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Characterization of a murine renal distal convoluted tubule cell line for the study of transcellular calcium transport

Robin J. W. Diepens, Rob den Dekker, Marcella Bens, A. Freek Weidema, Alain Vandewalle, René J. M. Bindels, and Joost G. J. Hoenderop.


CA2+ homeostasis is regulated by Ca2+ absorption by the intestine, storage in and release from bones, and by reabsorption and excretion by the kidney. In the kidney, 98% of the filtered Ca2+ is reabsorbed, whereas only 2% is excreted in the urine (10). The majority of Ca2+ reabsorption occurs by paracellular transport in the proximal part of the nephron. In the distal part of the nephron, mainly in the distal convoluted tubule (DCT) and the connecting tubule (CNT), transcellular Ca2+ transport is the main route for Ca2+ reabsorption, which accounts for only 10–20% of total Ca2+ reabsorption by the kidney. However, this represents a key portion for regulated Ca2+ reabsorption, because transcellular Ca2+ transport in the distal part of the nephron is tightly controlled by calcitropic hormones (3, 33, 34).

Transcellular Ca2+ transport can be divided into three processes: Ca2+ entry at the apical side, intracellular translocation, and extrusion at the basolateral side. The recently discovered highly selective Ca2+-channels TRPV5 and TRPV6 (also known as ECaC1 and ECaC2, respectively) at the apical side of cells are the main Ca2+ entry sites (15, 21, 23, 25). Calbindins-D9k and -D28k are intracellular Ca2+-binding proteins that are thought to participate in shuttling Ca2+ from the apical to the basolateral membrane (5, 12, 19, 36), whereas the Na+-Ca2+ exchanger (NCX1) and the plasma membrane Ca2+-ATPase 1b (PMCA1b) account for Ca2+ extrusion (24, 33, 40).

The calcitropic hormone 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] tightly regulates transcellular Ca2+ transport. It is active at all three processes necessary for transcellular Ca2+ transport. 1,25(OH)2D3 stimulates mRNA expression of the Ca2+ entry channels TRPV5 and TRPV6 (22), calbindins-D9k and -D28k (13, 22), and PMCA1b (18). Currently, the molecular regulation of transcellular Ca2+ transport in DCTs remains elusive, because in vivo transcellular Ca2+ transport assays are difficult to perform. We describe here for the first time a cell system, which approaches the endogenous physiological state of DCTs in mice, in which the regulation of transcellular Ca2+ transport can be studied.

MATERIALS AND METHODS

Cell culture. Studies were performed on mpkDCT cells (38) microdissected from the kidney of an SV-PK/Tag transgenic mouse carrying the SV-40 large T and small t antigens under control of the SV-40 enhancer placed in front of the −1,000-bp fragment of the rat L-L'-PK gene regulatory region in the 5'-flanking region (30). The murine distal convoluted tubule (mpkDCT) cells have been established using the same protocol described for the establishment of the differentiated cortical collecting duct (CD) principal mpkCCD1a cells (1, 11). mpkDCT cells were cultured in a defined medium with minor modifications: DMEM/Ham's F-12 medium (1:1 vol/vol; Invitrogen, Breda, The Netherlands) supplemented with 2% (vol/vol) heat-inactivated FCS, 5 mg/ml insulin (Sigma, St. Louis, MO), 5 nM deoxymethasone (Sigma), 90 nM sodium selenate (Sigma), 5 μg/ml transferrin (Sigma), 1 nM triiodothyronine (Sigma), 10 ng/ml EGF (Sigma), 0.2% n-glucose (Sigma), 20 mM HEPES (Biogenesis, Breda, The Netherlands), 4.5 mM glutamine (Biogenesis), and 45 μg/ml gentamicin at pH 7.4, equilibrated with 5% CO2-95% air at 37°C. For the cost of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
routine passage, cells were grown in T25 plastic culture flasks and passed every 3–4 days when 80% confluent. For $^{45}$Ca$^{2+}$ transport studies, and RNA isolation, $\sim$5$\times$10$^5$ cells were seeded on collagen-coated permeable membrane filter inserts (6.5-mm internal diameter, 0.4-$\mu$m pore size; Costar) and cultured for 6 days; medium was changed on day 3. Just before every study, the transepithelial electrical resistance (TEER) was determined as a measure of confluence of the monolayer, using a volt-ohm meter (Millipore, Eiten-Leur, The Netherlands). As controls, experiments were performed on confluent monolayers of primary cultured DCT cells. Isolated late DCT cells were microdissected from the kidneys of wild-type C57BL6 9- to 12-wk-old female mice using the same protocol used for primary cultures of microdissected cortical CDs (2). Briefly, mice were killed by cervical dislocation. Kidneys were rapidly removed under sterile conditions and incubated in the same medium described above and supplemented with collagenase A (0.1% wt/vol, Roche Diagnostic, Mannheim, Germany) for 45 min at 37°C. Distal tubules were then microdissected out under sterile condition as described before (2). Isolated DCTs (8–10 fragments, 0.2- to 0.5-mm long) were seeded on collagen-coated permeable membrane filter inserts and grown in the same defined medium described above. After 5 days, the medium was changed every 2 days. Experiments were carried out 2 wk after seeding, and just before every study, the TEER was determined as a measure for confluence of the monolayer, using a volt-ohm meter. All experiments were performed in accordance with the guidelines of the French and Dutch Agricultural Offices and in compliance with legislation governing animal studies.

$^{45}$Ca$^{2+}$ transport assay. Transport studies were conducted as described previously, with minor modifications (4, 13). Briefly, confluent monolayers were incubated in Krebs-Henseleit buffer containing 110 mM NaCl, 5 mM KCl, 1.2 mM MgCl$_2$, 10 mM sodium acetate, 20 mM HEPES, 2 mM NaH$_2$PO$_4$, 4 mM t-lactate, 10 mM D-glucose, 1 mM t-alanine adjusted to pH 7.4 using 1 M Tris, 4mM a2 ATP, and 10 mM HEPES/CsOH, pH 7.2. The external solution contained 1 mM CaCl$_2$, 0.1% BSA, 10 mM Na$_2$ATP, and 10 mM EDTA. All experiments were performed at 37°C; followed by 40 cycles for 1 min at 95°C, 1 min at the correct annealing temperature (see Table 1) and 1 min at 72°C; followed by a 10 min at 72°C. An actin RT$^\text{m}$ sample was used as a negative control and was run for 60 cycles at an annealing temperature of 50°C. Primers targeting the genes of interest are listed in Table 1.

Electrophysiology. Electrophysiological methods for measuring Na$^+$ and Ca$^{2+}$ currents related to TRPV5 and TRPV6 channel activity have been described previously in detail (39). Patch-clamp experiments were performed in the whole cell configuration, using an EPC-9 patch-clamp amplifier (HEKA Elektronik, Lambrecht, Germany). Patch pipettes had DC resistances of 2–4 MΩ when filled with intracellular solution. A ramp protocol, consisting of linear voltage ramps from +100 to −100 mV within 450 ms, was applied every 2 s from a holding potential of 0 mV. Current densities, expressed per unit membrane capacitance, were calculated from the current at −80 mV during ramp protocols. The internal (pipette) solution contained 20 mM CsCl, 100 mM Cs aspartate, 1 mM MgCl$_2$, 10 mM BaCl$_2$, 4 mM Na$_2$ATP, and 10 mM HEPES/CsOH, pH 7.2. The external solution contained 1 mM CaCl$_2$, 150 mM NaCl, 6 mM CsCl, 1 mM MgCl$_2$, 10 mM HEPES/NaOH (pH 7.4), 10 mM glucose, and different concentrations of RR (100 mM-100 μM). Divalent-free solutions did not contain added divalent cations, while trace amounts of divalent cations were removed with 100 μM EDTA. All experiments were performed at room temperature (20–24°C).

Statistics. In all experiments, data are expressed as means ± SE. Statistical significance was determined by Student’s t-test. Differences in means with $P < 0.05$ were considered statistically significant.

RESULTS

mpkDCT cell line possesses specific properties of DCTs. mpkDCT cells were derived from isolated DCTs, microdissected from the kidney of an adult transgenic mouse (SV-PK/CALP-1b).

Table 1. Sequences of primers for RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Product</th>
<th>Annealing Temp</th>
<th>No. of Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRPV5</td>
<td>5'-GTGGTGGTTGTTTGAAGGTGGTGAAC-3'</td>
<td>5'-GGGTGAGAGATGCAAGGCAGACATCTCTA-3'</td>
<td>168</td>
<td>50°C</td>
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<td>TRPV6</td>
<td>5'-ATCCGGCCCTAATGATGAC-3'</td>
<td>5'-ATGGCTTGTCCTCTCTGATTTTTTTCCTA-3'</td>
<td>80</td>
<td>50°C</td>
<td>40</td>
</tr>
<tr>
<td>CaBP-D$_{Da}$</td>
<td>5'-CCGGCAAGAGAAGGGAGCAACATTTT-3'</td>
<td>5'-TGATGACGTCTAGCAAGCCAT-3'</td>
<td>175</td>
<td>50°C</td>
<td>40</td>
</tr>
<tr>
<td>CaBP-D$_{Dh}$</td>
<td>5'-ACATGCAAGACAGAGGGAGCAATTTT-3'</td>
<td>5'-GAGAGTTTCTTCTCAGACAGATTTCTA-3'</td>
<td>87</td>
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<td>60</td>
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<tr>
<td>PMCA 1b</td>
<td>5'-CAGCAGGTCTGGGATTCATACCCAGTT-3'</td>
<td>5'-CACAGGTTTCTTACTTATCAAGAGTC-3'</td>
<td>109</td>
<td>50°C</td>
<td>60</td>
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<tr>
<td>NCX1</td>
<td>5'-TTCCTGCTTAACCAATTTGAGGACAGA-3'</td>
<td>5'-TTTCACTCATGTTTGGCTATGATTT-3'</td>
<td>142</td>
<td>50°C</td>
<td>60</td>
</tr>
<tr>
<td>CaSR</td>
<td>5'-TCTTTTCTTCTTCTCTTCTTCTTCTTGTGATTT-3'</td>
<td>5'-GCAAAATGATGCTTTATCAACAGA-3'</td>
<td>120</td>
<td>50°C</td>
<td>40</td>
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<tr>
<td>NCC</td>
<td>5'-TGCAAGGATGATGCTTATTTTACAGC-3'</td>
<td>5'-TTTCCAGTTACATGTTTACATCTGATGTT-3'</td>
<td>461</td>
<td>50°C</td>
<td>60</td>
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<tr>
<td>AQP2</td>
<td>5'-CCAGGGCGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG-3'</td>
<td>5'-GCAAGCATGCTTGTTTACATCTGATGTT-3'</td>
<td>555</td>
<td>50°C</td>
<td>60</td>
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<tr>
<td>PThr</td>
<td>5'-GGCGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG-3'</td>
<td>5'-GGGAGGTGATGCTTGTTTACATCTGATGTT-3'</td>
<td>531</td>
<td>54°C</td>
<td>40</td>
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<tr>
<td>β-Actin</td>
<td>5'-GTGGTGGTTGTTTGAAGGTGGTGAAC-3'</td>
<td>5'-GGGTGAGAGATGCAAGGCAGACATCTCTA-3'</td>
<td>201</td>
<td>50°C</td>
<td>40</td>
</tr>
</tbody>
</table>

PCR primers were purchased from Biolegio. Product size is in base pairs. CaBP-D$_{Da}$ and −D$_{Dh}$, calbindin −D$_{Da}$ and −D$_{Dh}$; PMCA 1b, plasma-membrane Ca$^{2+}$-ATPase 1b; NCX1, Na$^+/Ca^{2+}$ exchanger; CaSR, calcium-sensing receptor; NCC, Na$^+/Cl^-$ cotransporter; AQP2, aquaporin-2; PThr, parathyroid hormone receptor.

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(AQP2), known to be expressed in CNT and CD. To determine which is a marker for DCT, but did not express aquaporin-2 (AQP2) mRNA encoding the parathyroid hormone receptor (PTHr) in mpkDCT cells. Mean TEER across a monolayer of primary cultures of DCT cells, grown for 2 wk on collagen-coated permeable filter supports, was determined at 2,389 ± 148 Ω·cm² (n = 14), whereas the mean TEER across a monolayer of mpkDCT cells, cultured for 6 days on collagen-coated permeable filter supports, was determined at 1,790 ± 88 Ω·cm² (n = 23). Net Ca²⁺ transport was calculated by subtracting basolateral-to-apical Ca²⁺ transport from apical-to-basolateral Ca²⁺ transport. As shown in Fig. 2, both primary cultures of DCT cells and the mpkDCT cell line exhibited significantly higher apical-to-basolateral transport than basolateral-to-apical transport from 15 min onward. Net Ca²⁺ transport across primary cultures of DCT cells was 0.4 ± 0.1 nmol·h⁻¹·cm⁻² (Fig. 2A), whereas net Ca²⁺ transport across the mpkDCT cell line was significantly higher (2.4 ± 0.2 nmol·h⁻¹·cm⁻²; Fig. 2B). The calculated net Ca²⁺ transport for the mpkDCT cell line was linear for up to 4 h after addition of ⁴⁵Ca²⁺. In subsequent experiments, net Ca²⁺ transport was calculated using the 60-min time point.

"**Transcellular Ca²⁺ transport by cultured murine DCT cells.**" Primary cultures of isolated late DCT cells microdissected from the kidneys of wild-type C57BL6 mice and grown on permeable filters were used to assess the Ca²⁺ transport capacities of the murine DCT and compared with that of the established cultured mpkDCT cells. Mean TEER across a monolayer of primary cultures of DCT cells, grown for 2 wk on collagen-coated permeable filter supports, was determined at 2,389 ± 148 Ω·cm² (n = 14), whereas the mean TEER across a monolayer of mpkDCT cells, cultured for 6 days on collagen-coated permeable filter supports, was determined at 1,790 ± 88 Ω·cm² (n = 23). Net Ca²⁺ transport was calculated by subtracting basolateral-to-apical Ca²⁺ transport from apical-to-basolateral Ca²⁺ transport. As shown in Fig. 2, both primary cultures of DCT cells and the mpkDCT cell line exhibited significantly higher apical-to-basolateral transport than basolateral-to-apical transport from 15 min onward. Net Ca²⁺ transport across primary cultures of DCT cells was 0.4 ± 0.1 nmol·h⁻¹·cm⁻² (Fig. 2A), whereas net Ca²⁺ transport across the mpkDCT cell line was significantly higher (2.4 ± 0.2 nmol·h⁻¹·cm⁻²; Fig. 2B). The calculated net Ca²⁺ transport for the mpkDCT cell line was linear for up to 4 h after addition of ⁴⁵Ca²⁺. In subsequent experiments, net Ca²⁺ transport was calculated using the 60-min time point.

"**Calcium transport across distal convoluted tubule cells**" To study RR-sensitive transcellular Ca²⁺ transport independent of voltage-operated Ca²⁺ channels, verapamil (10 μM) and felodipine (10 μM) were added apically as well as basolaterally to the transport buffer in all experiments performed to block voltage-operated Ca²⁺ channels, and BaCl₂ (1 mM) was added to prevent changes in membrane potential. With the use of different concentrations of RR, the IC₅₀ of RR for the blockage of transcellular Ca²⁺ transport was determined. For mpkDCT cells, the IC₅₀ of RR on the Na⁺ current was 8.8 ± 0.3 μM (Fig. 3). In this study, the IC₅₀ of RR on the Na⁺ current of
TRPV5- and TRPV6-expressing human embryonic kidney 293 cells (HEK 293 cells) was determined at <0.1 and 17.5 ± 0.6 µM, respectively, as determined by patch-clamp analysis. Net 45Ca2+ transport measured in the mpkDCT cells was inhibited by RR with an IC50 of 2.2 ± 0.7 µM (Fig. 4), a value that is on the same order of magnitude as the value determined by patch-clamp analysis.

cAMP and 1,25(OH)2D3 increased transcellular Ca2+ transport in mpkDCT cells. It has been shown that an increase of intracellular cAMP triggers stimulation of Ca2+ reabsorption in the kidney. Other studies showed that Ca2+ reabsorption is stimulated on activation of the PKC pathway (14, 20). Therefore, the effect of a rise in cAMP and stimulation of PKC on transcellular Ca2+ transport was studied in the mouse mpkDCT cell line. Forskolin (10 µM) in combination with IBMX (100 µM) increased Ca2+ transport by 92 ± 13% compared with nontreated cells (Fig. 5). This rise was completely inhibited by 100 µM RR, indicating that the stimulation induced by the PKA pathway reflected is possibly mediated by TRPV5 and/or TRPV6. On the other hand, the PKC activator PMA did not induce a rise in Ca2+ transport across confluent monolayers of mpkDCT cells (Fig. 5).

To study the effect of physiologically relevant calcitropic hormones, confluent mpkDCT cells were incubated with either 1,25(OH)2D3 (100 nM), bPTH (1–34) (100 nM), or dDAVP (10 nM) during the Ca2+ transport experiment. mpkDCT cells were incubated for 72 h with 1,25(OH)2D3 (100 nM) to determine the long-term effects on Ca2+ transport. As shown in Fig. 6A, both dDAVP and long-term stimulation with 1,25(OH)2D3 increased Ca2+ transport with 119 ± 7 and 70 ± 21%, respectively, compared with nontreated cells. 1,25(OH)2D3- and dDAVP-stimulated Ca2+ transport was significantly inhibited by RR (Fig. 6A). Because bPTH had no significant effect on transcellular Ca2+ transport, PTH derived from rat (Sigma) and bovine (NIBSC, Herts, UK) was also tested. Although PThr was detected at the mRNA level (Fig. 1C), none of the tested PTH batches affected Ca2+ transport. Gesek and Friedman (17) showed a significant effect of PTH on 45Ca2+ influx in a mixture of DCT and cortical thick ascending limb (cTAL) cells, which was abolished by nifedipine. In the absence of felodipine, verapamil, and BaCl2, PTH did not stimulate transcellular Ca2+ transport in mpkDCT cells (Fig. 6B). Moreover, transcellular Ca2+ transport across mpkDCT cells is primarily regulated by RR-sensitive channels and not by dihydropyridine-sensitive channels.

**DISCUSSION**

The present study demonstrated that the mpkDCT cell line established from transgenic SV-PK/Tag mouse distal tubules is a valuable cell system in which to study transcellular Ca2+ transport in the DCT in vitro. This conclusion is based on the following observations: 1) mpkDCT cells express the key players necessary for transcellular Ca2+ transport: the epithelial Ca2+ channels TRPV5 and TRPV6, the intracellular Ca2+ translocators calbindin-D9k and calbindin-D28k, and the basolateral Ca2+ extrusion proteins NCX1 and PMCA1b; 2) mpkDCT cells exhibit a significant net apical-to-basolateral Ca2+ transport; 3) RR completely inhibits net Ca2+ transport, sug-
Fig. 6. Effect of 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], 1-deamino-8-D-arginine vasopressin (dDAVP), and parathyroid hormone (PTH) on Ca²⁺ transport across mpkDCT monolayers. A: confluent mpkDCT cells were incubated with RR (100 μM), 1,25(OH)₂D₃ (100 nM, 72 h), dDAVP (10 nM), or PTH (100 nM) in the presence of tracer ⁴⁵Ca²⁺ for 60 min at 37°C. Net Ca²⁺ transport in these experiments was 2.7 ± 0.3 nmol·h⁻¹·cm⁻² and set at 100%, to which all values are related. B: confluent mpkDCT cells in the presence (control) or absence (control⁻) of verapamil (10 μM), felodipine (10 μM), and BaCl₂ (1 mM) were stimulated with PTH (100 nM). Net Ca²⁺ transport across mpkDCT cells treated with verapamil, felodipine, and BaCl₂ was 4.6 ± 0.2 nmol·h⁻¹·cm⁻² and set at 100%, to which all values are related. Data are presented as means ± SE (n = 3 filters per condition). *P < 0.05 significantly different from control value.

suggesting a possible role for TRPV5 and/or TRPV6 in transcellular Ca²⁺ transport in mpkDCT cells; 4) stimulation of the cAMP/PKA pathway by forskolin in combination with IBMX increases net Ca²⁺ transport twofold; and 5) 1,25(OH)₂D₃ and dDAVP increase Ca²⁺ transport to the same extent as observed with cAMP/PKA pathway activation.

Confluent mpkDCT cells grown on filters developed high TEER, clearly indicating that the mpkDCT cell line forms tight monolayers. The observed TEER values are in line with observations reported by Peng et al. (32), who first described the mpkDCT cell line and demonstrated that the mpkDCT cells formed confluent monolayers of epithelial cells. Subsequently, Van Huyen et al. (38) clearly demonstrated a polarized monolayer of mpkDCT cells as assessed by staining for Na⁺-K⁺-ATPase in the basolateral membrane.

The distal part of the nephron consists of cTAL, DCT, CNT, and CD. To determine the origin of the murine mpkDCT cell line derived from late DCTs, AQP2 and NCC expression was determined. The CNT and CD marker AQP2 (9, 16) was not expressed in mpkDCT cells, whereas the DCT marker NCC (6, 28, 29) was present. These results demonstrated that the mpkDCT cell line possesses the expression profile of mouse DCT cells.

Ca²⁺ reabsorption in the distal part of the nephron is stimulated by several hormones including PTH, 1,25(OH)₂D₃, calcitonin, and vasopressin (26). PTH, calcitonin, and vasopressin bind to surface receptors that activate the trimeric G-protein Gₛ, leading to an increase in the cAMP level and subsequent activation of PKA (8, 37). However, other studies suggested that PKC activation is an important mechanism leading to the observed increase in Ca²⁺ reabsorption in the distal part of the nephron (14, 20). In the study of regulation of Ca²⁺ transport in confluent layers of mpkDCT cells, it was found that stimulation of PKC by PMA had no effect on transcellular Ca²⁺ transport, whereas a rise in cAMP caused by the addition of forskolin/IBMX significantly increased Ca²⁺ transport. Using RR, the increase in transcellular Ca²⁺ transport due to forskolin/IBMX stimulation could be completely inhibited, suggesting that the observed effect is due to RR-sensitive transcellular Ca²⁺ transport, possibly mediated by TRPV5 and/or TRPV6. These findings point to a role of the cAMP/PKA pathway, but not the PKC pathway, in regulating Ca²⁺ transport in DCT cells. This is in line with previous findings of our laboratory (20), that the dose-response curve for the increase in cAMP virtually matched that for transcellular Ca²⁺ transport in CNT and CD. The mechanism by which cAMP activates Ca²⁺ transport remains to be elucidated. It is unlikely that phosphorylation of TRPV5 and/or TRPV6 channels by PKA is involved, as the potential PKA phosphorylation sites are not species conserved. The mpkDCT cell line thus represents a valuable tool for unraveling the mechanism of hormone-mediated stimulation of transcellular Ca²⁺ transport.

1,25(OH)₂D₃ stimulated the Ca²⁺ transport by 70% across monolayers of mpkDCT cells grown on permeable filters. Previously, it was shown that 1,25(OH)₂D₃ increases the mRNA expression level of both Ca²⁺-transporting proteins, TRPV5 and TRPV6 (22, 27), calbindin-D₉k and calbindin-D₂₈k (13, 22), and PMCA1b (18). Together with the increased transport on stimulation by 1,25(OH)₂D₃, this clearly demonstrated that the effect of 1,25(OH)₂D₃ is due to transcriptional regulation of Ca²⁺-transporting proteins, which is consistent with previous results (22).

Transcellular Ca²⁺ transport across mpkDCT cells increased by ~100% on stimulation with dDAVP, whereas PTH had no significant effect on transcellular Ca²⁺ transport, which is in line with previous studies in which PTH does not stimulate adenylate cyclase in the DCT but does affect adenylate cyclase activity in the cTAL, CNT, and CD of the nephron (31). Another study also reported that PTH does not stimulate Ca²⁺ transport in the DCT, whereas ⁸(p-chlorophenylthio)-adenosine 3′,5′-cyclic monophosphate exhibits a stimulatory effect (35). On the other hand, Chabardes et al. (7) showed a PTH-dependent stimulation of adenylate cyclase activity in the mouse DCT and Peng et al. (32) demonstrated a minor but significant increase in the cAMP content after PTH stimulation in mpkDCT cells, whereas dDAVP increased cellular cAMP levels ~82-fold in these cells. Gesek and Friedman (17) demonstrated a stimulating effect of PTH on ⁴⁵Ca²⁺ influx in
a mixture of DCT and cTAL cells, which was abolished by nifedipine. The present study indicates that PTH has no significant effect on transepithelial Ca\(^{2+}\) transport in mpkDCT cells in the absence of nifedipine, verapamil, and BaCl\(_2\), suggesting that the described dihydropyridine- and PTH-sensitive Ca\(^{2+}\) channels (17) are not present in the mpkDCT cells.

The aim of the present study was to develop a cell line that can be of use in elucidating the molecular mechanism controlling transepithelial Ca\(^{2+}\) transport in the DCT. We postulate a possible role for TRPV5 and/or TRPV6 in mpkDCT cells, which is based on the following observations: 1) mpkDCT cells express TRPV5 and TRPV6 on mRNA level; 2) mpkDCT cells exhibit functional transepithelial Ca\(^{2+}\) transport, which is regulated by 1,25(OH)\(_2\)D\(_3\); 3) mpkDCT cells show an IC\(_{50}\) for RR, which is in line with the affinities for TRPV5/6; and 4) mpkDCT cells display strong inward rectifying currents (data not shown), which could be blocked by RR. Because specific blockers for TRPV5 and TRPV6 are not yet available, it is at present difficult to demonstrate conclusively that these Ca\(^{2+}\) channels are solely responsible for the transepithelial Ca\(^{2+}\) transport process observed in mpkDCT cells.

In conclusion, the findings of the present study clearly demonstrate that the transfected mpkDCT cell line exhibits transepithelial Ca\(^{2+}\) transport and has maintained many characteristics of native DCT cells, including expression of TRPV5 and TRPV6. This cell line thus provides a valuable tool for investigating short-term (regulatory) and long-term (transcriptional) control of renal transepithelial Ca\(^{2+}\) reabsorption.

GRANTS

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