Modeling of transcellular Ca transport in rat duodenum points to coexistence of two mechanisms of apical entry

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Slepchenko, Boris M., and Felix Bronner. Modeling of transcellular Ca transport in rat duodenum points to coexistence of two mechanisms of apical entry. Am J Physiol Cell Physiol 281: C270–C281, 2001.—Employing realistic parameters, we have demonstrated that a relatively simple mathematical model can reproduce key features of steady-state Ca transport with the assumption of two mechanisms of Ca entry: a channel-like flux and a carrier-mediated transport. At low luminal [Ca2+] (1–5 mM), facilitated entry dominates and saturates with Km = 0.4 mM. At luminal [Ca2+] of tens of millimolar, apical permeability is dominated by the channel flux that in turn is regulated by cytosolic Ca2+. The model reproduces the linear relationship between maximum Ca2+ transport rate and intestinal calbindin D9K (CaBP) content. At luminal [Ca2+] > 50 mM, local sensitivity analysis shows transcellular transport to be most sensitive to variations in CaBP. At low luminal [Ca2+], transport becomes sensitive to apical entry regulation. The simulations have been run within the Virtual Cell modeling environment, yielding the time course of external Ca2+ and spatiotemporal distributions of both intracellular Ca2+ and CaBP. Coexistence of two apical entry mechanisms accords with the properties of the duodenal Ca2+ transport protein CaT1 and the epithelial Ca2+ channel ECaC.

intestine; calcium entry; brush border; calbindin D9K; intracellular calcium diffusion

TRANSEPITHELIAL CALCIUM TRANSPORT in the small intestine follows two pathways: a transcellular, vitamin D-dependent route, localized largely to the duodenum but also present to a minor degree in the upper jejunum (2). The paracellular process is not subject to regulation beyond that affecting tight junction function generally. The transcellular pathway is saturable, requires metabolic energy, and is upregulated in pregnancy and calcium deficiency and downregulated with increasing calcium, with regulation mediated by the hormone-like actions of vitamin D (2, 5). Thus transcellular calcium movement plays a significant role under conditions of low calcium intake, transporting a substantial proportion of absorbed calcium (2, 5). When calcium intake goes up, an increasing proportion is absorbed via the paracellular route, predominantly in jejunum and ileum.

It is now widely recognized that transcellular calcium transport is a three-step process consisting of passive calcium entry at the brush border, facilitated diffusion through the cell, and active extrusion at the basolateral membrane (2, 7, 13) (see diagram in Fig. 1). Calcium extrusion is mediated by the Ca2+-ATPase (8) and, to a minor degree, by a Na+/Ca2+ exchanger (19). It has been shown experimentally (5) that the extrusion capacity is more than adequate, and therefore this step does not appear to be rate limiting in the overall transport. A major molecular expression of vitamin D regulation is calbindin D9K (CaBP), a cytosolic protein discovered by Wasserman and colleagues (28), which, by binding calcium, has been shown (5, 15, 29) to act like an intracellular ferry, thereby increasing the rate of intracellular calcium diffusion. The role of CaBP in facilitating intracellular calcium diffusion has been demonstrated by mathematical modeling (7). On the other hand, the molecular nature of the apical entry mechanism has remained obscure until recently identified structures, the epithelial Ca2+ channel (ECaC) in rabbit duodenum, jejunum, and kidney (11, 13) and the duodenal calcium transport protein CaT1 in rat duodenum and proximal jejunum (24), were shown to mediate calcium entry. These proteins have 75% structural similarity and exhibit similar calcium transport features. However, unlike ECaC, CaT1 is not responsive to 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] administration or to calcium deficiency (10, 24). Controversy still exists as to how these structures transport calcium ions. Both molecules have pore regions that would suggest a channel-type mechanism (11, 24). However, their macroscopic kinetic properties, as well as the fact that no single-channel calcium currents have been detected, favor a facilitated transporter mechanism that requires calcium binding to a transporter. Thus each of these two structures, CaT1 or ECaC, can act as a calcium channel and transporter.

In the present study, a relatively simple mathematical model is used to analyze the typical features of calcium transport in rat duodenum (21–23). On the
basis of the volume of the small intestine of rats and their calcium intake, one can estimate that, after a meal, calcium concentrations in the lumen can vary up to >50 mM. Experimentally, solutions of widely ranging calcium concentrations were instilled into intestinal loops, with calcium transport then measured in animals that had been on different calcium intakes and, therefore, had varying levels of CaBP (5). In experiments done at tens of millimolar of soluble luminal calcium, with the cellular component of transepithelial calcium transport was well approximated by a Michaelis-Menten relationship, with $V_m = 21 \mu$mol·h$^{-1}$·g$^{-1}$ and $K_m = 51$ mM, where $V_m$ is the maximum rate of luminal calcium efflux and $K_m$ is the calcium concentration at $V_m/2$. Moreover, $V_m$ is a positive, linear function of the cellular calbindin $D_{9K}$ content (5, 21). In the 1–5 mM range, however, as evident from intestinal sac experiments (5), transepithelial calcium saturates with $K_m = 0.35$ mM and $V_m = 2.2 \mu$mol·h$^{-1}$·g$^{-1}$, findings that translate into much higher brush-border permeability. Interestingly, a value of $K_m = 0.35$ mM is in agreement with the experimental data obtained recently in studies of CaT1 and ECaC (11, 24) done at low luminal calcium concentrations.

Employing realistic parameters, we have demonstrated here that the model reproduces the key experimental features of steady-state calcium transport if the coexistence of two mechanisms of calcium entry, a channel-like flux and a carrier-mediated transport, is assumed. The mechanism of facilitated entry dominates at low luminal calcium concentrations and saturates with $K_m = 0.4$ mM. At luminal calcium concentration in the range of tens of millimolar, channel-like flux regulated by cytosolic calcium at the inner brush border largely determines brush-border permeability. Given the similarity in the transport properties of ECaC and CaT1, both structures can, as mentioned above, function at low and high luminal calcium concentrations. In other words, each can function as a transporter at low luminal calcium concentrations and as a channel at high luminal calcium concentrations (see also DISCUSSION).

The model has been used to simulate the time dependence of luminal calcium in loop experiments (5) under various initial conditions. The simulations have been run within the Virtual Cell environment (25), which allows one to obtain spatiotemporal distributions of intracellular calcium and CaBP as well as the time course of external calcium. In addition, we have used the model to analyze the effect of vitamin D on calcium entry.

**MODEL DESCRIPTION**

To analyze transcellular calcium transport, we have used a one-dimensional model of intracellular calcium dynamics in the presence of a mobile buffer. We let $c$ denote the free calcium concentration and $b$ represent the concentration of calcium bound to CaBP. The model is then described by the equations

$$\frac{\partial c}{\partial t} = D_c \frac{\partial^2 c}{\partial x^2} + \cdots + R \quad (1)$$

$$\frac{\partial b}{\partial t} = D_b \frac{\partial^2 b}{\partial x^2} - R \quad (2)$$

where $D_c$ and $D_b$ are diffusion coefficients of calcium and CaBP, respectively, and $R$, the reaction rate of calcium binding to CaBP, is determined by simple mass action kinetics

$$R = -k_{on}(B_0 - b)c + k_{off}b \quad (3)$$

with $k_{on}$ and $k_{off}$ denoting kinetic constants and $B_0$, representing the total concentration of CaBP. The ellipsis in Eq. 1 represents the rates of all intracellular processes in which calcium can participate, such as binding to fixed binding sites or being pumped into internal calcium stores.

Equation 1 is subject to the boundary conditions that prescribe fluxes of apical calcium entry ($J_a$) and of calcium extrusion at the basolateral membrane ($J_b$)

$$-D_c \frac{\partial c}{\partial x} \bigg|_{x=0} = J_a, \quad -D_c \frac{\partial c}{\partial x} \bigg|_{x=L} = J_b \quad (4.1)$$

while the boundary conditions for Eq. 2 reflect zero flux of CaBP at the cell membrane

$$\frac{\partial b}{\partial x} \bigg|_{x=0} = 0, \quad \frac{\partial b}{\partial x} \bigg|_{x=L} = 0 \quad (4.2)$$

In Eqs. 4.1 and 4.2, $L$ denotes the length of the cell.

Assuming steady calcium flux and near-instantaneous buffering, the model reduces to a system of nonlinear algebraic equations for the total calcium flux density

$$J = -D_c \frac{\partial c}{\partial x} - D_b \frac{\partial b}{\partial x}$$

and the concentrations of intracellular calcium at the boundaries, $c_0 = (c)_{x=0}$ and $c_L = (c)_{x=L}$ (see APPENDIX A) such that

$$J = J_a \quad (5.1)$$
for apical calcium entry (1st stage)

\[ J = \frac{(c_\text{-} - c_\text{+})}{L} \left( D_{\text{c}} + \frac{D_{\text{p}} B_{\text{K}}}{(c_\text{-} + K)(c_\text{+} + K)} \right) \]  

(5.2)

for facilitated diffusion (2nd stage) and

\[ J = J_b \]  

(5.3)

for calcium extrusion (3rd stage). In Eq. 5.2, \( K \) is the dissociation constant for calcium binding to CaBP, \( K = k_{\text{off}}/k_{\text{on}} \). The experimentally determined value of \( K \) is 0.43 \( \mu \text{M} \) (7).

As mentioned above, calcium is extruded from the intestinal cell at the basolateral membrane, predominantly via \( \text{Ca}^{2+} \)-ATPase pumps. The rate of \( \text{Ca}^{2+} \)-ATPase pumping is commonly described by a Hill-type equation (17). It is known that in some cell types the plasma membrane pumps exhibit a threshold behavior as a function of cytosolic calcium, with the rate increasing in a near-linear fashion beyond the threshold (9).

Taking this into account, we use the following expression for \( J_b \)

\[ J_b = \begin{cases} V_p \left( c_\text{+} - \frac{c_\text{0}}{c_\text{+} + K_{\text{p}}} \right), & c_\text{+} > c_\text{0} \\ 0, & c_\text{+} \leq c_\text{0} \end{cases} \]  

(6)

The threshold calcium concentration, \( c_\text{0} \), in Eq. 6 corresponds to an average concentration of free intracellular calcium at rest, estimated as 0.07 \( \mu \text{M} \) (see also Table 1 for other parameter values). The maximum flux density of calcium extrusion, \( V_p \), has been determined as 150 \( \mu \text{mol} \cdot \text{h}^{-1} \cdot \text{g}^{-1} \), and the experimental value for the dissociation constant \( K_{\text{p}} \) of calcium binding to a pump is 0.25 \( \mu \text{M} \) (5). The second term in the upper line of Eq. 6 represents the leak of calcium into a cell across the basolateral membrane, a flux balanced by pumping under rest conditions.

In the following, the model for apical calcium entry is presented in detail. We show that the model of a channel regulated by intracellular calcium is consistent with the experimental data obtained at high luminal calcium. The model fails, however, in the physiological range of relatively low luminal calcium concentrations, where brush-border permeability appears to be much higher, whereas flux saturates at a much lower level. This can be explained by a transporter-mediated mechanism of calcium apical entry. The coexistence of both mechanisms then explains experimental findings over the full range of luminal calcium concentration.

**RESULTS**

*Transcellular calcium flux at high luminal calcium concentrations.* Because the \( V_p \) is more than seven times greater than the \( V_m \), extrusion is not a limiting factor, and therefore all calcium transported to the basolateral membrane can be readily pumped out of the cell (see below for sensitivity analysis). As a result, the intracellular calcium concentration at the basolateral membrane will be close to \( c_\text{0} \) at any time, and we can set \( c_\text{+} \approx c_\text{0} \) in Eq. 5.2. We also take into account that \( D_{\text{p}} B_{\text{K}}/D_{\text{c}} c_\text{0} \approx 1 \) when there is an adequate amount of CaBP. This means that only a small fraction of calcium flux is in the form of free calcium in the presence of CaBP. Equation 5.2 can therefore be reduced to

\[ J = \frac{D_{\text{p}} B_{\text{K}}}{L(c_\text{0} + K)} \frac{\Delta c}{(\Delta c + k_{\text{m}})} \]  

(7.1)

where \( \Delta c \) is by definition \( c_\text{-} - c_\text{0} \) and \( k_{\text{m}} \) is by definition \( c_\text{0} + K = 0.5 \mu \text{M} \). Equation 7.1 helps us understand why the experimental data obtained at high luminal calcium concentrations (\{\text{Ca}^{2+}\}_\text{lumen}\}) can be well approximated by a Michaelis-Menten function

\[ J = \frac{V_m \{\text{Ca}^{2+}\}_\text{lumen}}{\{\text{Ca}^{2+}\}_\text{lumen} + K_{\text{m}}} \]  

(7.2)

A comparison of Eqs. 7.1 and 7.2 immediately yields \( k_{\text{m}} = \beta k_{\text{m}} \) with \( \beta = 10^{-5} \) and

\[ \Delta c = \beta \{\text{Ca}^{2+}\}_\text{lumen} \]  

(8.1)

\[ V_m = \frac{D_{\text{p}} K}{L(K + c_\text{0})} B_\text{t} \]  

(8.2)

Equation 8.2 states, in agreement with experimental findings (5), that the maximum calcium transport is linearly dependent on the total amount of CaBP. The latter is therefore a limiting factor at high luminal calcium concentrations. We confirm this conclusion below by means of sensitivity analysis.

Interestingly, the slope of this dependence is determined by the CaBP diffusion coefficient. To estimate \( D_{\text{b}} \) from Eq. 8.2, we must evaluate the CaBP concentration, \( B_\text{t} \), and convert experimental flux values, ex-

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**Table 1. Parameters used in simulations**

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<th>Symbol/Value/Units</th>
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<tr>
<td>Maximum flux density of calcium extrusion</td>
<td>( V_p )</td>
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</tr>
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<tr>
<td>Dissociation constant for cytosolic calcium binding to calbindin ( D_{\text{OK}} )</td>
<td>( K )</td>
</tr>
<tr>
<td>Diffusion coefficient of calcium in cytosol</td>
<td>( D_c )</td>
</tr>
<tr>
<td>Diffusion coefficient of calbindin</td>
<td>( D_{\text{b}} )</td>
</tr>
<tr>
<td>Maximum transporter flux density</td>
<td>( V_{\text{tr}} )</td>
</tr>
<tr>
<td>Dissociation constant for luminal calcium binding to a transporter</td>
<td>( K_{\text{tr}} )</td>
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</table>

*Value used for Figs. 2 and 3 and Table 2, channel model; **value used for Figs. 5–9, complete model.*
pressed as \( \mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1} \), into the units of flux density, \( \mu\text{M}\cdot\mu\text{m}\cdot\text{s}^{-1} \). The conversion of units yields 3.6 \( \mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1} \) \( = (10^9/N\sigma_0) \mu\text{M}\cdot\mu\text{m}^3\cdot\text{s}^{-1} \), where \( N \) is the number of cells per gram of tissue and \( \sigma_0 \) is the cross-sectional area of a cell. Assuming \( N = 10^8 \) and \( \sigma_0 = 10^2 \mu\text{m}^2 \) \((5, 7)\), we arrive at the conversion relationship \( 1 \mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1} = (10^9/3.6) \mu\text{M}\cdot\mu\text{m}^3\cdot\text{s}^{-1} \), the value used below. The CaBP content can be estimated from the fact that rats on a low-calcium diet \( (0.06\% \text{ Ca, } 0.2\% \text{ P}) \) will have up to 100 nmol of Ca bound to CaBP per gram of mucosa \((6)\), with two calcium binding sites per one CaBP \((27)\). (However, the transport equations account for only one \( \text{Ca}^{2+} \)/CaBP, because the other is very tightly bound.) With the assumption, as above, of \( 10^8 \) cells per gram of mucosa, each with a volume of \( \sim 2,000 \mu\text{m}^3 \), \( B_1 \) equals 250 \( \mu\text{M} \). With \( K = 0.43 \mu\text{M} \), \( c_0 = 0.07 \mu\text{M} \), \( L = 20 \mu\text{m} \), and \( B_0 = 250 \mu\text{M} \), we then obtain for \( D_b \) the value of 56.8 \( \mu\text{m}^2\text{s}^{-1} \). Interestingly, the estimate of \( D_b \) from the comparison with the self-diffusion coefficient of calcium in cytoplasm yields a similar value. Indeed, \( D_b \) is estimated as 300 \( \mu\text{m}^2\text{s}^{-1} \) \((1)\). The diffusion coefficient of CaBP can then be roughly evaluated as \( D_i = D_i(m_{CaBP}m_{CaBP})^{1/3} = [1/6]D_c = 50 \mu\text{m}^2\text{s}^{-1} \), where \( m \) refers to molecular mass.

We next turn to \( \text{Eq. 5.1} \) to show that the hypothesis of calcium apical entry via channels, regulated by intracellular calcium, is consistent with the experimental data on calcium transport at high luminal calcium concentrations. At the brush border, the calcium flow through a pore would take place at a favorable electrical gradient and can be approximated by the Goldman-Hodgkin-Katz equation (Ref. 14, p. 53). This equation, because the membrane potential is normally positive and \( [\text{Ca}^{2+}]_{\text{lumen}} \) is greater than \( c_- \) by several orders of magnitude, can be simplified to

\[
J_s = P[\text{Ca}^{2+}]_{\text{lumen}}
\]  

\( (9) \)

with \( P = g(\phi/\phi_0)[1 - \exp(\phi/\phi_0)]^{-1} \), where \( \phi \) is the membrane potential, \( \phi_0 = RT2F (R \text{ is the gas constant}, \ T \text{ is the absolute temperature}, \ F \text{ is the Faraday number}) \), and the channel permeability \( g \) can be a function of the membrane potential and can be regulated by binding of intracellular calcium and/or some other molecules to a channel (channel gating). Although \( P \) is nominally a function of the membrane potential, \( \phi \) does not explicitly enter the equations below because we are concerned only with changes in the chemical potential.

There is increasing experimental evidence that the apical entry is regulated by intracellular calcium \((7, 12, 24)\). Mathematically, this means that \( P \) should be a function of \( c_- \). To determine this function, we use \( \text{Eq. 8.1} \) to rewrite \( \text{Eq. 7.2} \) as follows

\[
J = \frac{V_n\beta[\text{Ca}^{2+}]_{\text{lumen}}}{(c_- + K_i)}
\]  

\( (10) \)

where \( K_i = k_m - c_0 = K \). From \( \text{Eqs. 5.1} \) and \( 9 \), we then conclude that \( P \propto (c_- + K_i)^{-1} \). This can be interpreted as channel inhibition by the binding of intracellular calcium to the channel-inhibiting binding sites with a dissociation constant \( K_i \). In this case, channel permeability is proportional to the fraction of uninhibited channels

\[
P = \frac{P_0 K_i}{K_i + c_-}
\]

\( (11) \)

where \( P_0 \) is the maximum permeability. It is interesting to note that the functional form of \( \text{Eq. 11} \) with \( K_i = 0.5 \mu\text{M} \) was postulated earlier \((7)\). By combining \( \text{Eqs. 5.1, 9, 10, and 11} \), we find

\[
P_0 = \frac{V_n\beta}{K_m} \approx \frac{V_n}{K_m}
\]

We thus conclude that brush-border permeability determines the ratio of the Michaelis-Menten parameters. The value of \( P_0 \) obtained for \( V_n = 21 \mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1} \) is 0.01 \( \mu\text{m}^{-1}\text{s}^{-1} \).

The numerical solution of \( \text{Eqs. 5, 6, 9, and 11} \) supports the conclusions of the preliminary analysis above. Parameter values used in computations are given in Table 1. We first fixed the concentration of CaBP at 250 \( \mu\text{M} \) and computed transcellular calcium flux \( J \) \((\text{along with} \ c_- \text{ and} \ c_+) \) as a function of \( [\text{Ca}^{2+}]_{\text{lumen}} \). These results are presented in Table 2 and Fig. 2. \( J, c_- \), and \( c_+ \), as well as the fraction of flux carried by free calcium, were obtained by solving the system of nonlinear algebraic \( \text{Eqs. 5.1–5.3} \) by means of Newton iterations. The results are accurate to \( 10^{-5} \) (relative error). An average free calcium concentration was determined for selected values of \( [\text{Ca}^{2+}]_{\text{lumen}} \) by running full spatial simulations based on \( \text{Eqs. 1–4} \) for a sufficiently long time. The results are accurate within 1% of relative error.

Figure 2 shows that, as expected, the model gives a virtually perfect Michaelis-Menten curve, with \( V_n = 23.3 \mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1} \) and \( K_m = 48.7 \mu\text{M} \). The numerical results in Table 2 confirm the assumptions made in the preliminary analysis. The concentration of intracellular calcium at the basolateral membrane undergoes only slight changes (Table 2, \( c_- \)) and CaBP carries >90% of the transported calcium (Table 2, Free calcium flux as a fraction of total).

To better understand the key elements of the three-step mechanism of steady-state calcium transport, we performed a sensitivity analysis (the equations for sensitivities are derived in \textit{Appendix B}). The logarithmic sensitivity \( \delta \log J/\delta \log \alpha \), where \( \alpha \) stands for any model parameter, represents a relative change in calcium flux, \( J \), divided by a small relative change in the parameter. The results of the sensitivity analysis for the parameter set of Table 1 are given in Fig. 3. These results indicate that at high \( [\text{Ca}^{2+}]_{\text{lumen}} \), calcium transport is most sensitive to the CaBP content, while at low \( [\text{Ca}^{2+}]_{\text{lumen}} \) it is mainly controlled by the brush-border permeability. Thus, in the range of high luminal calcium concentrations, in agreement with the previous analysis, the Michaelis-Menten parameter \( V_m \) is determined by the properties of CaBP, while the ratio \( V_m/K_m \) depends largely on the brush-border permeability. Also, as expected, the sensitivity of calcium transport to the parameters of calcium extrusion is low over
the whole range of luminal calcium concentrations. Interestingly, there can be a change in sign of the sensitivity of the calcium transport with respect to the affinity $K$ of CaBP to calcium. This is consistent with the fact that $J$ is a non-monotone function of $K$ (see Eq. 5.2).

Calcium transport at low luminal calcium concentrations. Experiments with everted duodenal sacs, carried out at relatively low luminal calcium concentrations, indicated flux saturation with $K_m = 0.35$ mM and $V_m = 2.2 \mu$mol·h$^{-1}$·g$^{-1}$ (5). Experiments on ECaC and CaT1 also indicated a value of $K_m$ in the range of 0.2–0.4 mM (11, 24). These data yield a brush-border permeability of the order of $V_m/K_m = 0.2 \mu$m/s, a value that is 20 times greater than the estimate of $P_0$ obtained above. This difference indicates the presence of an additional entry mechanism with substantially higher brush-border permeability but saturating at relatively low luminal calcium. In principle, this could be a different channel, with much higher permeability and an inhibiting binding site for intracellular calcium with $K_i$ of the order of 0.01 mM; this is unlikely. In light of the reported macroscopic kinetic properties of CaT1 and ECaC, the mechanism is more likely to be a transporter, as previously suggested (30). With this hypothesis we show below that the higher brush-border permeability and the low-level saturation can both be accounted for, consistent with the results of the previous section. Figure 4 displays a diagram describing a transporter. In the two states on the left-hand side of Fig. 4, $T_0$ and $T_1$, the calcium binding site of a transporter is exposed to external calcium, and in states $T_2$ and $T_3$ on the right-hand side, it is exposed to internal calcium. To derive the expression for the transporter-mediated calcium flux, $J_{tr}$, we consider steady flux conditions. In addition, we assume that calcium binding on the luminal side of the membrane is near instantaneous. $J_{tr}$ can then be found from the following equations

$$J_{tr} = k_1[T_1] - k_3[T_2] = k_2[T_3] - k_3[T_0] = k_2(T_2 - \frac{c}{K_{i2}}T_3)$$

$$[T_0][Ca^{2+}]_{lumen} = K_i[T_1]$$

In Eqs. 12, $k_1$ and $k_3$ are the rates of transition between transporter states as shown in Fig. 4; $k_2$ is the rate of calcium dissociation from the transporter in the cytosol; and $K_i$ and $K_{i2}$ are the inside and outside transporter dissociation constants, respectively, for

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<th>$c_+, \mu$M</th>
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<tr>
<td>160</td>
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<td>0.127435</td>
<td>17.1193</td>
<td>0.059806</td>
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<tr>
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<td>0.128416</td>
<td>17.3665</td>
<td>0.062642</td>
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<tr>
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<td>0.129353</td>
<td>17.5958</td>
<td>0.06545</td>
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<tr>
<td>190</td>
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<td>0.130186</td>
<td>17.8094</td>
<td>0.068231</td>
<td></td>
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<tr>
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<td>0.130999</td>
<td>18.0093</td>
<td>0.070896</td>
<td>0.757238</td>
</tr>
</tbody>
</table>

[Ca$^{2+}]_{lumen}$, luminal calcium concentration; $c_-$, apical intracellular calcium concentration; $c_+$, basolateral membrane intracellular calcium concentration; $J$, total calcium flux.
binding calcium. Solving the system of Eqs. 12 for $J_{tr}$ (see APPENDIX C for details) yields

$$J_{tr} = \frac{V_{tr}[Ca^{2+}]_{\text{lumen}}}{K_{tr} + [Ca^{2+}]_{\text{lumen}}}$$  \hspace{1cm} (13)

where the parameter $V_{tr}$ is determined by the total density of transporter binding sites and a certain average of the kinetic constants $k_1^-$, $k_3^-$, and $k_2^+$, whereas the parameter $K_{tr}$ is determined by the dissociation constant $K_o$, modified by a ratio of kinetic constants as specified in APPENDIX C.

Eqs. 9, 11, and 13, combined, provide the complete description of calcium apical entry as

$$J_a = \left( P_0 \frac{K_i}{K_i + c_-} + \frac{V_{tr}[Ca^{2+}]_{\text{lumen}}}{K_{tr} + [Ca^{2+}]_{\text{lumen}}} \right) [Ca^{2+}]_{\text{lumen}}$$  \hspace{1cm} (14)

The expression inside the parentheses in Eq. 14 can be interpreted as the macroscopic permeability of the apical membrane, modulated by both intracellular and luminal calcium. With the parameters $V_{tr}$ and $K_{tr}$ set so that $P_0 \ll V_{tr}/K_{tr}$ and $V_{tr}/[Ca^{2+}]_{\text{lumen}} \ll P_0$ in the range of tens to milimolar of luminal calcium, then, at relatively low $[Ca^{2+}]_{\text{lumen}}$ (on the order of $K_{tr}$ or smaller), the brush-border permeability will be much greater than $P_0$, while tending to approach $P_0$ as $[Ca^{2+}]_{\text{lumen}}$ increases. At high luminal calcium concentrations, therefore, simulations based on the complete model of the apical entry (Eq. 14) will yield results similar to those described in the preceding section, whereas at low luminal calcium concentrations, entry saturates in accordance with Eq. 13. Figure 5 shows the results obtained with $V_{tr} = 2.2 \, \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$, $K_{tr} = 0.2 \, \text{mM}$, $P_0 = 0.01 \, \mu\text{m/s}$, and all other parameters as defined in Table 1. At high luminal calcium, the simulation results are again well approximated by a Michaelis-Menten equation, with $V_m = 21.3 \, \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$ and $K_m = 50.0 \, \text{mM}$. At low luminal calcium, a plateau is reached (see Fig. 5, inset), characterized by $V_m = 3.1 \, \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$ and $K_m = 0.4 \, \text{mM}$.

Finally, we used the complete model to determine the steady-state spatial distributions of free and bound
Calcium absorption and transport in duodenal loops. In loop experiments (21–23), the intestine is exteriorized from an anesthetized, laparotomized animal, the most proximal 10 cm of the small intestine is tied proximally and distally, and a calibrated quantity of a calcium-containing buffer is instilled into the previously rinsed intestinal loop. The decrease in calcium concentration as a function of time is established on the basis of measurements in a series of loops; a single time point is typically the mean of three to six loop measurements. Figure 7A represents typical results.

To simulate these findings, it is necessary to take into account both paracellular and transcellular calcium movement, with the rate of the former 0.13–0.16 h⁻¹ (5). Equation 15 describes the time-dependent changes in the luminal calcium concentration

$$ V_l \frac{\partial [Ca^{2+}]_{\text{lumen}}}{\partial t} = -S_l (J_{\text{para}} + J_a) \quad (15) $$

where $V_l$ and $S_l$ are the loop volume and internal surface area, respectively; $J_{\text{para}} = k[Ca^{2+}]_{\text{lumen}}$ is the flux density of the paracellular transport with the appropriate rate constant $k$, and $J_a$ is the flux density of calcium entry at the brush border from Eq. 14. To solve Eq. 15, we need to estimate the loop surface-to-volume ratio, $\sigma_l = S_l/V_l$. Because $J_a$ is, according to Eq. 14, significantly regulated internally, Eq. 15 has to be solved simultaneously with Eqs. 1–4, 6, and 14. To do this rigorously, one has to fill in the ellipsis of Eq. 1 by introducing the mechanisms of intracellular calcium uptake. This, however, leads to a myriad of additional parameters with uncertain values. If these mechanisms are ignored, it is still possible to obtain reasonable simulation results in the two limiting cases of high and low initial lumen calcium concentrations. Because the principal interest is to simulate events that have a time scale of minutes and hours (cf. Fig. 7A), the very fast component of intracellular calcium uptake, i.e., rapid binding to fixed sites, may be ignored inasmuch as equilibrium is attained very rapidly and the binding capacity is low.

At low initial luminal calcium concentration, the process of absorption is brief. Therefore, the relatively slow uptake by intracellular calcium stores is likely to have little effect on calcium transport. When the initial luminal calcium concentration is high, the other of the two limiting situations, intracellular calcium uptake by fixed sites and via endoplasmic calcium pumps, is bound to be small compared with total transcellular calcium transport and will have been completed well before transport is completed. Hence, it again can be ignored. At intermediate luminal calcium concentrations, however, results of simulation studies that do not take intracellular calcium uptake into account may deviate signficantly from the experimental findings.

The simulation results displayed in Fig. 7B have utilized the parameters of a complete model (Table 1).
plus two additional parameters, i.e., \( \sigma_1 = 0.015 \, \mu m^{-1} \) and \( k = 0.0022 \, \mu m/s \), which in turn yield \( k \cdot \sigma_1 = 0.12 \, h^{-1} \), a value comparable to what has been found experimentally (5). A typical radius of an intestinal loop is \( r \approx 2 \, mm \). This value would yield a surface-to-volume ratio of \( 2/r = 0.001 \, \mu m^{-1} \). However, intestinal villi markedly increase the available surface area. A 15-fold surface amplification factor is compatible with the estimate (7) of \( 10^8 \) cells/g duodenum used above.

At initial luminal calcium concentrations of 1 mM or 50–200 mM, there is good agreement between the experimental findings (Fig. 7A) and the simulation results (Fig. 7B). In the intermediate range of 10–25 mM, agreement between experiment and simulation is relatively poor, intracellular calcium uptake having been ignored. The discrepancy between simulation and experiment in this range of luminal calcium can, however, provide information on the rate and capacity of intracellular calcium uptake by duodenal cells. For instance, the discrepancy in calcium absorption at an initial luminal calcium concentration of 25 mM (Fig. 7) indicates that intracellular calcium uptake is complete at 45 min. A rough estimate of uptake capacity yields a value of 25 nmol/10^6 cells. Interestingly, these estimates are consistent with the experimental finding (4) that isolated duodenal cells immersed in 3 mM calcium have taken up calcium at the rate of 10 nmol (10^6 cells)^{-1}h^{-1}, with uptake not yet complete at 30 min.

**Effect of vitamin D on calcium entry.** As shown previously (5, 15, 29) and earlier in this paper, calbindin D<sub>9K</sub>, a 1,25(OH)<sub>2</sub>D<sub>3</sub>-dependent protein, significantly facilitates transcellular calcium transport. At the same time, calbindin D<sub>9K</sub> acts as a mobile calcium buffer that helps keep intracellular free calcium at a low level, even when there is a large steady-state calcium flux through the cytosol. In the case of vitamin D deficiency, therefore, when no calbindin D<sub>9K</sub> is expressed in the transporting duodenal cell, a prohibitively high concentration of free cytosolic calcium would build up unless vitamin D also upregulated calcium entry. Indeed, our model (Eqs. 5.1–5.3, 6, and 14) predicts that in the duodenal cells exposed to 50 mM of luminal calcium in the absence of CaBP, the concentration of free calcium at the inner brush border will rise to >6 \( \mu M \). To keep free calcium concentration <1 \( \mu M \), the brush-border permeability must be reduced to 7% of its nominal value. We thus conclude that the vitamin D-independent component of calcium entry, presumably mediated by CaT1, amounts to <10% of the total calcium entry that takes place in the presence of vitamin D, with the vitamin D-dependent ECaC then mediating the increase in calcium entry.

Experiments with brush-border vesicles (18) showed that in the case of vitamin D deficiency, calcium entry decreased by some 30–50%. This value was probably an underestimate because in vesicles, unlike intact cells, the calcium influx is substantially reduced as a result of calcium accumulation inside a vesicle. The vitamin D dependence of calcium entry can also be inferred from in situ experiments (21–23) in which both the saturable component of calcium transport and the CaBP content were evaluated. As shown above, the brush-border permeability can be estimated from the Michaelis-Menten parameters as being equal to \( V_m/K_m \), whereas the CaBP content is a measure of the degree of vitamin D deficiency or sufficiency. Our model also permits estimation of the effect of vitamin D on calcium entry if two additional assumptions are made: 1) the vitamin D-insensitive component constitutes 7% of the total of calcium entry (see the estimate above), and 2) transcellular calcium transport is a linear function of the CaBP content.

Figure 8 is a comparison of experimental findings with a modeling function. The experimental data for the transcellular transport with \( K_m \) values relatively close to 50 mM (Table 3) were obtained under similar conditions in animals of varying ages and varying levels of vitamin D sufficiency (22, 23). Permeability was calculated as \( V_m/K_m \). The permeability values of the modeling function were derived from the modeled linear relationship between calcium flux and CaBP content. To facilitate comparison, all values were normalized to their respective maxima. The reasonable agreement between the experimental data and the
modeling function supports the assumptions made above. In particular, taking into account the vitamin D dependence of calcium entry from Fig. 8, we can now compute transcellular calcium transport as a function of the luminal calcium concentration for a wide range of CaBP concentrations. The computation results in Fig. 9, A: simulated calcium flux, $J$, as a function of luminal calcium concentration at varying tissue concentrations of CaBP ($[\text{CaBP}]$). B: relationship between CaBP content and maximum calcium transport ($V_m$).

As shown above, at a macroscopic level both mechanisms result in a Michaelis-Menten-type equation for calcium transport, but with very different values of $K_m$. As a consequence, when luminal calcium is low (1–5 mM), the facilitated entry mechanism dominates calcium transport but saturates at a relatively low flux level. In the range of tens of millimolar of luminal calcium, apical transport is largely determined by the channel flow of calcium. Indeed, as follows from Table 2, the channel permeability of 0.015 μm/s, which fits the experimental data at high luminal calcium, yields only 0.5 μmol·h$^{-1}$·g$^{-1}$ of calcium flux at $[\text{Ca}^{2+}]_{\text{lumen}} = 1$ mM. With a reasonable channel density of $10^3$ per cell and the fraction of open channels of the order of 0.01 (20), this calcium flux translates into a single-channel current amplitude of 0.03 pA, well below the limits of detectability. This can explain why single-channel events associated with calcium influx have not been detected (24). The model, however, predicts that in the range of tens of millimolar of luminal calcium, these events should become detectable.

The channel mechanism assumes importance under conditions of calcium deficiency when an animal is faced with the opportunity of increased calcium intake. In the absence of a channel mechanism, the relatively low calcium concentrations that might be available for absorption from the gut are not sufficient to activate a facilitated transport system. Instead, calcium transport is largely controlled by the passive diffusion of calcium through a calcium-selective channel, as predicted by Tables 1 and 2.

**DISCUSSION**

The mathematical model of transcellular calcium transport in rat duodenum developed here indicates the coexistence of two regulatory mechanisms that operate at the brush border: a channel calcium flux regulated by intracellular calcium and a facilitated transporter mechanism with calcium binding to the transporter. This conclusion accords with the properties of the recently discovered epithelial calcium channel, ECaC (12), and the calcium transport protein, CaT1 (24). We believe that either of these molecular structures can accommodate the two mechanisms of calcium entry.

**Table 3. Saturable calcium transport as a function of CaBP in rats of varying ages**

<table>
<thead>
<tr>
<th>Age, days (Mean weight, g)</th>
<th>CaBP, nmol g$^{-1}$ mucosa</th>
<th>$V_m$, μmol·h$^{-1}$·g$^{-1}$·tissue$^{-1}$</th>
<th>$K_m$, mM</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>31 (77)</td>
<td>51 ± 4</td>
<td>12.4 ± 0.5</td>
<td>41 ± 5</td>
<td>23</td>
</tr>
<tr>
<td>40 (128)</td>
<td>73 ± 3</td>
<td>20.8 ± 3.6</td>
<td>51 ± 5</td>
<td>22</td>
</tr>
<tr>
<td>60 (225)</td>
<td>31 ± 2.6</td>
<td>7.2 ± 1.2</td>
<td>33 ± 4</td>
<td>22</td>
</tr>
<tr>
<td>150 (375)</td>
<td>9.6 ± 5.8</td>
<td>2.84 ± 1.56</td>
<td>45 ± 8</td>
<td>22</td>
</tr>
</tbody>
</table>

Data were obtained under similar experimental conditions. $V_m$, maximum rate of calcium transport; $K_m$, Michaelis-Menten constant.
low level of saturation of the transporter would limit calcium entry and, therefore, the amount absorbed. Because of the existence of the channel mechanism, calcium entry is no longer limited when calcium intake goes up, inasmuch as the channel permits calcium to enter in proportion to the luminal calcium concentration.

The model also allows estimation of the density of transporter binding sites, \( T \) (see APPENDIX C). With the typical average transporter rate of \( 10^3 \) s\(^{-1}\) and the maximum transporter flux \( V_{tr} = 2.2 \, \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{g}^{-1}\), the value for \( T \) is \( \approx 40 \, \mu\text{m}^{-2} \), or 4,000 transporter binding sites per cell, a not unreasonable number.

It has been shown that intracellular calcium can inhibit both ECaC (12) and CaT1 (24). The model predicts that the channel mechanism is inhibited by binding intracellular calcium with a dissociation constant of \( \approx 0.5 \, \mu\text{M} \) (see Eq. 11). Note that to describe the detailed kinetics on a fraction-of-a-second time scale, this equation should be replaced with \( P = P_0 h_t \), where the fraction of uninhibited channels, \( h_t \), should be described by an additional equation, \( h_t = k^+ [K_c - (K_c + c_-)] \), with \( k^+ \) being an on-rate constant (16). The transporter mechanism also slows down at high intracellular calcium, with \( V_{tr} \) being generally a decreasing function of \( c_- \) (see APPENDIX C).

Both contributions to the apical calcium flux are voltage dependent. The channel permeability is naturally sensitive to membrane potential (see Eq. 9 and related text). The rates of facilitated transport, \( k_1^+ \), are also affected by the intramembrane electric field. This is in agreement with the observed ECaC activation by hyperpolarization (12) and the voltage dependency of CaT1-mediated currents (24). The detailed quantitative description of the response of each of the mechanisms to the change in the electrochemical potential requires further experiments and additional model development.

Although similar in mediating calcium transport, ECaC and CaT1 differ in that only the former is vitamin D dependent (10, 24). Transcellular, i.e., active, calcium transport assumes physiological importance under conditions of low calcium intake, which lead to an increase in vitamin D-dependent processes in the cell, including neosynthesis of CaBP. This upregulates transcellular movement. Inasmuch as ECaC is vitamin D dependent, its contribution to calcium entry increases in proportion to the increase in \( 1,25(\text{OH})_2\text{D}_3 \) in the circulation.

At high luminal calcium, the ability of the epithelial cell to restrict calcium entry is increasingly challenged, and downregulation of facilitated diffusion is likely accompanied by a shutting down of the ECaC entry system. The epithelial cell thus increasingly depends on the CaT1 entry system, a logical response to the need of the cell to maintain a low intracellular free calcium concentration. In view of the fact that even when the luminal calcium concentration is 1 mM the intracellular free calcium concentration is being maintained below 1 \( \mu\text{M} \), severe entry restriction is needed at all times to prevent cellular flooding with calcium.

When suitably modified, the model developed here can be applied to calbindin-mediated transcellular calcium transport in other tissues, e.g., proximal jejunum (23), kidney (10), cecum (3), and colon (3). For example, in the proximal jejunum (23), with the calbindin D\(_{9K}\) content found to be 45% of that in the duodenum, transcellular transport was well approximated by a Michaelis-Menten function with \( V_m = 8 \, \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{g}^{-1} \) and \( K_m = 20 \, \mu\text{M} \). In reproducing these findings, our model yields a permeability value for the jejunum equal to 65% of that in the duodenum.

In summary, a simple model of transcellular calcium transport in rat duodenal cells has been formulated. On the assumption of the coexistence of two mechanisms of calcium apical entry, the model faithfully reproduces the relationship between luminal calcium and calcium transport. At high luminal calcium, transcellular calcium transport is largely a function of the intracellular concentration of calbindin D\(_{9K}\), a vitamin-D-dependent mobile calcium binding protein that acts like a calcium ferry. The model reproduces the positive, linear dependence of \( V_m \), the maximum flux rate derived from a Michaelis-Menten relationship, on the calbindin D\(_{9K}\) content, with the slope of this relationship being a function of the rate of calbindin D\(_{9K}\) diffusion through the cell.

At low luminal calcium, the regulation of apical entry is, according to the model, a major limiting factor for transcellular calcium transport. This regulation can be described in terms of facilitated entry saturated at relatively low luminal calcium. The coexistence of both mechanisms is required for a consistent description of transcellular calcium transport in the full range of luminal calcium concentration.

The model qualitatively describes the calcium transient in duodenal loops. The simulation results are reasonable in the limiting cases of the high and low initial concentrations of luminal calcium and allow for approximate estimates of the characteristics of calcium uptake.

In addition, when applied to an analysis of available experimental data, the model shows that the vitamin D-insensitive component of calcium entry, presumably CaT1, accounts for \(<10\%\) of calcium flux, with the vitamin D-dependent ECaC then mediating the remainder.

**APPENDIX A**

We derived Eqs. 5.1–5.3 from Eqs. 1–4 in the text by applying steady-state conditions and assuming near-instantaneous calcium binding to calbindin D\(_{9K}\) (CaBP). Under steady-state conditions, all intracellular processes of calcium uptake are completed, and the corresponding terms in Eq. 1 in the text (denoted by the ellipsis) cancel out. Because the time derivatives on the left-hand side of Eqs. 1 and 2 disappear at steady state, adding them up yields

\[
\frac{\partial}{\partial x} \left( -D_x \frac{\partial c}{\partial x} - D_b \frac{\partial b}{\partial x} \right) = 0 \quad (A1)
\]
The expression in the parentheses in Eq. A1 is the total flux of free and bound calcium, \( J \), which, as follows from Eq. A1, is constant in time and independent of \( x \)

\[
J = -D \frac{\partial c}{\partial x} - D \frac{\partial b}{\partial x} \quad (A2)
\]

Applying Eq. A2 to \( x = 0 \) and \( x = L \) and using boundary conditions Eqs. 3 and 4 in the text, we obtain Eqs. 5.1 and 5.3. Integration of Eq. A2 over the segment \([0, L]\) yields

\[
JL = D_r(c_\infty - c_\infty) + D_r(b_\infty - b_\infty) \quad (A3)
\]

We now use the near-instantaneous approximation for calcium binding to CaBP by assuming \( R = 0 \) in Eq. 3. This yields

\[
b = \frac{B_ck}{c_\infty + K} \quad (A4)
\]

where \( K = k_{\text{off}}/k_{\text{on}} \). Applying Eq. 4 to the boundary points, we get

\[
b_\infty = \frac{B_ck}{c_\infty + K}, \quad b_\infty = \frac{B_ck}{c_\infty + K} \quad (A5)
\]

Substituting Eqs. A5 into Eq. A3, we obtain Eq. 5.2.

**APPENDIX B**

Here we derive equations for the steady-state sensitivity analysis of the model described by Eqs. 5.1–5.3 in the text. In the general case of a system of \( n \) algebraic equations with \( n \) variables \( x_i (i = 1, \ldots, n) \) and \( m \) parameters \( a_j (j = 1, \ldots, m) \)

\[
F(x_1, \ldots, x_n; a_1, \ldots, a_m) = 0, \quad i = 1, \ldots, n \quad (B1)
\]

we introduce the sensitivity matrix \( \alpha_{ij} = \partial F/\partial a_j \). The sensitivities \( \alpha_{ij} \) are to be found by solving the linear system of equations, which is derived by differentiating Eqs. B1 with respect to parameters

\[
\sum_{i=1}^{n} J_{ij}\alpha_{ij} = R_{ij} \quad (B2)
\]

where \( J_{ij} = \partial F_i/\partial a_j \) is the Jacobian matrix of the system in Eqs. B1 and \( R_{ij} = \partial F_i/\partial a_j \). In our case, we deal with the system of Eqs. 5.1–5.3 (n = 3) with the variables \( x_0 = J, x_1 = c_\infty \), and \( x_2 = c_\infty \)

\[
x_0 - \frac{P}{K} \frac{[Ca^{2+}]_{\text{human}}}{(c_\infty + K)} = 0
\]

\[
x_0 - \frac{VJK(x_1 - c_\infty)}{(x_1 + K)(c_\infty + K)} = 0
\]

\[
x_0 - \frac{x_2 - x_1}{L} \left( D_r + \frac{D_r BK}{(x_1 + K)(x_2 + K)} \right) = 0
\]

The Jacobian matrix obtained from Eqs. B3 is

\[
J = \begin{bmatrix}
1 & 0 & \frac{PJK[Ca^{2+}]_{\text{human}}}{(K_i + x_3)^2} \\
\frac{K}{K_i + x_2} - \frac{PJK[Ca^{2+}]_{\text{human}}}{(K_i + x_2)^2} & 0 & 0 \\
0 & 0 & 0 \\
\frac{DJK(x_2 - x_1)}{L(K + x_1)^2(K + x_2)^2} & \frac{DJK(x_2 - x_1)}{L(K + x_1)(K + x_2)^2} & 0 \\
\end{bmatrix}
\]

We now enumerate parameters as \( a_0 = P_0, a_1 = K_i, a_2 = V_T, a_3 = K_p, a_4 = K, \) and \( a_5 = B_i \). The matrix \( R = [R_{ij}] \) then is

\[
The sensitivities \( \alpha_{ij} \) can then be transformed into the logarithmic sensitivities \( x_i^{-1}a_j \alpha_{ij} \). The results for \( x_0^{-1}a_j \alpha_{ij} \) are presented in Fig. 3.

**APPENDIX C**

Solving the system of Eqs. 12, accompanied by a thermodynamic constraint, \( k_1^3 k_2^3 K_i^3 = k_1^3 k_2^3 K_i^3 \), yields

\[
J = \frac{V_T ([Ca^{2+}]_{\text{human}} - c_\infty)}{K_i + [Ca^{2+}]_{\text{human}}} \quad (C1)
\]

with

\[
V_T = \frac{k_1^3 k_2^3 T}{k_1^3 (k_1^3 + k_1^3 + k_1^3 + k_1^3 + k_1^3 + k_1^3 + k_1^3)c_\infty} \quad (C2)
\]

where \( T \) is the density of transporter binding sites, \( T = [T_0] + [T_1] + [T_2] + [T_3] \), and

\[
K_T = K_s \frac{(k_3 + k_3)(1 + k_1) + (k_5 + k_5)c_\infty}{k_1^3 + k_1^3 + k_1^3 + k_1^3 + k_1^3 + k_1^3 + k_1^3} \quad (C3)
\]

Note that, generally, both \( V_T \) and \( K_T \) are functions of the intracellular calcium concentration, \( c_\infty \). In particular, \( V_T \) is a decreasing function of \( c_\infty \), which means that a transporter can in effect be inhibited by elevation of intracellular calcium. However, with the simplifying assumption that once calcium gets inside the cell, it can be readily released from the transporter, i.e., \( c_\infty/K_i^3 \ll 1 \), a characteristic of any effective transporter under normal physiological conditions, the parameters \( V_T \) and \( K_T \) can be regarded as constants determined by kinetic rates \( k_1^3, k_5^3 \), and \( k_2^3 \).
Finally, taking into account \([Ca^{2+}]_{\text{lumen}} \gg c^-, \text{Eq. C3.1 reduces to Eq. 13 of the text.}"

\[ k_i \]^{-1}.

**NOTE ADDED IN PROOF**

According to recent evidence (E. M. Brown, personal communication), duodenal EC\textit{A}C is now thought to be essentially the same molecule as CaT1. Our model then predicts that calcium entry mediated by this molecule should be at least partly vitamin D responsive.

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