Vitamin D increases tight-junction conductance and paracellular Ca\(^{2+}\) transport in Caco-2 cell cultures

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Chirayath, Mary V., Leszek Gajdzik, Wolfgang Hulla, Jürg Graf, Heide S. Cross, and Meinrad Peterlik. Vitamin D increases tight-junction conductance and paracellular Ca\(^{2+}\) transport in Caco-2 cell cultures. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G389–G396, 1998.—We investigated the effects of 1α,25-dihydroxyvitamin D\(_3\) [1,25(OH)\(_2\)D\(_3\)] on paracellular intestinal Ca\(^{2+}\) absorption by determination of transepithelial electric resistance (TER), as a measurement of tight-junction permeability and bidirectional transepithelial \(^{45}\)Ca\(^{2+}\) fluxes in confluent Caco-2 cell cultures. The rise of TER to steady-state levels of \(\sim 2,000\ \Omega\cdot cm^2\) was significantly attenuated by 1,25(OH)\(_2\)D\(_3\) (by up to 50%) in a dose-dependent fashion between 10\(^{–11}\) and 10\(^{–6}\) M. Synthetic analogs of 1,25(OH)\(_2\)D\(_3\), namely, 1α,25-dihydroxy-26,27-hexafluoro-16-ene-23-yne-vitamin D\(_3\) and 1α,25-dihydroxy-16-ene-23-yne-vitamin D\(_3\), exhibited similar biopotency, whereas their genonomically inactive 1-deoxy congeners were only marginally effective. Enhancement of transepithelial conductance of Caco-2 cell monolayers by vitamin D was accompanied by a significant increase in bidirectional transepithelial \(^{45}\)Ca\(^{2+}\) fluxes. Although 1,25(OH)\(_2\)D\(_3\) also induced cellular \(^{45}\)Ca\(^{2+}\) uptake from the apical aspect of Caco-2 cell layers and upregulated the expression of calbindin-9kDa mRNA, no significant contribution of the Ca\(^{2+}\)-adenosinetriphosphatase-mediated transcellular pathway to transepithelial Ca\(^{2+}\) transport could be detected. Therefore stimulation of Ca\(^{2+}\) fluxes across confluent Caco-2 cells very likely results from a genomic effect of vitamin D sterols on assembly and permeability of tight-junctional complexes.

**Cell culture:** Caco-2/AQ cells (between passages 20 and 50) were grown either in 24-well Falcon culture plates (Becton-Dickinson, Bedford, MA) or on filters with 0.4-µm pore size (Falcon cell culture inserts), as appropriate, in Dulbecco’s modified Eagle’s medium [supplemented with 10% fetal calf serum, 4.0 mM glutamine, 20 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 50 U/ml penicillin, and 50 µg/ml streptomycin] at 37°C in a 5% CO\(_2\)-95% air atmosphere.

**Vitamin D compounds** were dissolved in ethanol and added to cultures so that the final solvent concentration in the medium did not exceed 0.01%.

**Measurement of transepithelial electrical resistance.** Transepithelial electrical resistance (TER) of the Caco-2 monolayers was measured by a high-precision technique as described previously in detail (30). Current pulses of 55 µA, 0.5-s duration, were passed across the monolayers with Ag-AgCl electrodes from an ESCOM 486 SX computer equipped with a high-performance Labcard (PCL-818). Resulting voltages were recorded with the aid of a differential amplifier with a high input resistance. Data were corrected for well area (given in \(\Omega\cdot cm^2\)).

**Transport studies.** If not indicated otherwise, the medium used for determination of transepithelial transport of \(^{45}\)Ca\(^{2+}\), \(^{86}\)Rb\(^{+}\), \(^{14}\)C\([\]mannitol, or \(^{14}\)C\]inulin, respectively, contained (in mM) 134 NaCl, 4.1 KCl, 2.1 CaCl\(_2\), 1.0 KH\(_2\)PO\(_4\), 1.0 MgSO\(_4\), and 12.3 HEPES. pH was adjusted to 7.4 with 1.0 N NaOH. Specific activity of radiotracers was 0.5 µCi/ml.

Transepithelial transport across confluent Caco-2 cells was evaluated as described by Giuliano and Wood (21). Briefly, at time 0, transport buffer containing the radiolabeled solute at an appropriate concentration, i.e., 2.1 mM \(^{45}\)Ca\(^{2+}\), 0.1 mM \(^{86}\)Rb\(^{+}\), 1.0 mM \(^{14}\)C\]mannitol, or 36 µM \(^{14}\)C\]inulin, respectively, was filled into the filter well (1.0 ml) or the outside compartment (3.0 ml) of the filter unit as appropriate. In each case, the concentration of the solute under investigation in the contralateral compartment was zero. The filter plates were shaken horizontally at a frequency of 50 oscillations/min...
at room temperature for 60 min. Linearity of transport rates was monitored by determination of radioactivity in 20-µl aliquots drawn from the contralateral solutions at 15-min intervals.

Uptake studies. Caco-2 cells grown in 24-well plates were allowed to equilibrate with room temperature for 30 min before experimentation. After aspiration of the culture medium, 0.5 ml of a "low-sodium" mannitol buffer containing 145Ca2+ (0.5 µCi/ml) was added into each well. The buffer composition was (in mM) 198 mannitol, 25 KCl, 1.2 NaH2PO4, 1.2 MgSO4, 0.25 CaCl2, and 20 glucose (24). The uptake experiment was carried out for 10 min under horizontal shaking (50 oscillations/min) at room temperature. For termination of uptake, the transport medium was sucked off, and the cells were washed three times with 1.0 ml of ice-cold phosphate-buffered saline (pH 7.4). Cells were then suspended in 1.0 ml of 1.0 N NaOH and allowed to solubilize by overnight standing at 4°C.

Calbindin-9kDa mRNA isolation and Northern blotting. Total RNA was prepared from cells grown on filters in six-well plates until day 15 according to Ref. 11; 20 µg were used for Northern blotting (as described in Ref. 2). There was no obvious need for the use of semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) as performed by Fleet and Wood (19), since specific message was sufficient for conventional Northern analysis of calbindin-9kDa (CaBP-9kDa) mRNA expression. Probes for human (h) CaBP-9kDa were generated by RT-PCR. The following primers were selected from hCaBP-9kDa cDNA: Cal9.1.1 (1. for coding), CCAAGTCAAT; Cal9.3.0 (0. for reverse), CACCGACT; Cal9.2.1 (1. for coding), CCAGAATGAG; Cal9.4.0 (0. for reverse), TAACTGAGTCCACAGGCGAG. The primers' specificity was determined by searching data bases with the respective sequences (FASTA), and the PCR product was cloned into a pCRII vector (Invitrogen) and cDNA yielded no product and thus served as a negative control. The PCR product was then reamplified with the inside primers. The expected length together obtained after 30 cycles. The longer PCR product was then used as probe for the expression of hCaBP-9kDa mRNA thereaf-

RESULTS
Development of paracellular permeability in confluent Caco-2 cells. Mucosal-to-serosal transepithelial transport of extracellular markers, namely, [14C]inulin (∼5,000 mol wt) and [14C]mannitol (182.2 mol wt), was determined in parallel to 45Ca2+ transfer across confluent Caco-2 cell layers. As shown in Fig. 1, exposure of Caco-2 cells to 10−8 M 1,25(OH)2D3 for 2 wk past confluence had a distinct effect on the extent to which extracellular markers of different molecular weight could penetrate the Caco-2 cell layer. Although exposure to the steroid hormone had no effect whatsoever on transfer of the high molecular weight compound inulin, a small but significant vitamin D-related increment of transport of the considerably smaller molecule mannitol could be observed. Expectedly, 1,25(OH)2D3 elicited an approximately threefold rise in transepithelial transport of 45Ca2+ (Fig. 1).

This observation suggested to us that 1,25(OH)2D3 could attenuate the closure of intercellular junctions during postconfluent differentiation to such an extent that the passage of bulky extracellular markers such as inulin would be severely restricted, whereas smaller molecules and ions such as mannitol, or even more importantly Ca2+, could still traverse the cell layer on a paracellular route.

To substantiate this notion, we measured TEER of confluent Caco-2 cell layers as a highly sensitive parameter of paracellullar permeability simultaneously with mucosal-to-serosal transepithelial transport of [14C]mannitol and 45Ca2+ at different time points after confluence. Figure 2A shows that 1,25(OH)2D3 markedly retarded the development of TEER of Caco-2 cell layers during postconfluent growth. A significant effect of the sterol becomes visible at day 4. Figure 2B shows that the rate
of $[^{14}C]$mannitol is inversely related to the development of TEER (32). Consequently, treatment with 1,25(OH)$_2$D$_3$ made Caco-2 cells to a small but significant extent ($P < 0.05$) more permeable to $[^{14}C]$mannitol (Fig. 2B). A similar relation to TEER was observed in the case of transepithelial $^{45}$Ca$^{2+}$ transport (Fig. 2C). The $\sim 50\%$ reduction of TEER observed in 1,25(OH)$_2$D$_3$-treated Caco-2 cultures between days 10 and 15 past confluence (Fig. 2A) was accompanied by an about threefold increase in the amount of mucosal-to-serosal Ca$^{2+}$ transfer (Fig. 2C).

A dose-response study was carried out at two different time points after Caco-2 cells reached confluence (Fig. 3). On day 4, a significant reduction of TEER and a concomitant increase in Ca$^{2+}$ transport was observed only in cells exposed to the highest 1,25(OH)$_2$D$_3$ concentration tested, i.e., $10^{-8}$ M, whereas, on day 12, linear dose responses were observed at sterol concentrations between $10^{-10}$ and $10^{-8}$ M.

A plot of transport rate vs. conductance (i.e., 1/resistance) generated from single measurement values at various time points after confluence (Fig. 4), yielded identical curves for both 1,25(OH)$_2$D$_3$-treated and untreated Caco-2 cell layers. The absence of any vitamin D-related transport increment at a given conductance value strongly indicates that 1,25(OH)$_2$D$_3$ had no visible influence on apical-to-basolateral Ca$^{2+}$ transfer other than on the paracellular route.

If transepithelial Ca$^{2+}$ transport as measured is solely determined by the capacity of the paracellular route, then Ca$^{2+}$ transfer both in the apical-to-basolateral and in the opposite direction should occur to an equal extent. In some experiments, we measured also the influence of 1,25(OH)$_2$D$_3$ on basolateral-to-apical transepithelial Ca$^{2+}$ transport. At any time point, transport rates across either 1,25(OH)$_2$D$_3$-treated or untreated Caco-2 cell layers were equal to respective flux rates in the opposite direction (cf. also Table 1). Again, an identical relationship between Ca$^{2+}$ transport rates and conductance as measure of paracellular permeability was observed in both hormone-treated and control groups (Fig. 4).

Effect of 1,25(OH)$_2$D$_3$ analogs on TEER of confluent Caco-2 cells. Caco-2 cells respond not only to 1,25(OH)$_2$D$_3$ but also to a number of its synthetic analogs by changes in growth and morphological appearance, provided that those compounds bear a 1-$\alpha$-hydroxy group (5), which is a prerequisite for binding to the vitamin D receptor (VDR) and hence for genomic activity. We therefore tested the effect of 1,25(OH)$_2$D$_3$...

Table 1. Short-term effect of 1,25(OH)2D3 on TEER and bidirectional 45Ca2+ fluxes across confluent Caco-2 cell layers

<table>
<thead>
<tr>
<th>Duration of Treatment, h</th>
<th>Addition of 1,25(OH)2D3 (10-8 M)</th>
<th>TEER, cm2</th>
<th>Transepithelial Ca2+ Transport, nmol·h-1·well-1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Apical to basolateral</td>
<td>Basolateral to apical</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>14.3 ± 1.11</td>
<td>11.2 ± 0.04</td>
</tr>
<tr>
<td>24</td>
<td>–</td>
<td>14.3 ± 1.11</td>
<td>11.2 ± 0.04</td>
</tr>
<tr>
<td>48</td>
<td>+</td>
<td>9.0 ± 0.36</td>
<td>10.3 ± 0.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.0 ± 0.36</td>
<td>10.3 ± 0.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.0 ± 0.36</td>
<td>10.3 ± 0.33</td>
</tr>
</tbody>
</table>

Data are means ± SE (n = 6) from a typical experiment. 1,25(OH)2D3, 1a,25-dihydroxyvitamin D3; TEER, transepithelial resistance.

development of TEER of postconfluent Caco-2 cell layers. Although 25(OH)D3, with the exception of day 5, had no significant effect, 25(OH)-16ene,23yne-D3 and 25(OH)-26,27-F6-16ene,23yne-D3 from day 8 or 5 on, respectively, significantly reduced TEER by an average of ≤10%. In contrast, 1,25(OH)2D3 and both synthetic 1a-hydroxylated analogs reduced TEER to ≤50% of control levels at any time point. It should be noted that the decrement in TEER induced by the synthetic 1a-hydroxyvitamin D compounds tended to be even higher than that induced by 1,25(OH)2D3.

Time course of 1,25(OH)2D3 effects on TEER and transepithelial Ca2+ transport. The increase in TEER during growth of confluent Caco-2 cells in all likelihood reflects the development of tight junctions (cf. Ref. 28). It was therefore of interest to know whether vitamin D would affect not only the assembly of intercellular junctions during confluent cell growth but also influence their barrier function in a more advanced state of development. Thus, in another series of experiments, Caco-2 cells were allowed to grow for 12 days past confluence before treatment with 1,25(OH)2D3 was begun. Table 1 shows that, after 48–72 h, a highly significant reduction of TEER with a concomitant rise should be noted that Ca2+ transport in the mucosal-to-serosal as well as in the opposite direction was influenced by the hormone to the same extent (Table 1).

Effect of 1,25(OH)2D3 on cellular 45Ca2+ uptake and CaBP-9kDa mRNA expression. To evaluate a possible contribution of the transcellular route to vitamin D-related transepithelial Ca2+ transport as measured, we
determined the effect of 1,25(OH)₂D₃ and analogs on Ca²⁺ uptake by Caco-2 cells at different growth stages (Fig. 6). Basal cellular Ca²⁺ uptake in vitamin D-free control cultures conspicuously increased during transition from the log growth phase into the confluent state. During this time period, the 1α-hydroxylated vitamin D compounds under investigation were most effective in raising cellular ⁴⁵Ca²⁺ accumulation, whereas the 1α-deoxy compound, 25(OH)-16ene,23yne-D₃, had no effect at all.

In another experiment, the effect of 1,25(OH)₂D₃ on cellular Ca²⁺ accumulation was studied in confluent filter-grown Caco-2 cells, which were selectively exposed to the radiotracer ⁴⁵Ca²⁺ either on their apical (i.e., mucosal) or basolateral (i.e., serosal) aspect, respectively. As expected, 1,25(OH)₂D₃, when added on day 12 past confluence at 10⁻⁸ M, after 72 h raised cellular ⁴⁵Ca²⁺ accumulation due to uptake from the mucosal compartment from 6.8 ± 0.04 to 15.2 ± 0.3 nmol·h⁻¹·well⁻¹ (n = 6, P < 0.01) but had no significant effect on uptake from the contralateral compartment, which was raised from 18.0 ± 1.8 to only 21.4 ± 0.3 nmol·h⁻¹·well⁻¹ (n = 6, P > 0.05).

In the same experiment, we determined CaBP-9kDa mRNA levels by Northern blot analysis (Fig. 7). Caco-2 cells were able to express CaBP-9kDa message even in the absence of 1,25(OH)₂D₃. As expected (cf. Refs. 18, 19), a two- to threefold rise in mRNA levels was induced by 10⁻⁸ to 10⁻⁷ M 1,25(OH)₂D₃ within 48–72 h.

Ca²⁺-adenosinetriphosphatase inhibition and transepithelial Ca²⁺ transport. Because the last step of mucosal-to-serosal transepithelial Ca²⁺ transport involves extrusion of Ca²⁺ across the basolateral aspect of the cell by the Ca²⁺-adenosinetriphosphatase (ATPase), we sought to evaluate the contribution of active Ca²⁺ pumping to net transepithelial transport by blocking the activity with a potent inhibitor, calmidazolium. The data collated in Table 2 show that, apart from the fact that pretreatment with the inhibitor had no influence on TEER in either controls or 1,25(OH)₂D₃-treated Caco-2 cells, a block of the Ca²⁺ pump did not change the extent of basal mucosal-to-serosal transepithelial Ca²⁺ transfer as measured but, even more important, by no means reduced its 1,25(OH)₂D₃-related increment.

1,25(OH)₂D₃ and transepithelial ⁸⁶Rb⁺ transport. Further proof for the assumption that 1,25(OH)₂D₃ can modulate ion transport on the paracellular route was obtained when we measured transepithelial ⁸⁶Rb⁺ transport across confluent Caco-2 cell layers (Table 3). ⁸⁶Rb⁺ is widely used as a substitute for K⁺ for assessment of Na⁺-K⁺-ATPase activity in whole cell preparations. It should be noted that, under the experimental conditions employed, transepithelial ⁸⁶Rb⁺ transport was completely insensitive to ouabain treatment (Table 3). Because this excludes any contribution from the Na⁺-K⁺-ATPase-mediated transepithelial pathway, ⁸⁶Rb⁺ transport as measured mainly reflects ion flux on a paracellular route. The data collated in Table 3 therefore strongly suggest that stimulation of ⁸⁶Rb⁺ transport across confluent Caco-2 cell layers in the serosal-to-mucosal direction by the steroid hormone occurs in parallel with reduction of TEER during postconfluent cell growth.

<table>
<thead>
<tr>
<th>Addition to Culture</th>
<th>Calmidazolium Pretreatment</th>
<th>TEER, Ω·cm⁻²</th>
<th>Transepithelial ⁴⁵Ca²⁺ Transport, nmol·h⁻¹·well⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>−</td>
<td>1.949 ± 0.61</td>
<td>11.4 ± 0.4</td>
</tr>
<tr>
<td>1,25(OH)₂D₃</td>
<td>−</td>
<td>1.935 ± 0.32</td>
<td>11.1 ± 0.5</td>
</tr>
<tr>
<td>(10⁻⁸ M)</td>
<td>+</td>
<td>1.955 ± 0.21</td>
<td>28.3 ± 0.2</td>
</tr>
<tr>
<td>25(OH)-16ene,23yne-D₃</td>
<td>+</td>
<td>1.021 ± 0.32</td>
<td>26.6 ± 1.2</td>
</tr>
</tbody>
</table>

Data are means ± SE (n = 6) from a typical experiment. Pretreatment with 100 µM calmidazolium was for 30 min before initiation of ⁴⁵Ca²⁺ transport. Inhibitor was included at the same concentration that was also in outer, i.e., basolateral compartment of filter unit (see EXPERIMENTAL METHODS).
Table 3. Effect of 1,25(OH)2D3 on paracellular 86Rb+ flux and TEER in confluent Caco-2 cells

<table>
<thead>
<tr>
<th>Day Past Confluence</th>
<th>1,25(OH)2D3 in Culture Medium</th>
<th>Ouabain</th>
<th>TEER, Ω·cm²</th>
<th>Serosal-to-Mucosal 86Rb+ Transport, pmol·min⁻¹·well⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>1.487 ± 0.75</td>
<td>37.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>1.504 ± 0.37</td>
<td>37.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>1.063 ± 0.21</td>
<td>49.2 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>1.058 ± 0.25</td>
<td>52.0 ± 0.6</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>1.609 ± 0.15</td>
<td>39.3 ± 1.0</td>
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<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>1.861 ± 0.55</td>
<td>37.8 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>798 ± 58</td>
<td>59.6 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>794 ± 50</td>
<td>59.9 ± 1.2</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>2.121 ± 0.25</td>
<td>25.2 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>1.022 ± 0.48</td>
<td>42.5 ± 1.4</td>
</tr>
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</table>

Data are means ± SE (n = 6) from a typical experiment. 1,25(OH)2D3 concentration in culture medium was 10⁻⁸ M. Preincubation with 1.0 mM ouabain was for 1 h before initiation of 86Rb+ transport. Inhibitor was included at same concentration also in transport buffer.

DISCUSSION

Caco-2 cells, though originally derived from a human colon adenocarcinoma, are still able to undergo spontaneous differentiation into enterocyte-like cells. Thereby Caco-2 cells form confluent monolayers consisting of well-polarized cells with tight junctions and a typical apical brush border (28).

It is generally accepted that transepithelial electric conductance across Caco-2 cell layers is mainly determined by the ionic permeability of the intercellular junctions which develop during postconfluent cell growth (28). The fact that the paracellular route is the predominant pathway of transepithelial ion flux can be inferred from the observations that ~80% of total electrical resistance of Caco-2 cells is located in the mucosal membrane and that, in addition, its Na⁺ conductance is very limited (22). Changes in TEER can also not be explained by activation of Na⁺-α-glucose cotransport, because the Caco-2 cell clone used in the present study is devoid of any Na⁺-dependent α-glucose transport activity (10, 22), and in addition, no α-glucose was present in the incubation medium. Thus the decline in TEER largely reflects an effect on tight junction-mediated paracellular ion permeability (27). The present study documents that 1,25(OH)2D3 as well as its synthetic D-ring and side-chain-modified analogs substantially reduce TEER of confluent Caco-2 cells. This must be considered as clear evidence for the ability of genomically active vitamin D compounds to increase bidirectional paracellular flux of all ion species including Ca²⁺. In this respect, it is interesting to note that Favus et al. (16) had observed that 1,25(OH)2D3 caused a significant increase of tissue conductance and, most notably, also of bidirectional mannitol fluxes in the duodenum and descending colon of rats, whereas Cross et al. (12) reported on stimulation of paracellular ion transport, i.e., Na⁺, K⁺, and Rb⁺, in organ-cultured embryonic chick small intestine.

Caco-2 cells express VDR mRNA and protein during the log growth phase as well as after confluence (20, 23). This is apparently the basis for the action on paracellular ion permeability of vitamin D compounds, since all 1α-deoxysteroid analogs under investigation were either completely ineffective in reducing TEER, namely, 25(OH)-D3, or, like the two synthetic compounds, 25(OH)-16ene,23yne-D3 and 25(OH)-26,27-F6-16ene,23yne-D3, showed only marginal activity compared with their 1α-hydroxylated congeners (cf. Fig. 5). Because the 1α-hydroxy group mediates high-affinity binding to the VDR (cf. Ref. 6), it is reasonable to assume that the observed effects on TEER of 1,25(OH)2D3 and its two synthetic side-chain- and D-ring-modified analogs result from a genomic rather than from a nongenomic action. The latter possibility seems unlikely also for a number of other reasons. First, typical nongenomic effects of 1,25(OH)2D3 (for review see, e.g., Ref. 7) involve interactions with plasma membrane activities and are observed within seconds or minutes, whereas reduction of TEER requires at least 48-h exposure to the hormone (cf. Table 1). Second, a rapid membrane action of 1,25(OH)2D3 cannot be easily reconciled with the observation that the sensitivity of Caco-2 cells varies with ongoing differentiation between days 4 and 12 post confluence (cf. Fig. 3). It has been shown, however, that the expression of genomic effects of 1,25(OH)2D3, particularly in enterocytes, can depend to a large extent on the degree of their differentiation (13). Third, 25(OH)-16ene,23yne-D3 was shown to be most potent in eliciting nongenomic effects such as activation of voltage-gated Ca²⁺ channels in rat osteosarcoma cells (15), whereas the same analog was only weakly effective in attenuating TEER (cf. Fig. 5). Fourth, it is conceivable that the observed small effects of synthetic 1-deoxysteroid compounds on TEER of Caco-2 cells reflect their small genomic potency due to the ability to bind weakly to the VDR (6, 15) or, fifth, result from conversion into genomically active 1-hydroxy compounds. Although substantial 25-hydroxyvitamin D3-1-hydroxylase activity has been observed only in serum-free cultures of Caco-2 cells (14), it is conceivable that, even under the culture conditions employed in the present study, a small fraction of the 25-hydroxy compounds tested is converted to respective 1α-hydroxy derivatives, which could then be responsible for the observed effects on TEER (Fig. 5).

Both assembly and barrier properties of tight junctions depend on the formation of a bipartite functional complex with adjacent adherens junctions as well as on an appropriate organization of the latter with the actin cytoskeleton (1, 26). Fialka et al. (17) showed that estrogen-related upregulation of the c-jun oncogene diminishes TEER in mammary epithelial cells and, at the same time, disrupts the polarized expression of the tight junction-associated protein zonin-1 as well as of the constituents of adherens junctions, E-cadherin and β-catenin. Because the c-jun protooncogene is also a well-known target for signaling from the VDR (9, 25), we suggest that upregulation of c-jun expression could also explain the observed effects of genomically active vitamin D compounds on tight-junctional permeability.
of Caco-2 cells. In fact, we have obtained evidence from Western blot analysis that treatment with 10^{-8} M 1,25(OH)_2D_3 for 5 days leads to reduced expression of E-cadherin in Caco-2 cells (unpublished results).

A strong argument for the notion that vitamin D stimulates transepithelial Ca^{2+} transport by an increase in junctional ion permeability rather than by stimulation of transcellular calbindin-mediated transport, as suggested by Fleet et al. (18, 19), can be derived from the following observations: 1) an identical relationship between TEER or conductance, respectively, and Ca^{2+} transport exists in untreated and vitamin D-treated Caco-2 cell cultures, and hence no conductance-independent vitamin D-related increment exists; 2) vitamin D has an identical effect on apical-to-basolateral as well as on basolateral-to-apical Ca^{2+} fluxes, which would not be the case if there were a major contribution from vectorial transcellular calbindin-mediated transport that proceeds exclusively in the apical-to-basolateral direction; and 3) the effect of vitamin D is not specific for Ca^{2+} transport but is visible also on bidirectional Rb^{+} fluxes, which are certainly not calbindin mediated.

As far as the existence of a major transcellular Ca^{2+} path in confluent Caco-2 cells is concerned, we were able to confirm the observation of Surendran et al. (29) that blocking Ca^{2+} extrusion across the basolateral membrane by Ca^{2+}-ATPase inhibition does not alter the extent of transepithelial Ca^{2+} transport. Because this is valid also for 1,25(OH)_2D_3-treated Caco-2 cells (cf. Table 2), this observation must be considered as additional support for the assumption that vitamin D affects Ca^{2+} transport mainly through its effect on tight-junctional ion permeability.

In probing the vitamin D sensitivity of the consecutive steps of apical-to-basolateral transcellular Ca^{2+} transport, we could show that cellular Ca^{2+} uptake from the apical aspect of confluent Caco-2 cell layers involves a genomic action of vitamin D sterols. Furthermore, consistent with the results of Fleet et al. (18, 19), 1,25(OH)_2D_3 upregulates CaBP-9kDa mRNA levels (Fig. 7). However, it must be borne in mind that as long as direct measurement of human CaBP-9kDa protein in Caco-2 cells is not available, it remains questionable whether the vitamin D actions on mucosal Ca^{2+} influx and CaBP-9kDa mRNA are of a magnitude to efficiently raise the rate of transcellular transport of Ca^{2+}.

Another explanation for the difference in the interpretation of our results and those of Fleet et al. (18, 19) lies in the fact that these authors did not observe any effect of vitamin D sterols on transepithelial transfer of phenol red, which they used as a marker for paracellular permeability. However, the relatively high molecular weight and negative charge of this compound may have compromised its use to detect changes in paracellular permeability of ions with a much smaller atomic radius, such as Ca^{2+}. Because of the lack of any substantial contribution of the transcellular route to transepithelial Ca^{2+} transport, the Caco-2 system could serve as an excellent model for the study of vitamin D effects on intestinal Ca^{2+} absorption via the paracellular route (for review, see Ref. 31).

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