has been reduced to 63.5 % of the baseline value (Table 3).

**Figure 7: Acute hypertension in rats induces the development of local pH microdomains in the proximal tubule.**
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A) Intravital confocal fluorescence images show that prior to an increase in blood pressure the signal from BCECF was uniform in the PT cells. Pseudocolor images are shown based on the ratio of BCECF fluorescence intensities at 496/458 nm excitation reflecting regional differences in pH as indicated. The lumen of the proximal tubule is indicated. B) Following an increase in blood pressure the BCECF fluorescence ratio (local pH) increased near the base of the apical (brush border) membrane structure. Selected areas from A) and B) are magnified in C) and D), respectively, as indicated. D) The arrows point at the base of the brush border. Small sub-apical vesicles are visible near the brush border indicating that the increase in BCECF fluorescence ratio occurs near the base of the brush border. E) Blood pressure was recorded continuously throughout the experiment and the images in A-D were acquired at the indicated time points. Blood pressure was increased by ligating the lower aorta, mesenteric and celiac arteries as indicated by the black horizontal bar.

Figure 8: The pH microdomains are sensitive to NHE inhibition with EIPA.

The left kidney was loaded with BCECF and blood pressure was measured as described in Fig. 7. A) Low-power BCECF ratio image of a larger renal cortical area taken after blood pressure elevation. Four adjacent proximal tubules (indicated by PT in the lumen) are shown. The arrows point at the high BCECEF fluorescence microdomain at the base of the brush border in each PT. B) Following an increase in blood pressure we observed that BCECF fluorescence ratio (local pH) was increased near the base of the apical brush border of the PT (arrows). C) In the same PT tubule segment the administration of EIPA (1.5 mg/kg iv via the jugular vein) caused a reduction of pH gradients (arrows). The selected same area from B), before EIPA, and C), after EIPA are highlighted in D) and E), respectively, as indicated.
Table 1. Fluxes and transports.

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<th>Flux</th>
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<tr>
<td>$CO_{2,\text{lumen}} \leftrightarrow CO_{2,\text{c}_{\text{yt}}}$</td>
<td>$P_{CO_2} (CO_{2,\text{lumen}} - CO_{2,\text{c}_{\text{yt}}})$ (52)</td>
<td></td>
</tr>
<tr>
<td><strong>Basolateral</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Na^+<em>\text{c}</em>{\text{yt}} + 3HCO_{3,\text{c}<em>{\text{yt}}} \rightarrow Na^+</em>\text{inter} + 3HCO_{3,\text{inter}}$</td>
<td>$J_{NaHCO_3}$ (17)</td>
<td></td>
</tr>
<tr>
<td>$Na^+<em>\text{c}</em>{\text{yt}} \rightarrow Na^+_\text{inter}$</td>
<td>$J_{Na_{-ATPase}}$ (52)</td>
<td></td>
</tr>
</tbody>
</table>

See Table 2 for the full equations and Table 3 for the parameters.
Table 2 The equations for the active fluxes

**NHE3:**

\[
J_{Na} = \frac{1}{A_{surface}} x_T \left( P_{Na} P_H \alpha \beta + P_{Na} P_{NH4} \alpha \gamma \right) \mu_{Na} - P_{Na} P_H \alpha \beta \mu_H - P_{Na} P_{NH4} \alpha \gamma \mu_{NH4}
\]

\[
J_{H} = \frac{1}{A_{surface}} x_T RT \Sigma \left( -P_{Na} P_H \alpha \beta \mu_{Na} + \left( P_{Na} P_H \alpha \beta + P_{H} P_{NH4} \beta \gamma \right) \mu_H - P_{H} P_{NH4} \beta \gamma \mu_{NH4} \right)
\]

\[
J_{NH4} = \frac{1}{A_{surface}} x_T RT \Sigma \left( -P_{Na} P_{NH4} \alpha \gamma \mu_{Na} + \left( P_{Na} P_{NH4} \alpha \gamma + P_{H} P_{NH4} \beta \gamma \right) \mu_{NH4} \right)
\]

\[
\Sigma = \left( 1 + \frac{Na_{lumen}^{+}}{K_{Na}} + \frac{H_{lumen}^{+}}{K_{H}} + \frac{NH4_{lumen}^{+}}{K_{NH4}} \right) \left( P_{Na} \frac{Na_{cyst}^{+}}{K_{Na}} + P_{H} \frac{H_{cyst}^{+}}{K_{H}} + P_{NH4} \frac{NH4_{cyst}^{+}}{K_{NH4}} \right)
\]

\[
\Sigma = \left( 1 + \frac{Na_{cyst}^{+}}{K_{Na}} + \frac{H_{cyst}^{+}}{K_{H}} + \frac{NH4_{cyst}^{+}}{K_{NH4}} \right) \left( P_{Na} \frac{Na_{lumen}^{+}}{K_{Na}} + P_{H} \frac{H_{lumen}^{+}}{K_{H}} + P_{NH4} \frac{NH4_{lumen}^{+}}{K_{NH4}} \right)
\]

\[
\overline{\alpha \beta} = \left( \frac{Na_{lumen}^{+}}{K_{Na}} \frac{H_{lumen}^{+}}{K_{H}} + \frac{Na_{cyst}^{+}}{K_{Na}} \frac{H_{cyst}^{+}}{K_{H}} \right) / 2
\]

\[
\overline{\alpha \gamma} = \left( \frac{Na_{lumen}^{+}}{K_{Na}} \frac{NH4_{lumen}^{+}}{K_{NH4}} + \frac{Na_{cyst}^{+}}{K_{Na}} \frac{NH4_{cyst}^{+}}{K_{NH4}} \right) / 2
\]

\[
\overline{\beta \gamma} = \left( \frac{H_{lumen}^{+}}{K_{H}} \frac{NH4_{lumen}^{+}}{K_{NH4}} + \frac{H_{cyst}^{+}}{K_{H}} \frac{NH4_{cyst}^{+}}{K_{NH4}} \right) / 2
\]

\[
\mu_{Na} = RT \ln \left( \frac{Na_{cyst}^{+}}{Na_{lumen}^{+}} \right)
\]

\[
\mu_{H} = RT \ln \left( \frac{H_{cyst}^{+}}{H_{lumen}^{+}} \right)
\]

\[
\mu_{NH4} = RT \ln \left( \frac{NH4_{cyst}^{+}}{NH4_{lumen}^{+}} \right)
\]

\[
P_{Na} = \frac{P_{Na}^0 f^M H_{cyst}^{+} + f^M K_{l}}{H_{cyst}^{+} + K_{l}}
\]

\[
P_{H} = \frac{P_{H}^0 f^M H_{cyst}^{+} + f^M K_{l}}{H_{cyst}^{+} + K_{l}}
\]

\[
P_{NH4} = \frac{P_{NH4}^0 f^M H_{cyst}^{+} + f^M K_{l}}{H_{cyst}^{+} + K_{l}}
\]
Local pH domains in the renal proximal tubule

876 **Na\(^+\)-HCO\(_3^-\) co-transporter:**

877 \[ J_{Na\text{HCO}_3} = J_{Na\text{HCO}_3,\text{MAX}} \frac{\left( HCO_3^- \right)^3 \text{Na}_{\text{cyt}}}{\left( K_{\text{HCO}_3,Na\text{HCO}_3} \right)^3 + \left( HCO_3^- \right)^3 \left( K_{Na,Na\text{HCO}_3} + \text{Na}_{\text{cyt}}^+ \right)} \]

878 **Na\(^+\)/K\(^+\) pump:**

879 \[ J_{\text{Na}_{\text{ATPase}}} = J_{\text{Na}_{\text{ATPase,MAX}}} \left( \frac{\text{Na}_{\text{cyt}}^+}{\text{Na}_{\text{cyt}}^+ + K_{\text{Na,ATPase}}} \right)^3 \]
Table 3 Flux parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Definition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_{\text{surface}}$ (normal) (Pressure natriuresis)</td>
<td>$6.63 \times 10^{-9} \text{ cm}^2$ $1.13 \times 10^{-10} \text{ cm}^2$</td>
<td>Effective area NHE3 is distributed in</td>
<td>Fitted</td>
</tr>
<tr>
<td>$x_T$</td>
<td>495 mM</td>
<td>Amount of NHE3</td>
<td>(51; 52)</td>
</tr>
<tr>
<td>$R$</td>
<td>8.31 J/mol/K</td>
<td>Gas constant</td>
<td>(51; 52)</td>
</tr>
<tr>
<td>$T$</td>
<td>310 K</td>
<td>Temperature</td>
<td></td>
</tr>
<tr>
<td>$K_{\text{Na}}$</td>
<td>30 mM</td>
<td>NHE3 Na+ affinity</td>
<td>(51; 52)</td>
</tr>
<tr>
<td>$K_H$</td>
<td>72 nM</td>
<td>NHE3 H+ affinity</td>
<td>(51; 52)</td>
</tr>
<tr>
<td>$K_{\text{NH}_4}$</td>
<td>0.2 mM</td>
<td>NHE3 NH4 affinity</td>
<td>(51; 52)</td>
</tr>
<tr>
<td>$P_{\text{Na}}^0$</td>
<td>$1.6 \times 10^{-3} \text{ cm/s}$</td>
<td>NHE3 Na+ permeability</td>
<td>(51; 52)</td>
</tr>
<tr>
<td>$P_H^0$</td>
<td>$0.48 \times 10^{-3} \text{ cm/s}$</td>
<td>NHE3 H+ permeability</td>
<td>(51; 52)</td>
</tr>
<tr>
<td>$P_{\text{NH}_4}^0$</td>
<td>$1.6 \times 10^{-3} \text{ cm/s}$</td>
<td>NHE3 NH4+ permeability</td>
<td>(51; 52)</td>
</tr>
<tr>
<td>$f^M$</td>
<td>2</td>
<td>NHE3 pH modifier</td>
<td>(51; 52)</td>
</tr>
<tr>
<td>$f^m$</td>
<td>0</td>
<td>NHE3 pH modifier</td>
<td>(51; 52)</td>
</tr>
<tr>
<td>$K_I$</td>
<td>1 $\mu$M</td>
<td>NHE3 pH modifier</td>
<td>(51; 52)</td>
</tr>
</tbody>
</table>
Local pH domains in the renal proximal tubule

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Expression</th>
<th>Description</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$J_{\text{Na}_{\text{ATPase}, \text{MAX}}}$</td>
<td>$2.109\times10^{-9} \text{ mol/cm}^2/\text{s}$</td>
<td>Na$^+$ ATPase max flux</td>
<td>(51), fitted</td>
</tr>
<tr>
<td>$K_{\text{Na}_{\text{ATPase}}}$</td>
<td>$3.31 \text{ mM}$</td>
<td>Na$^+$ ATPase Na$^+$ affinity</td>
<td>(51)</td>
</tr>
<tr>
<td>$J_{\text{NaHCO}_3, \text{MAX}}$</td>
<td>$7.088\times10^{-10} \text{ mol/cm}^2/\text{s}$</td>
<td>Na$^+$-HCO$_3^-$ co-transporter max flux</td>
<td>(17), fitted</td>
</tr>
<tr>
<td>$K_{\text{HCO}_3^-, \text{NaHCO}_3}$</td>
<td>$19 \text{ mM}$</td>
<td>Na$^+$-HCO$_3^-$ co-transporter, HCO$_3^-$ affinity</td>
<td>(17)</td>
</tr>
<tr>
<td>$K_{\text{Na}, \text{NaHCO}_3}$</td>
<td>$14 \text{ mM}$</td>
<td>Na$^+$-HCO$_3^-$ co-transporter, Na$^+$ affinity</td>
<td>(17)</td>
</tr>
<tr>
<td>$P_{\text{NH}_4}$</td>
<td>$3.06\times10^{-2} \text{ cm/s}$</td>
<td>Membrane NH$_4^+$ permeability</td>
<td>(51)</td>
</tr>
<tr>
<td>$P_{\text{CO}_2}$</td>
<td>$0.5 \text{ cm/s}$</td>
<td>Membrane CO$_2$ permeability</td>
<td>(51)</td>
</tr>
<tr>
<td>$D_{\text{proton}}$</td>
<td>$12 \times10^{-5} \text{ cm}^2/\text{s}$</td>
<td>Diffusion coefficient of protons</td>
<td>(44; 45)</td>
</tr>
<tr>
<td>$D_{\text{Na}}$</td>
<td>$1.334 \times10^{-5} \text{ cm}^2/\text{s}$</td>
<td>Diffusion coefficient of Na$^+$</td>
<td>(44; 45)</td>
</tr>
</tbody>
</table>
### Local pH domains in the renal proximal tubule

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Value</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_{\text{buffer}}$</td>
<td>$33 \times 10^{-7}$ cm$^2$/s</td>
<td>Diffusion coefficient of pH buffer</td>
<td>(44; 45)</td>
</tr>
<tr>
<td>$D_{\text{NH}_3}$</td>
<td>$1.27 \times 10^{-5}$ cm$^2$/s</td>
<td>Diffusion coefficient of NH$_3$</td>
<td>(44; 45)</td>
</tr>
<tr>
<td>$D_{\text{NH}_4}$</td>
<td>$1.27 \times 10^{-5}$ cm$^2$/s</td>
<td>Diffusion coefficient of NH$_4^+$</td>
<td>(44; 45)</td>
</tr>
<tr>
<td>$D_{\text{CHCO}_3}$</td>
<td>$1.3 \times 10^{-5}$ cm$^2$/s</td>
<td>Diffusion coefficient of CHCO$_3$</td>
<td>(44; 45)</td>
</tr>
<tr>
<td>$D_{\text{CO}_2}$</td>
<td>$1.105 \times 10^{-5}$ cm$^2$/s</td>
<td>Diffusion coefficient of CO$_2$</td>
<td>(44; 45)</td>
</tr>
</tbody>
</table>
Local pH domains in the renal proximal tubule

### Table 4: Reactions

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Rate constants</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H^+ + B \xrightleftharpoons[k_{Buffer1}]{k_{Buffer2}} B^+$</td>
<td>$k_{Buffer1} = 10^{10} \text{ M}^{-1} s^{-1}$</td>
<td>(44; 52)</td>
</tr>
<tr>
<td></td>
<td>$k_{Buffer2} = 269 \text{ s}^{-1}$ (pKa 7.57)</td>
<td></td>
</tr>
<tr>
<td>$CO_2 + H_2O \xrightleftharpoons[k_{CA1}]{k_{CA2}} H^+ + HCO_3^-$</td>
<td>$k_{CA1} = 370 \text{ s}^{-1}$</td>
<td>(23; 32)</td>
</tr>
<tr>
<td></td>
<td>$k_{Buffer2} = 8.28 \times 10^8 \text{ M}^{-1} s^{-1}$ (pKa 6.35)</td>
<td></td>
</tr>
<tr>
<td>$H^+ + NH_3 \xrightleftharpoons[k_{Amm1}]{k_{Amm2}} NH_4^+$</td>
<td>$k_{Amm1} = 4.3 \times 10^{10} \text{ M}^{-1} s^{-1}$</td>
<td>(44; 52)</td>
</tr>
<tr>
<td></td>
<td>$k_{Amm2} = 40 \text{ s}^{-1}$ (pKa 9.03)</td>
<td></td>
</tr>
<tr>
<td>Lumen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$CO_2 + H_2O \xrightleftharpoons[k_{CA1,L}]{k_{CA2,L}} H^+ + HCO_3^-$</td>
<td>$k_{CA1} = 370 \text{ s}^{-1}$</td>
<td>(23; 32)</td>
</tr>
<tr>
<td></td>
<td>$k_{Buffer2} = 8.28 \times 10^8 \text{ M}^{-1} s^{-1}$ (pKa 6.35)</td>
<td></td>
</tr>
<tr>
<td>$H^+ + NH_3 \xrightleftharpoons[k_{Amm1,L}]{k_{Amm2,L}} NH_4^+$</td>
<td>$k_{Amm1} = 4.3 \times 10^{10} \text{ M}^{-1} s^{-1}$</td>
<td>(44; 52)</td>
</tr>
<tr>
<td></td>
<td>$k_{Amm2} = 40 \text{ s}^{-1}$ (pKa 9.03)</td>
<td></td>
</tr>
</tbody>
</table>
Table 5. Boundary and initial conditions

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boundary in lumen and initial condition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$CO_2$</td>
<td>$1.5 \text{ mM}$</td>
<td>(52)</td>
</tr>
<tr>
<td>$H^+$</td>
<td>$4.9431 \times 10^{-5} \text{ mM}$</td>
<td>(52)</td>
</tr>
<tr>
<td>$Na^+$</td>
<td>$140 \text{ mM}$</td>
<td>(52)</td>
</tr>
<tr>
<td>$NH_4^+$</td>
<td>$0.2 \text{ mM}$</td>
<td>(52)</td>
</tr>
</tbody>
</table>

| Initial condition in lumen       |               |           |
| $HCO_3^-$                        | $13.55 \text{ mM}$ | (52)     |
| $NH_3$                           | $4.775 \mu M$    | (52)     |

| Initial condition in cytosol     |               |           |
| $CO_2$                          | $1.4995 \text{ mM}$ | (52)     |
| $H^+$                            | $4.67 \times 10^{-5} \text{ mM}$ | (52)     |
| $Na^+$                           | $20.9 \text{ mM}$ | (52)     |
| $NH_4^+$                         | $0.14 \text{ mM}$  | (52)     |
| $HCO_3^-$                        | $14.265 \text{ mM}$ | (52)     |
Local pH domains in the renal proximal tubule

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$NH_3$</td>
<td>2.8 $\mu M$</td>
<td>(52)</td>
</tr>
<tr>
<td>$B^+$</td>
<td>16.494 mM</td>
<td>(15; 21)</td>
</tr>
<tr>
<td>$B$</td>
<td>9.5062 mM</td>
<td>(15; 21)</td>
</tr>
</tbody>
</table>
Cytosolic pH Normotensive

- Microvilli
- Lumen
- Cytosol

Luminal pH Normotensive

- Microvilli
- Lumen
- Cytosol

Cytosolic pH Hypertensive

- Microvilli
- Lumen
- Cytosol

Luminal pH Hypertensive

- Microvilli
- Lumen
- Cytosol