Transepithelial glucose transport and Na⁺/K⁺ homeostasis in enterocytes: an integrative model

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Abstract

The uptake and transport of glucose by the epithelial enterocyte (cell), and the inner extracellular site (serosal), is a highly regulated process. Much of the glucose uptake in the small intestine is apical and coupled with the basolateral Na-K-ATPase pump rate. The enterocyte also faces the challenge of maintaining ionic Na⁺ homeostasis. In doing its job, the epithelial enterocyte is able to deal with the rapid influx of Na⁺ and Cl⁻, basolateral Na-K-ATPase (the sodium potassium pump), basolateral GLUT2 (the facilitated glucose transporter), and diffusive flows of the three included ionic species: Na⁺, K⁺, and Cl⁻. We will test the hypothesis that these responses, which enable homeostasis of intracellular Na⁺ and K⁺, are achieved by a direct regulation of Na-K-ATPase production by intracellular Na⁺ together with a regulation of the basolateral permeability for K⁺ based on the Na-K-ATPase pump rate. The enterocyte also faces the same challenges as other transporting epithelia in maintaining homeostasis in enterocytes: an integrative model.

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other properties such as cell volume and pH value, but we are in this work primarily interested in ionic homeostasis. It should be mentioned that some of the other models of epithelial cells have also been used to investigate homeostatic mechanisms (93, 95), although not for enterocytes.

We consider our enterocyte model as a valuable tool for assessment of hypothetical regulatory mechanisms. The model provides a quantitative framework where plausible mechanisms for regulation and adaptation to changes, like altered nutrient load or cell aging, can be tested. Our model is, to our knowledge, the first integrative approach to model both short and long-term phenomena in enterocytes.

**Physiological Work Mode**

In the small intestine, carbohydrates are hydrolyzed to disaccharides by pancreatic enzymes and further hydrolyzed into glucose, galactose, and fructose by enzymes at the brush border membrane of the epithelial enterocytes.

Glucose and galactose transport from the intestine consists of both a saturable component and a nonsaturable component (32). It is generally agreed upon that the saturable component stems from active transport via SGLT1, whereas the nonsaturable component is argued to stem from either solvent drag and paracellular flow a theory advocated by Pappenheimer and Reiss (63) or from trafficking of GLUT2 to the apical membrane at higher luminal glucose concentrations (36). In this work we only consider the saturable component, which contributes most to the transport at low glucose concentrations.

Figure 1 gives an overview of important transporter proteins in the enterocyte. Glucose is actively transported against its chemical gradient into the epithelial enterocytes by cotransport of sodium in SGLT1 (61, 97). Galactose can also bind to SGLT1 in the same way as glucose. Fructose enters by facilitated diffusion through GLUT5. The sodium concentration inside the enterocyte ([Na\textsubscript{i}]) is kept low by basolateral Na-K-ATPase, which cleaves ATP to transport sodium ions against their concentration gradient out of the cell and potassium ions against their gradient into the cell (23, 67). The sodium concentration in the intestinal chyme ([Na\textsubscript{m}]) is roughly the same as in extracellular fluid and plasma, i.e., ~140 mM. This is much higher than the concentration inside the enterocytes, i.e., ~50 mM (23). K channels give the basolateral membrane a high K permeability, thus providing a return path for the potassium ions that are imported into the cell by Na-K-ATPase. This allows the absorption of nutrients to continue without a buildup of positive ions (28, 67). The imported glucose, galactose, and fructose are transported out of the enterocytes and into the serosal extracellular area by GLUT2, except for the part of it that is metabolized by the enterocyte. Movement of galactose and fructose is always favorable as their concentrations in blood are low (they are swiftly taken up by the liver). The cotransport of glucose through SGLT1 is utilized to up-concentrate glucose in the enterocyte so that it also can diffuse through GLUT2 into the extracellular area and further into the capillaries.

Whereas nonabsorbing enterocytes have a cylindrical form, the presence of glucose or amino acids causes the basolateral part of the enterocytes to shrink, so that it forms a truncated cone, as shown in Fig. 1. By doing this the enterocytes open up intercellular spaces, which are claimed to increase fluid and nutrient uptake (61–63). The intercellular spaces make it possible for large fluxes to flow without having to pass the less permeable nuclei and mitochondria in the lower part of the enterocyte. In this work we have used the spatial dimensions of rat enterocytes in absorbing state (see Spatial Dimensions of an Absorbing Enterocyte).

**METHODS: MATHEMATICAL MODELING**

To provide more insight into the modeling process, we include a short review of the important aspects for each of the transporter proteins before setting up the mathematical rate expressions.

In our modeling, we have followed the principle that all parameters should be identifiable from experiments reported in the literature. Using rate expressions where the proteins are considered to be in steady state (i.e., we do not consider dynamic distribution of different conformations of the same protein) keeps the mathematical model on a moderate level of complexity.

**SGLT1**

The SGLT1 cotransporter is responsible for the active uptake of glucose from digested food, and it is primarily expressed on the apical, brush border, membrane of intestinal epithelial enterocytes.
(97, 99). SGLT1 works by undergoing a cycle of conformational changes, translocating its binding site from the outside to the inside of the cell.

From the work done by Wright and others the kinetics and rate of SGLT1 transport is well known (37, 64, 65, 97). The saturated stoichiometry of the SGLT1 cotransporter is two Na⁺ ions for each glucose molecule (31, 64). Studies by Wright et al., revised in Ref. 97, have shown that the flow of glucose and Na⁺ through SGLT1 can be adequately described by a cotransport carrier model with six states and ordered binding (65, 97) (see Fig. 2). The model, first introduced by Parent et al. in 1992 (65), describes each subreaction by its corresponding forward and reverse rates. At the outer (mucosal) face, the empty carrier binds first to two Na⁺ ions, modeled as one step, and then to one glucose molecule. On the inner (cell) face, glucose is released before Na⁺ (last in first out). In the model the empty carrier is assumed to have a charge of $-2$, making the carrier neutral when bound to two Na⁺ ions. The translocation of the empty carrier across the membrane and the binding/release of Na⁺ on either sides are thus the only steps that are dependent on the membrane potential.

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mM. Likewise, a change in external K⁺ was found to scale the turnover with 1.3·|Kc|/(|Na₀| + |Kc|), where |Kc| is the serosal concentration of K⁺, and |Na₀| is the concentration of Na⁺ in the cell inside, so fast that it does not contribute significantly to alter the inflow of ions per time unit, hence the maximum flow of NaCl transport for the whole enterocyte.

GLUT2

The glucose absorbed into the enterocytes from the lumen is transported out of the enterocytes by facilitated diffusion through GLUT2 transporter proteins. The glucose exits to the extracellular serosal space near blood capillaries.

To model the flow of glucose through GLUT2 we use an expression based on the so-called fixed-site carrier model, suggested by Baker and Widdas (3). This model treats the transporter protein as a pore with two binding sites, one on the inward facing side (c) and one on the outward facing side (s). The net flow from the inside to the outside of the cell is then given as

\[ J_{GLUT} = \frac{V_{GLUT}}{R_{GLUT}} \left( \frac{K_{Gc}^{max} V_{Gc}}{K_{Gc}^{s} + [G]_{s}} \right) \]

where \( V_{GLUT} \) and \( V_{Gc}^{max} \) are the maximum transport rate out of and into the enterocyte for one single GLUT2 transporter. \( K_{Gc}^{max} \) and \( K_{Gc}^{s} \) are the half saturation constants for the inside facing and the outside facing binding sites, respectively. [G] and [G] are the concentrations of glucose inside and outside of the enterocyte, respectively, and \( R_{GLUT} \) is the number of GLUT2 transporter proteins in the basolateral membrane.

At zero-trans conditions this rate expression reduces to a Michaelis-Menten form, hence making the constants easy identifiable from the studies of Maenz and Cheeseman (52). However, we had to make some adjustments of the parameters to ensure zero net flow at equal concentrations, in keeping with the Haldane relationship, \( V_{GLUT}^{max} K_{Gc}^{s} = V_{Gc}^{max} K_{Gc}^{max} \), These adjustments are discussed in Adjustment of Kinetic Parameters for GLUT2.

Diffusive Flows

Diffusive flows of Na⁺, K⁺, and Cl⁻ are also included in our model. We use the Goldman-Hodgkin-Katz (GHK) flux equation (70).
to describe the diffusional flows across the apical and the basolateral membrane. The equation has the form:

\[ j^D = -\frac{P_i z_i F \psi}{RT} \left( \frac{i_{\text{out}} - i_{\text{in}} \exp(z_i F \psi/RT)}{1 - \exp(z_i F \psi/RT)} \right) \cdot A \]  

(5)

The subscript \( i \) denotes the ion (Na\(^+\), K\(^+\), or Cl\(^-\)), \( P_i \) is the membrane permeability of ion \( i \), \( z_i \) is the valence of the ion, \( [i_{\text{out}}] \) and \( [i_{\text{in}}] \) are the outer and inner concentrations of the ion, and \( \psi \) is the membrane potential (\( \psi_{\text{apic}} \) for the apical and \( \psi_{\text{basal}} \) for the basolateral membrane). \( F \), \( R \), and \( T \) are the Faraday constant, the gas constant, and the temperature, respectively. The GHK flux-equation gives the diffusive flow as a flux, i.e., flow per membrane area, so we multiply with the membrane area \( A \) (see Table A2) to find the total diffusional flow over the membrane. The diffusional flows given by Eq. 5 are positive when directed into the cell.

The permeability coefficients account for the total diffusional flow, including diffusion through ion channels. The most notable type of ion channels in enterocytes is the basolateral K channels (28, 86). The K channels give the enterocyte a high permeability for K\(^+\) and provide a return path for the K\(^+\) ions imported into the cell by the Na-K-ATPase. Regulation of the K permeability also plays an important part in the ionic homeostasis in enterocytes. This is further discussed in Regulation of basolateral K permeability and potassium homeostasis.

In addition to diffusional flows into the cell, we also include diffusional paracellular flows that allow ions to travel directly from the mucosal to the serosal side through the tight junctions. These paracellular flows are calculated by Eq. 5, using the mucosal concentration of the ion as the outer concentration and the serosal concentration as the inner concentration and \( \psi_{\text{muc}} \), as the membrane potential from the mucosal to the serosal side. The paracellular flows are positive when directed from the mucosal to the serosal side.

Cell Membrane Potential

The SGLT1 and the Na-K-ATPase are both electrogenic transporters, i.e., the transport of ions through these proteins constitute electrical currents flowing in and out of the enterocyte. Diffusional ion flows are also electrical currents, and this, together with the fact that the flows are driven by processes that are dependent on the membrane potential, makes it essential to include the membrane potential of the enterocyte in our model.

Our enterocyte model includes the mucosal to cell (apical) membrane potential \( \psi_{\text{muc}} \), the serosal to cell (basolateral) membrane potential \( \psi_{\text{basal}} \), and the potential over the whole epithelial layer from mucosal to serosal \( \psi_{\text{muc, basal}} \). See Fig. 1. Only two of these are independent as the potentials are related by the equation:

\[ \psi_{\text{muc, basal}} = \psi_{\text{basal}} - \psi_{\text{muc}} \]  

(6)

An important principle for any form of transport of ions into and out of cells is the principle of electroneutrality (42, 70), which states that the bulk or macroscopic concentrations of positive and negative ions has to be equal at all times. The reason behind this principle is that only a very small amount of charge separation is needed to create a large electric field. A charge difference of 1 mM more positively charged ions than negatively charged ions will for a cell with the dimensions used in this work (see Spatial Dimensions of an Absorbing Enterocyte) give an electric field inside the membrane with a magnitude of about 2-10\(^9\) V/m. With this field strength and a membrane thickness of 7.5 nm (77), the corresponding membrane potential is in the order of 10 V/meq of charge difference. Thus a membrane potential in the range of millivolts corresponds to a negligible difference in concentration, which is too small to be measured by most chemical methods (42).

Cell electroneutrality holds when there is no net current into the cell, i.e.,

\[ \sum_i z_i(j_i^\text{in} + j_i^\text{out}) = 0 \]  

(7)

where \( j_i^\text{in} \) and \( j_i^\text{out} \) are the inflow of species \( i \), with valence \( z_i \), over the apical and basolateral membrane, respectively. For the same reasons as above, any buildup of charge on either the mucosal or the serosal side would result in unphysical high values for the transepithelial potential (\( \psi_{\text{muc, basal}} \)). To preserve mucosal and serosal bath electroneutrality the net epithelial current must thus also be zero,

\[ \sum_i z_i(j_i^\text{muc} - j_i^\text{seros}) = 0 \]  

(8)

where \( j_i^\text{muc} \) is the paracellular inflow into the serosal space of species \( i \), with valence \( z_i \).

The principle of electroneutrality, in the form of Eqs. 7 and 8, is often used as a condition to help calculate the membrane potentials in models of epithelial transport [see for instance Stephenson and Weinsteins models of renal epithelium (87, 93, 96)]. The idea is to compute a value for the membrane potential that, when used to calculate the individual flows, gives a zero net current.

We have chosen to use an iterative approach as outlined in Ref. 43. At each time step we start with guess values for the membrane potentials and calculate the flows; the estimate for the membrane potential is then refined using the Newton-Raphson method and the flows are recalculated until the net cell current (Eq. 7) and the net epithelial current (Eq. 8) are sufficiently close to zero (<10\(^{-9}\) pmol/ h).

Once the flows are in keeping with Eqs. 7 and 8 we integrate the net flow of each species over the length of the time step to update the concentrations. This iterative calculation of the membrane potentials is well proven for epithelial models (25, 43, 85). A detailed description of our implementation and validation of the iterative method is given in Iterative Calculation of Membrane Potentials.

Integrative Model

We build our model by combining the reaction kinetic expressions for SGLT1, Na-K-ATPase, coupled NaCl inflow, GLUT2, and diffusive flows into a set of nonlinear differential equations, shown in Model Equations. As described above the flows are calculated together with the membrane potentials at each time step in the simulation by an iterative method. The model describes the dynamical behavior of flows, as well as the concentration of intracellular chemical species (cell glucose [\( G_c \]), cell sodium [\( Na_c \]), cell potassium [\( K_c \]), and cell chloride [\( Cl_c \]], and the cell membrane potentials (\( \psi_{\text{muc, basal}}, \psi_{\text{muc}}, \text{and } \psi_{\text{basal}} \)). The model does not consider the amount of glucose metabolized by the enterocyte itself.

The model was implemented and solved numerically by using Matlab/Simulink from MathWorks. Numerical integration of the differential equations is done by the Matlab/Simulink solver ode23tb. The solver uses an absolute and relative tolerance of 10\(^{-6}\) and a variable time step length between 3.6 and 360 ms. Simulations run quickly on an ordinary desktop computer; it takes about half a minute to simulate a 4-min short-term response to a transient change in mucosal glucose and about a minute to simulate a 40-min long-term response to a stepwise (constant) change in glucose (see Short-Term Response to Mucosal Glucose and Long-Term Response with Controller Motifs, respectively).

The parameters used are listed in the Appendix (see Table A2) and are as discussed based on experimental data and evidence. However, some adjustments of the ionic permeabilities had to be done as we found little usable data on permeability coefficients for enterocytes. Studies exists (4, 17), but they are old and unreliable. Measurements are typically done for whole tissue layers without blocking nondiffusive pathways for active transport and cotransport. For the paracellular permeabilities we started with values reported for rabbit ileum (17), and for the mucosal and serosal membrane we started with permeability coefficients from a Necturus gallbladder model (1). The coefficients were then adjusted by trial and error until reasonable re-
sponses were observed, primarily in the membrane potential. The most prominent adjustments were made in the apical permeability for K⁺ ($P_{K_a}$), which was reduced to be in the same order of magnitude as the apical permeability for Na⁺ ($P_{Na_a}$) as reported for rat small intestine (4), and the basolateral Na⁺ permeability ($P_{Na_bl}$), which was increased since it in Ref. 1 was set to zero. We kept the ratio between the paracellular permeabilities as in Ref. 17 but had to increase the magnitude to reduce the transepithelial potential to ∼3 mV as reported in Refs. 59 and 69.

RESULTS

We first tested the short-term behavior of the model, without adding additional regulatory mechanisms for ionic homeostasis. During a short timeframe (up to 5 min), it is reasonable to assume that the amount of transporter protein in the membrane remains constant, as regulation of transporter protein expression by transcriptional and translational pathways may have little influence.

Short-Term Response to Mucosal Glucose

Short-term response to changes in mucosal glucose is well studied in vitro (7, 30, 69), and thus, provides a sound basis for testing our model. We have simulated its response to a short-term increase in mucosal glucose ([Gm]) similar to the experimental studies performed on rabbit ileum by Rose and Schultz (69). Mimicking the conditions of Rose and Schultz, we abruptly increase the mucosal glucose concentration from 100 μM to 20 mM and then let it fall back to 100 μM in an exponential manner, as to simulate glucose being flushed out (see Fig. 3A). Using parameters from the literature as described in METHODS: MATHEMATICAL MODELING (see Table A2 for list of parameters), the response in state variables from the model is shown in Fig. 3, B–H, whereas the flows are shown in Fig. 4.

The simulation shows that the addition of mucosal glucose leads to a less negative mucosal to cell membrane potential.
(\(\psi_{\text{ms}}\); Fig. 3F) and a more positive mucosal to serosal membrane potential (\(\psi_{\text{ms}}\); Fig. 3H), in good agreement with the experimental findings of Rose and Schultz (69). A direct comparison of the simulated membrane potential response and the experimental findings in Ref. 69 is shown in the DISCUSSION (see Fig. 11).

The steady-state levels of cell Na\(^+\), K\(^+\), and Cl\(^-\) are in the expected range (55, 57). The short-term increase in cell Na\(^+\) (see Fig. 3C) is supported by experiments (30), but is reported to fall over time if the mucosal concentration is kept high instead of being flushed out, indicating a slower regulatory mechanism that increases the number of Na-K-ATPase transporters (30). This is further discussed in Regulation of Na-K-ATPase and sodium homeostasis.

The reduced concentration of intracellular K\(^+\) (Fig. 3D) is a result of an increased outflow of K\(^+\) (Fig. 4E) due to membrane depolarization. The change in membrane potential towards more positive values increases the diffusive K\(^+\) outflow, in agreement with Eq. 5. K\(^+\) is removed faster than the Na-K-ATPase imports K\(^+\), a property that is anticipated based on experimental results (7, 22).

Net epithelial flows of Na\(^+\), K\(^+\), and Cl\(^-\) are found by summing the basolateral and paracellular flows. Figure 5 shows the net flows during the short-term increase in mucosal glucose. Focusing on the steady state before and after the step in mucosal glucose, we see that Na\(^+\) and Cl\(^-\) are absorbed from the intestine, in keeping with enterocytes being Na\(^+\)/H\(^+\) and sodium homeostasis. See Fig. 11.

The enterocyte response to short-term changes in mucosal Na\(^+\) is well studied (57, 59, 69). We simulated short-term changes in the concentration of mucosal Na\(^+\) ([Na\(_{\text{m}}\)]), by changing the mucosal Na\(^+\) from its base value of 140 to 100 mM and back again in a stepwise manner. The concentration of mucosal glucose was kept constant at 0.5 mM. Figure 6 shows the simulated model response. When the mucosal sodium concentration is kept at 100 mM, the model reaches a new steady state; the reduced availability of mucosal Na\(^+\) leads to a decrease in the intracellular Na\(^+\) concentration ([Na\(_{\text{i}}\)]). This decrease in intracellular Na\(^+\) is balanced by

\[
\Delta C_{\text{Na}^+} \approx \Delta C_{\text{Cl}^-} \approx \frac{1}{2} \Delta C_{\text{Na}^+} + \Delta C_{\text{K}^+}.
\]

\(\Delta C_{\text{Na}^+}\) is removed faster than the (Fig. 3D). There is a small leak of K\(^+\) in the opposite direction, which is expected because some of the K\(^+\) pumped into the cell at the basolateral membrane will escape through K conductance in the apical membrane (Fig. 4E). The rate of K\(^+\) secretion is very low (0.14 pmol/h) compared with the inflow of Na\(^+\) and Cl\(^-\) (1.82 and 1.68 pmol/h), ~8% of the Na\(^+\) inflow, and is believed to be of little physiological significance (71). Although there is an increase in Na\(^+\) pumped by the Na-K-ATPase (Fig. 4C), the net epithelial Na\(^+\) inflow decreases during the transient phase (Fig. 5A). This is caused by the increase in paracellular back flow of Na\(^+\) into the lumen (Fig. 4F); a more positive transepithelial potential \(\psi_{\text{ms}}\) (Fig. 3H) drives this paracellular movement of Na\(^+\).

Short-Term Response to Mucosal Na\(^+\)

\begin{align*}
\text{AJP-Cell Physiol} & \cdot \text{doi:10.1152/ajpcell.00068.2013} \cdot \text{www.ajpcell.org}
\end{align*}
decrease in intracellular Cl\(^-\) (Fig. 6C) and an increase in intracellular K\(^+\) (Fig. 6B).

The simulation also shows cell hyperpolarization (see Fig. 6, D–F) as a result of decreased mucosal Na\(^+\). The reversal of polarity from positive to negative of the transepithelial potential \(\psi_{\text{trans}}\) is particularly noteworthy. These results are similar to what have been observed in rat duodenum (59). The change in \(\psi_{\text{bas}}\) and \(\psi_{\text{trans}}\) of about –6 mV is in the same range as reported for the same change in mucosal Na\(^+\). Changing the mucosal Na\(^+\) concentration back to 140 mM causes the enterocyte to return to its initial state.

There is a continuous net absorbance of Na\(^+\) and Cl\(^-\) from the intestine during the whole simulated response to this change in mucosal Na\(^+\). The net inflow of Na\(^+\) goes from 1.86 to 0.85 pmol/h when mucosal Na\(^+\) changes from 140 to 100 mM (results not shown).

Extension of the Model to Test Regulatory Mechanisms

Having shown that the enterocyte model behaves as expected for short-term dynamics, we continue to test our hypothesis by adding regulatory mechanisms to the model.

Regulation of Na-K-ATPase and sodium homeostasis. Experiments show that when mucosal glucose is increased and kept high, the increase in intracellular Na\(^+\) ([Na\(_i\)]) is followed by a slow decrease back to basal values, indicating a mechanism that increases the number of Na-K-ATPase transporter proteins to maintain Na\(^+\) homeostasis (30). This increase may stem from increased synthesis as it has been shown to do in central neurons and cardiocytes (74, 98) or from a cytoplasmic holding area of available Na-K-ATPase proteins (73).

Our hypothesis is that the observed homeostasis can be explained by a production of Na-K-ATPase directly regulated by intracellular Na\(^+\). Intracellular Na\(^+\) has been shown to directly regulate the Na-K-ATPase gene expression in other cell types (74, 98).

We have earlier presented a set of simple controller motifs that can bring about robust homeostasis (10, 83). Because Na-K-ATPase can be said to work as an outflow controller by transporting Na\(^+\) out of the cell, the activating outflow controller (type 5) from our work is a possible candidate for the mechanism that regulates Na\(^+\) by adjusting the number of Na-K-ATPase proteins. The controller motif is shown in Fig. 7.

Our motif treats the concentration of intracellular Na\(^+\) as an activator for the synthesis/insertion of Na-K-ATPase proteins, modeled as a first order process, where the insertion rate is given as \(k_{\text{NaK}}[\text{Na}_i]\). The degradation (or removal) of Na-K-ATPase is assumed to be similar to an enzymatic process where the degradation enzyme \(E_{\text{NaK}}^{\text{degrade}}\) is saturated. This controller motif extends the model with the following differential equation:

\[
\dot{n}_{\text{NaK}} = k_{\text{NaK}} \cdot [\text{Na}_i] - \frac{V_{\text{NaK}_{\text{max}}} \cdot n_{\text{NaK}}}{K_{\text{NaK}_{0.5}} + n_{\text{NaK}}} \tag{9}
\]

where \(V_{\text{NaK}_{\text{max}}}\) and \(K_{\text{NaK}_{0.5}}\) are the maximum rate and the half saturation constant for the enzyme/process that removes Na-K-ATPase. Saturation of the Na-K-ATPase removing process ensures regulation of intracellular Na\(^+\) to the setpoint \(V_{\text{NaK}_{\text{max}}} / k_{\text{NaK}}\), because saturation provides near zero-order kinetics. In detail, the setpoint for intracellular Na\(^+\) is determined by the steady-state condition of Eq. 9. With \(K_{\text{NaK}_{0.5}} \ll n_{\text{NaK}}\) (saturation) the degradation term is approximately constant at \(V_{\text{NaK}_{\text{max}}} / k_{\text{NaK}}\), and thus \(\dot{n}_{\text{NaK}}\) is zero when \([\text{Na}_i]\) is at the setpoint \(V_{\text{NaK}_{\text{max}}} / k_{\text{NaK}}\). We have previously shown that zero-order kinetics is a sufficient condition for integral control (58). The outflow of Na\(^+\) is still given by the \(J_{\text{NaK}}^{\text{Na}}\) expression (Eq. 2), but the amount of Na-K-ATPase (\(n_{\text{NaK}}\)) will now change dynamically according to Eq. 9 to counteract the perturbations in Na\(^+\) inflow. A transient increase in intracellular Na\(^+\) (from increased apical inflow) causes the amount of Na-K-ATPase to increase (\(\dot{n}_{\text{NaK}} > 0\)); this increase continues until the outflow of Na\(^+\) through Na-K-ATPase is so large that the concentration of intracellular Na\(^+\) is brought back down to its setpoint.

Regulation of basolateral K permeability and potassium homeostasis. The pump-leak parallelism between K permeability and the stimulation of basolateral Na-K-ATPase is well studied (7, 28, 73). An increase in the Na-K-ATPase pump rate, as seen under the uptake of nutrients, is accompanied by an increase in the basolateral K permeability. Whether this increase stems from an increased expression of K channels or from nontranscriptional mechanisms is not yet fully understood as little is known about changes in K-channel expression in enterocytes (28).

Most works have focused on elucidating nontranscriptional regulation and one prominent feature is an apparent Ca\(^{2+}\) activation of the K channels (75). Other nontranscriptional regulatory mechanisms include activation by cell swelling, membrane stretch, and also an interesting inverse dependence on cytosolic ATP (28, 84).
Our attention in this work is on the regulation of K permeability by intracellular ATP. Basolateral K<sub>ATP</sub> channels have been found in enterocytes (12) and other epithelia (84). The increased usage of ATP by Na-K-ATPase during absorption lowers the intracellular concentration of ATP; this reduction in intracellular ATP relieves the inhibition of basolateral K<sub>ATP</sub> channels, thus increasing the K permeability.

Structuring this into a controller motif we have that intracellular ATP acts as an outflow controller of [K<sub>c</sub>] (see Fig. 8). ATP is used by the Na-K-ATPase at a rate of \( J_{NaK} \). We assume that the synthesis of ATP can be modeled by an expression on the form \( k_1^\text{ATP} \{K_{0.5}^\text{ATP} / (K_{0.5}^\text{ATP} / [ATP])\} \), i.e., that the synthesis of ATP increases when the concentration of ATP decreases. The theoretical maximum rate of synthesis is \( k_1^\text{ATP} \) when [ATP] is zero, and \( K_{0.5}^\text{ATP} \) is the concentration of ATP where the rate of synthesis is half of \( k_1^\text{ATP} \). The synthesis of ATP must increase as the concentration of intracellular ATP is reduced to reach a new steady state, otherwise an increase in \( J_{NaK} \) would simply deplete the cell of ATP as the synthesis would never catch up with the increased consumption.

Fig. 6. Simulated model response to a short-term change in mucosal sodium using model parameters in Table A2. At \( t = 1 \) min, the mucosal side Na\(^+\) concentration ([Nam]) is decreased from 140 to 100 mM (step), and at \( t = 6 \) min, the Na\(^+\) concentration is changed back to 140 mM. A: cell sodium concentration ([Na<sub>c</sub>]). B: concentration of cell potassium ([K<sub>c</sub>]). C: concentration of cell chloride ([Cl<sub>c</sub>]). D: mucosal to cell membrane potential (\( \phi_{mc} \)). E: serosal to cell membrane potential (\( \phi_{sc} \)). F: mucosal to serosal membrane potential (\( \phi_{ms} \)).

Fig. 7. Controller motif proposed for the regulation of intracellular Na\(^+\) by production of Na-K-ATPase.

The inverse relation between the concentration of intracellular ATP and current through K<sub>ATP</sub> channels has been quantified in cardiac myocytes (35). The current through the K channels is inhibited by [ATP] with an inhibition constant \( K^\text{I}_{ATP} \) of 0.8 mM. From this basis we express the relationship between ATP and basolateral K permeability on a standard inhibition form as:

\[
P_{K_{bl}}/K_{max_{bl}} \frac{[ATP]}{K_{I_{ATP}} / [ATP]}.
\]

By doing this we end up with a slightly modified controller motif of type 6 (see Ref. 10). The degradation of the controller species, which is intracellular ATP, is proportional to the inflow, rather than the concentration, of the controlled species [K<sub>c</sub>].

Fig. 8. Controller motif proposed for the regulation of intracellular K\(^+\). The concentration of the controller species, which is intracellular ATP, is proportional to the inflow, rather than the concentration, of the controlled species [K<sub>c</sub>].
This controller motif leads to a differential equation describing the dynamics in [ATP], and an expression for the basolateral K permeability as

\[
\frac{\dot{[ATP]}}{V_c} = \frac{1}{V_c} \left( \frac{k_{ATP}^{ATP}}{K_{ATP} + [ATP]} - J_{NaK} \right)
\]

(10)

\[
P_{K_{\text{bl}}} = P_{K_{\text{bl}}}^{\text{max}} \left( \frac{K_{ATP}^{ATP}}{K_{ATP}^{ATP} + [ATP]} \right)
\]

(11)

where \( V_c \) is the cell (cytoplasm) volume and \( K_{ATP}^{ATP} = 0.8 \text{ mM} \). \( P_{K_{\text{bl}}}^{\text{max}} \) can be found from \( P_{K_{\text{bl}}} \) and the steady-state level of [ATP], which is \( \sim 4.3 \text{ mM} \) in enterocytes (9). We have adjusted \( k_{ATP} \) so that [ATP] is in steady state (4 mM in our simulations) before the addition of nutrients; \( K_{ATP}^{ATP} \) is set to 0.5 mM.

**Long-Term Response with Controller Motifs**

Adding these two controller motifs to the model gives the long-term responses shown in Figs. 9 and 10 (solid lines) for a stepwise increase in mucosal glucose (note the time scale). The results show adaptation in the concentration of intracellular Na\(^+\) (Fig. 9C) and K\(^+\) (Fig. 9D) where the concentrations are regulated towards their setpoints of \( \sim 49 \) and 130 mM, respectively. Comparing this to the simulation results of an enterocyte without the regulatory mechanisms (dashed lines in Fig.

\[
\begin{align*}
A & : \text{at time } t = 4 \text{ min mucosal glucose concentration } ([G_m]) \text{ is stepped from 0.1 to 10 mM.} \\
B & : \text{cell glucose } ([G_c]) \\
C & : \text{cell sodium } ([Na_c]) \\
D & : \text{cell potassium } ([K_c]) \\
E & : \text{cell chloride } ([Cl_c]) \\
F & : \text{mucosal to cell membrane potential } (\psi_{mc}) \\
G & : \text{serosal to cell membrane potential } (\psi_{sc}) \\
H & : \text{mucosal to serosal membrane potential } (\psi_{ms}) \\
\end{align*}
\]

\[
\begin{align*}
A & : \text{amount of Na-K-ATPase } (n_{NaK}) \\
B & : \text{concentration of ATP } ([ATP])
\end{align*}
\]
has to do with the accuracy of the controller motif for
than without. This fits with the higher total ionic flow from
step in mucosal glucose and is higher with regulatory motifs
Na-K-ATPase by intracellular Na
More specifically, we wanted to test whether regulation of
regulatory mechanisms for ionic homeostasis in enterocytes.
combined to form a mathematical model of glucose absorption in
the enterocyte would be flooded by either Na
or K
. The adaptation is not perfect in the sense that exactly the
same ionic concentrations are achieved before and after the
step; the concentration of K
is for instance close to 129 mM
before the step in mucosal glucose and 136 mM after the
step. There is a slight overadaptation (11) (Fig. 9D). This
has to do with the accuracy of the controller motif for
intracellular K
(10). As expected there is no adaptation in
the concentration of Cl
(Fig. 9E), as there is no specific
controller motif regulating Cl
.

The amount of Na-K-ATPase and ATP is shown in Fig. 10.
The reduction in ATP from 4 to 2.8 mM fits well with reported
values for absorbing epithelial cells in the renal proximal
tubule (84).

In addition to maintaining the ionic concentration at their
setpoints, the regulation also enables a higher total flow
through the enterocyte (data not shown). The net epithelial
flow of Na
increases by 20% after the step in mucosal glucose
with regulation (from 1.8 to 2.2 pmol/h) whereas it without
regulation only increases by 5% (from 1.8 to 1.9 pmol/h). The
net flow of glucose is 13% higher with regulation than without.

The results also show a hyperpolarization of the membrane
potential compared with the situation with no regulation (Fig.
9, F–H). The transepithelial potential (\(\psi_{\text{m}}\)) increases with the
step in mucosal glucose and is higher with regulatory motifs
than without. This fits with the higher total ionic flow from
mucosal to serosal with regulation.

**DISCUSSION**

We asked whether the available kinetic data could be com-
bined to form a mathematical model of glucose absorption in
enterocytes and whether such a model could be used to predict
regulatory mechanisms for ionic homeostasis in enterocytes.
More specifically, we wanted to test whether regulation of
Na-K-ATPase by intracellular Na
 together with an ATP-
based regulation of the basolateral permeability for K
could explain how enterocytes adapt to changing inflow of Na
.

**Model and Short-Term Response**

The short-term response of our model shows that the mod-
eled enterocyte behaves as expected from experiments reported
in literature. This confirms that reaction rates, and other results,
from studies done on specific enterocyte transporter proteins in
isolation can be combined to form an integrated model that
describes the transport of glucose, and the cell homeostasis of
ions, in a satisfactory manner.

Figure 11 shows the membrane potential response from our
simulated change in mucosal glucose (from Fig. 3) together
with the experimental data from the study by Rose and Schultz
(69) on rabbit ileum. In our view the match is remarkable
considering the fact that most of our parameters are gathered
from the literature of single transporter type studies, done with
proteins from different organisms including human type
SGLT1 expressed in oocytes (97), guinea pig Na-K-ATPase
from ventricular myocytes (19, 54), and GLUT2 from rat
enterocytes (52). The spikier simulated response in membrane
potential (Fig. 11) may be related to the effect of unstirred
layers around the microvilli. A gradual equilibrating between
the mucosal bath and the microvilli area will effectively drag
out the response.

Because our kinetic flow expressions and physiological
parameters are based on single transporter type studies, param-
eter fitting would probably be needed if we were to evaluate the
model’s ability to exactly match quantitative results from
studies done on whole enterocytes. Cell variance would in that
case also have to be considered, e.g., the membrane potential of
rabbit enterocytes bathed in a control medium was in the study
of Rose and Schultz (69) shown to range from \(-15\) to \(-55\)
mV, indicating considerable variance among cells.

Whereas our results indicate that use of parameter values
from reported experimental data lead to a reasonable response
in our model, it is not always possible to find a unanimous
value for every parameter. Experimentalists do not always
agree on one value, and the uncertainty can in some cases be
significant. As an example, although several experiments have
been done to estimate the turnover rate of SGLT1, there is little
agreement on a consensus value (45). The rate in our model
(1–3 s\(^{-1}\)), which is based on parameters from Ref. 97, is in the
lower region of reported values. A recent detailed modeling of
the SGLT1 transport cycle (46) suggests that the rate should be
higher (35 \(^{-1}\)). Similar discussion can be made for all the flow
expressions used in this study. We have in general chosen to
not include uncertain aspects in the rate expressions when good
empirical data for various reasons have been lacking.

**Regulatory Mechanisms and Na\(^+/K\(^+\) Homeostasis**

The presented model provides insight into how enterocytes
maintain intracellular homeostasis even when confronted with
changing rates of Na
inflow. It provides a useful framework
for quantitative testing of plausible regulatory mechanisms in
the enterocyte, in which the two controller motifs for Na-K-
ATPase and K permeability presented here are examples of
such.

When the inflow of Na
increases due to absorption of
glucose, the enterocyte has to adapt the Na-K-ATPase-driven
compensatory outflow of Na
. Since the Na-K-ATPase also
imports K
into the cell, an increase in Na-K-ATPase Na
outflow must be followed by an increase in K
outflow. If not,
the enterocyte would be flooded by either Na
or K
. The
earlier mentioned experiments show that besides a transient

---

**Fig. 11. Simulated model response in membrane potential (solid) and replotted experimental values (dashed). [Reprinted from Rose and Schultz (69) with permission.]**
increase there is no sustained higher concentration of intracellular Na\(^+\) ([Na\(_i\)]) during absorption of nutrients (30, 72). This means that the Na-K-ATPase-driven outflow has to increase by other means than mass action kinetics. The explanation suggested by our controller motif is that the transient increase in intracellular Na\(^+\) brings about an increase in the number of active Na-K-ATPase proteins in the basolateral membrane. This can explain the results of Rokaw et al. (68), which show that the amount of Na-K-ATPase in A6 model cells for kidney collecting ducts epithelium is regulated in correspondence to inflow of Na\(^+\).

The intracellular Na\(^+\) activation of Na-K-ATPase synthesis has been shown for other cell types, where it increases the amount of mRNA coding for both the α- and β-subunits that combines into Na-K-ATPase (74, 98). Yamamoto et al. (98) showed that the increase in cardiocytes, was directly caused by Na\(^+\) due to Na\(^+\)-responsive sequences located within the 5′-flanking regions of the α-gene (α\(_1\)-, α\(_2\)-, and α\(_3\)-isoforms). Parallels can be drawn to the intestinal enterocytes as they express the α\(_1\)-isoform (44). Our simulations confirm that the regulation of Na-K-ATPase by intracellular Na\(^+\) (Fig. 7) is a controller motif that is able to achieve homeostasis (10, 83), thus being a plausible mechanism for how the enterocytes manage to adapt and survive in a changing environment.

In addition to synthesis of Na-K-ATPase, posttranslational regulation by the insertion of preexisting transporter molecules from cytoplasmic storage pools can also be a contributing factor. Such regulation in response to intracellular Na\(^+\) has been indicated in rabbit cortical collecting tubules (6). Given the many similarities between kidney and intestine epithelial cells, we cannot rule out the possibility that the abundance of membrane bound Na-K-ATPase in enterocytes is regulated by both translational and posttranslational pathways that are dependent on intracellular Na\(^+\). Although we have focused on regulation by synthesis of new Na-K-ATPase in our argument, the ability of the controller to achieve homeostasis is coupled to the action where intracellular Na\(^+\) regulates the number of active transporters and not to whether this happens by de novo synthesis or by insertion/activation of latent transporters.

Earlier work on sodium-transporting epithelial cells has speculated whether the relationship between the Na-K-ATPase turnover and intracellular Na\(^+\) can be so steep that small changes in the latter may result in very large changes in the former (30, 72), enabling Na\(^+\) outflow adaptation to occur without an increase in the amount of Na-K-ATPase. This alternative hypothesis is, however, considered unlikely and has also been shown to be incompatible with the experimental measurement of Na-K-ATPase kinetics (19, 54).

Nevertheless, an increase in the amount of Na-K-ATPase can possibly be induced by other means than direct activation by intracellular Na\(^+\). Other mechanisms for the regulation of Na-K-ATPase are known; many studies have focused on the hormonal regulation through protein kinase A and C (PKA and PKC) pathways (see Ref. 80 for a review). Although important, hormonal mechanisms (involving sensing or prediction of the Na\(^+\) inflow in enterocytes and secretion of hormones by some organ distant to the enterocyte) may be slower than the direct regulation by intracellular Na\(^+\) and might thus play a secondary role. The effects of hormones on Na-K-ATPase activity have also been shown to depend on intracellular Na\(^+\) in proximal tubule cells, suggesting that the level of intracellular Na\(^+\) modulates whether hormones stimulate, inhibit, or have no effect on Na-K-ATPase levels (13).

A coupling between the activity of the Na-K-ATPase and the basolateral K permeability has been experimentally shown (7, 22). While this coupling has been confirmed for decades, it is still not clear what mechanism lies behind it (28). Our proposed regulatory motif (Fig. 8) is a minimal mechanism where ATP acts as a regulator. Since intracellular ATP concentration is dependent on the current ATP usage by Na-K-ATPase, i.e., the pump rate, the ATP concentration contains information about the inflow of K\(^+\). ATP regulates the intracellular K\(^+\) concentration by acting as an outflow controller modulating the outflow of K\(^+\) through K\(_{ATP}\) channels, which are inhibited by ATP. This mechanism has only one intermediate between Na-K-ATPase pump rate and the basolateral K permeability.

Tschiya et al. (84) have reported that ATP is the main coupling modulator between Na-K-ATPase and K-channel activity in epithelial cells in the renal proximal tubule; they showed that an increase in Na-K-ATPase activity due to luminal addition of glucose and alanine leads to a 57% decrease in intracellular ATP, from 3.7 to 2.1 mM, followed by an increase in K conductance. Similar experiments (5) report of a reduction from 4.4 to 2.7 mM. These studies show that ATP-sensitive K\(_{ATP}\) channels react to a decrease in ATP caused by an increase in Na-K-ATPase activity during transcellular Na\(^+\) transport. The role of ATP as a regulator is supported by the observation that an increase in K permeability is not seen in ATP-loaded proximal tubule cells (84). The kinetics of the inhibition by ATP has a K\(_i\) of 0.8 mM (in cardiac myocytes) (35). ATP-sensitive K channels have also been found in enterocytes (12). Our controller motif is based on the same type of coupling as is reported for ATP, and our results confirm that this is a mechanism that can provide homeostasis.

For simplicity we did only include consumption of ATP by the Na-K-ATPase. Other usage of ATP can, however, be included without changing the main results as long as these consumptions are approximately constant or increasing during nutrient transport. The only change is that the k\(_{ATP}\) parameter must be adjusted so that the synthesis and consumption balance each other at steady state.

The synthesis of ATP (see Eq. 10) must increase when the intracellular ATP concentration falls in order for the ATP concentration to reach a new steady state. If not, an increase in J\(_{NaK}^\text{in}^\) will deplete the cell from ATP; the rate of consumption is in this case always greater than the rate of production. The rate of consumption can be modeled as a cyclic function (20) with an inhibition of 1.5 mM. The reduction in ATP during transport can thus regulate the K permeability and the outflow of K\(^+\) without impeding the net transport by lowering the Na-K-ATPase pump rate. We have assumed that Na-K-ATPase is saturated...
with respect to ATP in our model, i.e., the flow expression, Eq. 2, does not include [ATP].

Intracellular Ca\(^{2+}\) is another candidate for the regulation of K permeability; Ca\(^{2+}\) is known to increase the opening probability of enterocyte K channels (75), but no clear evidence has been given for a direct link between Na-K-ATPase activity and Ca\(^{2+}\). Cell swelling has, however, been shown to increase the K permeability and interestingly this increase seems to be mediated by an increase in intracellular Ca\(^{2+}\) concentration together with a cell acidification (50). Cell swelling is not included in our enterocyte model, and we have therefore not examined this mechanism.

Other Regulatory Mechanisms

The regulation of cell volume is for enterocytes closely related to cell survivability during transport. The enterocyte has to react not only to an altered luminal nutrient load but also to a changing osmolarity. Exposing enterocytes to hypertonic additions of L-alanine and D-glucose elicits cell swelling followed by regulating volume decrease causing the cell to shrink to a smaller than initial size (50). Volume-activated Cl\(^{-}\) channels play an important part in this regulation, but volume regulation is not directly dependent on active transepithelial Na\(^{+}\) transport or the operation of Na-K-ATPase (57). Many of the transporter proteins are also water transporters, e.g., SGLT1 (101) and GLUT2 (102). We kept the cell volume fixed in our model to make the modeling task feasible.

Being vital for cell survival, the regulation of intracellular volume homeostasis must from the enterocyte’s viewpoint have high priority. For the whole organism that the enterocyte is a part of, however, other transport-related regulatory mechanisms may have just as crucial implications. Regulation of the capacity of nutrient uptake, and the focus of nutrient uptake (sugar, proteins, or lipids), play major roles in securing organ-capacity of nutrient uptake, and the focus of nutrient uptake (sugar, proteins, or lipids), play major roles in securing organ-

Estimation the Amount of SGLT1

To estimate the amount, or number, of SGLT1 transporters in enterocytes, we have used reported maximum absorption rates for active SGLT1-mediated uptake of glucose in jejunal sleeves, reviewed in Ref. 60. This rate varies between 8 to 23 \(\mu\text{mol} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}\) for small animals including mouse, rat, cat, and rabbit, where the smaller animals have higher absorption rates per surface area than larger animals. The absorption rates are given per centimeter squared of smooth mucosal area, which according to Pappenheimer (60) can be thought of as the area of an intestinal segment that has been distended to a circular cylinder. The effective surface area including the villi is three to eight times greater than the smooth surface area. For rats the absorption rate is between 12 to 20 \(\mu\text{mol} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}\) (60). Taking into account the increased surface due to the villi, using a factor of 5, and assuming that most of the villus area is covered with enterocytes, we estimate an absorption rate of 2.4 to 4 \(\mu\text{mol} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}\) of enterocyte covered surface area. A typical rat enterocyte has an apical radii of 3.65 \(\mu\text{m}\) (62), corresponding to a mucosal exposed surface of \(\sim 42 \mu\text{m}^2\) (see Spatial Dimensions of an Absorbing Enterocyte).

From the above, and using a SGLT1 glucose transport rate of 1.5 \(\text{s}^{-1} \cdot (9\cdot 10^{-21} \text{ mol/h})\), we estimate that a single enterocyte contains around 100 to 250 million SGLT1 transporters. The estimate can be considered an average value over all enterocytes. We use 180 million SGLT1 transporters in our simulations.

APPENDIX

Spatial Dimensions of an Absorbing Enterocyte

In its absorbing state the enterocytes have a form as shown in Fig. A1. The upper part is formed like a cylinder, and the lower part is formed like a truncated cone. The cross section of the cells are not perfectly rounded but shaped more like a polygonal giving the enterocytes a honeycomb shape that enables tighter packing than a circular shape (62). For calculations, however, it is adequate to assume a circular shape. For a rat enterocyte the typical dimensions are as in Ref. 62: \(r_m\), radius at the mucosal top = 3.65 \(\mu\text{m}\); \(r_s\), radius at the serosal bottom = 1.9 \(\mu\text{m}\); \(h_o\), height of the tight junctions = 5 \(\mu\text{m}\); and \(h_c\), height of the cone = 28 \(\mu\text{m}\). From these values we get an apical surface of 42 \(\mu\text{m}^2\) (the effective absorptive area is roughly 20 times greater because of the microvilli), a basolateral surface area of the cone and the bottom of 500 \(\mu\text{m}^2\), and a whole cell volume of roughly 900 \(\mu\text{m}^3\). From the composition of enterocytes seen in electron micrographs (51), it is reasonable to assume that the cytoplasm occupies \(\sim 50\%\) of the total cell volume (450 \(\mu\text{m}^3\)).

![Fig. A1. The spatial dimensions of an enterocyte in absorbing state. The apical part of the cell has a cylindrical shape, and the lower part has is shaped like a truncated cone. See text for typical values of \(r_m\), \(r_s\), \(h_o\), and \(h_c\).](https://www.ajpcell.org/doi/10.1152/ajpcell.00068.2013/fig1.png)
Maenz and Cheeseman further uses $^3$H-cytochalasin B binding to the basolateral membrane as an estimate for the number of GLUT2 transporters. At saturated conditions the vesicles was found to bind 8.3 pmol/mg protein. Assuming a one-to-one relationship between the $^3$H-cytochalasin B binding and the number of GLUT2 transporters we get the following: maximum inflow $(V_{\text{in}})$: 137 molecules of glucose per second (8.19·10$^{-13}$ μmol/h); and maximum (corrected) outflow $(V_{\text{out}})$: 66 molecules of glucose per second (3.95·10$^{-13}$ μmol/h) for one single GLUT2 transporter protein.

**Iterative Calculation of Membrane Potentials**

The iterative calculation of membrane potentials is based on the procedure outlined in Ref. 43. It utilizes two nested Newton-Raphson steps to refine the estimates until the conditions for cell and epithelial electroneutrality are met. The goal of the iterative calculation is to adjust the two independent membrane potentials so that both the cell current $I_c = \sum c_i J_i$ (Eq. 7) and the net epithelial current $I_e = \sum e_i J_i$ (Eq. 8) are sufficiently close to zero. The two loops are organized into an inner loop which adjusts $\psi_{in}$ so that $F(\psi_{in}) = 0$, and an outer loop which adjusts $\psi_{out}$ so that $F(\psi_{out}) = 0$.

The Newton-Raphson adjustments require the computation of the derivatives $dF(\psi_{in})/d\psi_{in}$ and $dF(\psi_{out})/d\psi_{out}$. We estimate these by calculating polynomial approximations of $F(\psi_{in})$, treating $\psi_{in}$ and all the other parameters as constants, and $F(\psi_{out})$, treating $\psi_{out}$ and all the other parameters as constants.

The procedure for the iterative method is as follows for each time step in the simulation:

1) Use the membrane potentials from the last time step as starting guesses.

2) Estimate $\psi_{in}$ and $\psi_{out}$ by two nested Newton-Raphson loops.

   a) Find a polynomial approximation of the net cell current $F$ as a function of $\psi_{in}$. This is done by calculating the individual flows for a range of different $\psi_{in}$ values.

   b) Estimate $\psi_{out}$ by an inner loop.

      i) Calculate the individual flows for the current $\psi_{in}$ and $\psi_{out}$.

      ii) Calculate the apical and the basolateral membrane currents,

         $$P = \sum_i c_i J_i$$

         $$P^0 = \sum_i c_i J_i$$

      iii) Calculate the net cell current $I_c = P^0 + P$.

     iv) If $|I_c| = |\sum_i c_i J_i|$ < 10$^{-9}$ pmol/h exit the inner loop with the current $\psi_{in}$ value. Otherwise update $\psi_{in}$ by a Newton-Raphson step: $\psi_{in} = \psi_{in} - F(\psi_{in})/dF(\psi_{in})|_{\psi_{in}}$ where the polynomial approximation of $F(\psi_{in})$ is used to estimate the derivative and then restart the inner loop.

   c) Find a polynomial approximation of the net epithelial current as a function of $\psi_{out}$. This is done by calculating the individual flows for a range of different $\psi_{out}$ values.

   d) Calculate the individual flows for the current $\psi_{in}$ and $\psi_{out}$.

<table>
<thead>
<tr>
<th>Table A1. Flow expressions</th>
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<tbody>
<tr>
<td><strong>Protein</strong></td>
</tr>
<tr>
<td>SGLT1</td>
</tr>
<tr>
<td>Na-K-ATPase</td>
</tr>
<tr>
<td>Coupled NaCl</td>
</tr>
<tr>
<td>GLUT2</td>
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<td>Diffusive flows</td>
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Table A2. Parameters used in the simulations (if not stated otherwise)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>Cell size</td>
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</tr>
<tr>
<td>V₀, cell (cytoplasm volume)</td>
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</tr>
<tr>
<td>A⁺, apical surface area</td>
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<tr>
<td>A⁻, apical absorptive membrane area</td>
<td>500 μm²</td>
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<tr>
<td>Δ⁴, basolateral absorptive membrane area</td>
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SGLT1

<table>
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<tr>
<td>α₂</td>
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<tr>
<td>δ</td>
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Rate constants

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<td>k₆₉</td>
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The macro constants α, β, γ, ε, and θ are given by Eqs. A22–A28 in Ref. 65.

Na-K-ATPase

<table>
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<tr>
<td>KₑGlut</td>
<td>48 mM</td>
</tr>
</tbody>
</table>

Permeabilities

<table>
<thead>
<tr>
<th>Permeability</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PₑNa</td>
<td>3.4 · 10⁻⁴ cm/h</td>
</tr>
<tr>
<td>PₑK</td>
<td>3.0 · 10⁻⁴ cm/h</td>
</tr>
<tr>
<td>PₑCl</td>
<td>3.6 · 10⁻⁴ cm/h</td>
</tr>
<tr>
<td>PₑNaCl</td>
<td>16 · 10⁻⁴ cm/h</td>
</tr>
<tr>
<td>PₑKCl</td>
<td>18 · 10⁻⁴ cm/h</td>
</tr>
<tr>
<td>PₑNaCl</td>
<td>9.0 · 10⁻⁵ cm/h</td>
</tr>
<tr>
<td>PₑKCl</td>
<td>3.6 · 10⁻⁴ cm/h</td>
</tr>
<tr>
<td>PₑCl</td>
<td>14.6 · 10⁻⁴ cm/h</td>
</tr>
<tr>
<td>PₑCl</td>
<td>2.6 · 10⁻³ cm/h</td>
</tr>
</tbody>
</table>

Mucosal and serosal concentrations

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>[G₄₆]</td>
<td>0.1–20 mM</td>
</tr>
<tr>
<td>[K₄₆]</td>
<td>5.4 mM</td>
</tr>
<tr>
<td>[G₄₇]</td>
<td>10 mM</td>
</tr>
<tr>
<td>[K₄₇]</td>
<td>5.4 mM</td>
</tr>
<tr>
<td>[N₄₆]</td>
<td>140 mM</td>
</tr>
<tr>
<td>[C₄₆]</td>
<td>140 mM</td>
</tr>
<tr>
<td>[N₄₇]</td>
<td>140 mM</td>
</tr>
<tr>
<td>[C₄₇]</td>
<td>103 mM</td>
</tr>
</tbody>
</table>

Cell variables

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>[G₄₆]</td>
<td>12 mM</td>
</tr>
<tr>
<td>[K₄₆]</td>
<td>129 mM</td>
</tr>
<tr>
<td>[ATP₄₆]</td>
<td>4 mM</td>
</tr>
<tr>
<td>[ADP₄₆]</td>
<td>49 mM</td>
</tr>
<tr>
<td>[Cl₄₆]</td>
<td>57 mM</td>
</tr>
</tbody>
</table>

---

Table A2.—Continued

<table>
<thead>
<tr>
<th>Amount of protein</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ψNaCl</td>
<td>180 · 10⁶</td>
</tr>
<tr>
<td>ψKCl</td>
<td>13.2 · 10⁶</td>
</tr>
<tr>
<td>ψNa₄₆</td>
<td>49.2 · 10⁶</td>
</tr>
</tbody>
</table>

Regulatory parameters

<table>
<thead>
<tr>
<th>Regulation of Na-K-ATPase</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>k₄₅</td>
<td>5.6 · 10⁴ M⁻¹ · h⁻¹</td>
</tr>
<tr>
<td>k₅₆</td>
<td>10¹⁴</td>
</tr>
<tr>
<td>k₆₅</td>
<td>2.74 · 10² h⁻¹</td>
</tr>
</tbody>
</table>

Regulation of basolateral K-permeability

<table>
<thead>
<tr>
<th>Regulation of basolateral K-permeability</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>k₆₅</td>
<td>10.2 pmol/h</td>
</tr>
<tr>
<td>k₆₅</td>
<td>0.8 mM</td>
</tr>
<tr>
<td>k₆₅</td>
<td>0.5 mM</td>
</tr>
<tr>
<td>k₆₅</td>
<td>87.6 · 10⁻³ cm/h</td>
</tr>
</tbody>
</table>

Other parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>96,485 C/mol</td>
</tr>
<tr>
<td>T</td>
<td>310.15 K</td>
</tr>
<tr>
<td>R</td>
<td>8.314 JK⁻¹ · mol⁻¹</td>
</tr>
</tbody>
</table>

Table footnote: *See Spatial Dimensions of an Absorbing Enterocyte. Parameters are taken from Ref. 97. However, we had to adjust the kₛₐ value to keep microscopic reversibility. The macro constants α, β, γ, ε, and θ are functions of the rate constants, the concentration of intracellular Na⁺ ([Nac]), intracellular glucose ([Gc]), and the membrane potential (ψₑc). They thus change during simulation, but typical values are α, 1.2 · 10⁻⁶; β, 0.8 · 10⁻⁶; γ, 2 · 10⁻⁷; ε, −0.94; θ, 0; ψₑc, 2.2 · 10⁻⁸ (at the end of the long-term simulation). +VₑNaCl, see Ref. 54. *See Ref. 55. VₑNaCl is adjusted so that the coupled NaCl transport makes up ~30% of the total Na⁺ inflow during glucose absorption ([Gac] = 10 mM). *See Ref. 52 and Adjustment of Kinetic Parameters for GLUT2 Integrative Model. *See Refs. 19 and 23. The serosal glucose concentration [Gs] is assumed to be 10 mM under light glucose load; the value is around twice that of arteriolar blood in a near fastening state. (According to Ref. 61 the concentration of glucose in villus capillary can reach up to 30 mM under heavy glucose load.) Initial concentrations used in all simulation except the simulation of short-term change in mucosal sodium where the following was used: [G₄₆] = 15 mM, [N₄₆] = 63 mM, [K₄₆] = 116 mM, and [Cl₄₆] = 60 mM. The initial concentrations are selected close to the steady-state concentrations to avoid startup transients. The figures in RESULTS do not show the startup transients. See Estimation the Amount of SGLT1 for nGlc; the other amounts are estimated based on numbers needed to respond to the inflow of glucose and sodium. The amount of Na-K-ATPase is in the range reported for rabbit kidney cells (34). These parameters are selected to achieve reasonable setpoints and dynamic response (10).

e) Calculate the current over the basolateral membrane, qBasolateral = ΣBasolateral i, and the paracellular current, F = ΣParacellular i.
f) Calculate the net epithelial current F = F⁺ – F⁻.
g) If |F⁺| = |F⁻| + |F| < 10⁻⁹ pmol/h exit the outer loop with the current ψₑm. Otherwise update ψₑm by a Newton-Raphson step: ψₑm = ψₑm – F/[dF(ψₑm)/dψₑm], where the polynomial approximation of F(ψₑm) is used to estimate the derivative and then restart the outer loop.

3) The conditions for electroneutrality are now met. Calculate ψₑc = ψₑm – ψₑm. Output the flows so that they can be integrated to the next time step.

The Newton-Raphson seek is stopped once the net cell current and the net epithelial current are sufficiently close to zero. We have used the following criteria:

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 10⁻⁹ pmol/h</td>
</tr>
<tr>
<td></td>
<td>&lt; 10⁻⁹ pmol/h</td>
</tr>
</tbody>
</table>

For our enterocyte, which has a volume of 450 μm³, these criteria correspond to a maximum charge difference of 2.22 nM/h (monovalent ions). A charge imbalance in this order will only affect the membrane potential by a few tens of a microvolt.

The iterative procedure was tested and verified by simulating the enterocyte model under conditions where the membrane potential can...
be analytically calculated by the Goldman-Hodgkin-Katz equation for membrane potential (\(70\)). The paracellular permeability was set to zero, and only diffusion of Na\(^+\), K\(^+\), and Cl\(^-\) was considered, i.e., no active transport or cotransport. Simulation of these conditions produced the exact same membrane potentials as the analytical solution (results not shown).

The nested procedure can be time consuming since \(\psi_{int}\) has to be fully optimized for each new and improved estimate of \(\psi_{int}\). Nevertheless, in practice the Newton-Raphson method quickly converges and needs mostly less than a total of 10 iterations of the inner loop in our simulations.

**Model Equations**

The following differential equations describes the dynamical states

\[
\begin{align*}
\dot{\Delta} \left( \frac{G_c}{V_c} \right) &= \frac{1}{V_c} \left( \frac{1}{2} J_{\text{Na}^+} - J_{\text{GLUT}} \right) \quad (A2) \\
\dot{[\text{Na}^+]_c} &= \frac{1}{V_c} \left( J_{\text{GLUT}}^{\text{Na}^+} - 3 J_{\text{Na}^+}^{\text{Na}^+} + J_{\text{Cl}^-}^{\text{Na}^+} + J_{\text{Na}^+}^{\text{Na}^+} + J_{\text{Na}^+}^{\text{Na}^+} \right) \quad (A3) \\
\dot{[\text{K}^+]_c} &= \frac{1}{V_c} \left( 2 J_{\text{Na}^+}^{\text{K}^+} + J_{\text{Na}^+}^{\text{K}^+} + J_{\text{Na}^+}^{\text{K}^+} \right) \quad (A4) \\
\dot{[\text{Cl}^-]_c} &= \frac{1}{V_c} \left( J_{\text{Na}^+}^{\text{Cl}^-} + J_{\text{Na}^+}^{\text{Cl}^-} + J_{\text{Na}^+}^{\text{Cl}^-} \right) \quad (A5)
\end{align*}
\]

where the different flow expressions \((J)\) are given in Table A1, and the parameters are given in Table A2. The model is expanded by Eqs. 9 to 11 in the main text to account for regulation of intracellular Na\(^+\) and K\(^+\).

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: K.T. conception and design of research; K.T. and T.D. performed experiments; K.T., T.D., and P.R. analyzed data; K.T. and P.R. approved final version of manuscript.

**REFERENCES**

AN INTEGRATIVE ENTEROCYTE MODEL


