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

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


This article cites 39 articles, 24 of which can be accessed free at:
http://ajprenal.physiology.org/content/286/3/F483.full.html#ref-list-1

This article has been cited by 5 other HighWire hosted articles

Klotho: a novel phosphaturic substance acting as an autocrine enzyme in the renal proximal tubule
Ming Chang Hu, Mingjun Shi, Jianning Zhang, Johanne Pastor, Teruyo Nakatani, Beate Lanske, M. Shawkat Razzaque, Kevin P. Rosenblatt, Michel G. Baum, Makoto Kuro-o and Orson W. Moe
FASEB J, September 2010; 24 (9): 3438-3450. [Abstract] [Full Text] [PDF]

K⁺-dependent Na⁺/Ca²⁺ exchanger 3 is involved in renal active calcium transport and is differentially expressed in the mouse kidney
Geun-Shik Lee, Kyung-Chul Choi and Eui-Bae Jeung
Am J Physiol Renal Physiol 2009; 297 (2): F371-F379. [Abstract] [Full Text] [PDF]

Renal expression of exchange protein directly activated by cAMP (Epac) 1 and 2
Yuedan Li, Irene B. M. Konings, Jun Zhao, Leo S. Price, Emile de Heer and Peter M. T. Deen
Am J Physiol Renal Physiol 2008; 295 (2): F525-F533. [Abstract] [Full Text] [PDF]

Protein kinase C inhibits caveolae-mediated endocytosis of TRPV5
Seung-Kyu Cha, Tao Wu and Chou-Long Huang
Am J Physiol Renal Physiol, May 2008; 294 (5): F1212-F1221. [Abstract] [Full Text] [PDF]

Defect in parathyroid-hormone-induced luminal calcium absorption in connecting tubules of Klotho mice
Shuichi Tsuruoka, Kenta Nishiki, Takashi Ioka, Hitoshi Ando, Yuichiro Saito, Masahiko Kurabayashi, Ryozo Nagai and Akio Fujimura
Nephrol. Dial. Transplant., October 2006; 21 (10): 2762-2767. [Abstract] [Full Text] [PDF]

Updated information and services including high resolution figures, can be found at:
http://ajprenal.physiology.org/content/286/3/F483.full.html

Additional material and information about AJP - Renal Physiology can be found at:
http://www.the-aps.org/publications/ajprenal

This information is current as of July 10, 2012.

AJP - Renal Physiology publishes original manuscripts on a broad range of subjects relating to the kidney, urinary tract, and their respective cells and vasculature, as well as to the control of body fluid volume and composition. It is published 12 times a year (monthly) by the American Physiological Society, 9650 Rockville Pike, Bethesda MD 20814-3991. Copyright © 2004 by the American Physiological Society. ISSN: 1931-857X, ESSN: 1522-1466. Visit our website at http://www.the-aps.org/.
Characterization of a murine renal distal convoluted tubule cell line for the study of transcellular calcium transport

Robin J. W. Diepens,1 Els den Dekker,1 Marcella Bens, A. Freek Weidema,1 Alain Vandewalle,2 René J. M. Bindels,3 and Joost G. J. Hoenderop1

1Department of Physiology, Nijmegen Center for Molecular Life Sciences, University Medical Center Nijmegen, NL-6500 HB Nijmegen, The Netherlands; and 2Institut National de la Santé et de la Recherche Médicale, U 478, Faculté de Médecine Xavier Bichat, 75870 Paris Cedex 18, France

Submitted 24 June 2003; accepted in final form 11 November 2003

Diepens, Robin J. W., Els den Dekker, Marcella Bens, A. Freek Weidema, Alain Vandewalle, René J. M. Bindels, and Joost G. J. Hoenderop. Characterization of a murine renal distal convoluted tubule cell line for the study of transcellular calcium transport. Am J Physiol Renal Physiol 286: F483–F489, 2004.—To unravel the molecular regulation of renal transcellular Ca2+ transport, a murine distal convoluted tubule (mpkDCT) cell line derived from the kidney of an SV-PK/Tag transgenic mouse was characterized. This cell line originated from DCT only, as mRNA encoding for the DCT marker thiazide-sensitive Na+/Cl− cotransporter was expressed, whereas mRNA encoding for the connecting tubule and collecting duct marker aquaporin-2 was not detected, as determined by reverse-transcriptase PCR. mpkDCT cells expressed mRNA encoding the Ca2+ channels TRPV5 and TRPV6 and other key players necessary for transcellular Ca2+ transport, i.e., calbindin-D9k, calbindin-D28k, plasma membrane Ca2+-ATPase isoform 1b, and Na+/Ca2+ exchanger 1. Primary cultures of DCT cells exhibited net tranacellular Ca2+ transport of 0.4 ± 0.1 nmol·h−1·cm−2, whereas net transcellular Ca2+ transport across mpkDCT cells was significantly higher at 2.4 ± 0.4 nmol·h−1·cm−2. Transcellular Ca2+ transport across mpkDCT cells was completely inhibited by ruthenium red, an inhibitor of TRPV5 and TRPV6, but not by the voltage-operated 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), where the Na+/Ca2+ exchanger (NCX1) and the plasma membrane Ca2+-ATPase 1b (PMCA1b) account for Ca2+ extrusion (24, 33, 40).

The calciotropic hormone 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) tightly regulates tranacellular Ca2+ transport. It is active at all three processes necessary for tranacellular 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 tranacellular 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 tranacellular 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 mpkCCD5,6 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 μg/ml insulin (Sigma, St. Louis, MO), 5 nM dexamethasone (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 ( Gibco, Breda, The Netherlands), 4.5 mM glutamine (GIBCO), and 45 μg/ml gentamicin at pH 7.4, equilibrated with 5% CO2-95% air at 37°C. For

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 tranacellular Ca2+ transport in the distal part of the nephron is tightly controlled by calciotropic 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), where the Na+/Ca2+ exchanger (NCX1) and the plasma membrane Ca2+-ATPase 1b (PMCA1b) account for Ca2+ extrusion (24, 33, 40).

The calciotropic hormone 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) tightly regulates tranellular Ca2+ transport. It is active at all three processes necessary for tranellular 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 tranellular Ca2+ transport in DCTs remains elusive, because in vivo tranellular 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 tranellular 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 mpkCCD5,6 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 μg/ml insulin (Sigma, St. Louis, MO), 5 nM dexamethasone (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 ( Gibco, Breda, The Netherlands), 4.5 mM glutamine (GIBCO), and 45 μg/ml gentamicin at pH 7.4, equilibrated with 5% CO2-95% air at 37°C.

The costs 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 \( ^{45}\text{Ca}^{2+} \) transport studies, and RNA isolation, \( \sim 5 \times 10^4 \) 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 C57BL/6 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 collagenses 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 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.

\( ^{45}\text{Ca}^{2+} \) 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 \( \delta \)-glucose, 1 mM t-alanine adjusted to pH 7.4 using 1 M Tris, supplemented with 1 mM CaCl\(_2\), 10 \( \mu \)M feldipine, 10 \( \mu \)M verapamil, 1 mM BaCl\(_2\), 2 \( \mu \)Ci \( ^{45}\text{Ca}^{2+} \)/ml, and with the drug of choice: 10 \( \mu \)M forskolin (Sigma) and 100 \( \mu \)M IBMX (Sigma), 100 mM PMA (Sigma), 100 mM 1,25(OH)\(_2\)D\(_3\) (Solvay, Weesp, The Netherlands), ruthenium red (RR; 100 nM Tris-HCl, 50 mM KCl, 0.01% (vol/vol) gelatin, and 2.5 mM MgCl\(_2\)), 1 mM dNTPs, and 0.2 \( \mu \)M of each primer. For RT-PCR, the following protocol was used: 1 min at 95°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 10 min at 72°C. An actin RT \(^\text{mRNA}\) 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 \( \text{Na}^+ \) and \( \text{Ca}^{2+} \) currents related to TRPV5 and TRPV6 channel activity have been described previously in detail (39). Patch-clamp experiments were performed with a whole-cell configuration, using an EPC-9 patch-clamp amplifier (HEKA Elektronik, Lambrecht, Germany). Patch pipettes had DC resistances of 2–4 M\( \Omega \) 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 +20 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 BPM, 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 \( \mu \)M glucose, and different concentrations of RR (100 nM-100 \( \mu \)M). Divalent-free solutions did not contain added divalent cations, while trace amounts of divalent cations were added with 100 \( \mu \)M 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

\( \text{mpkDCT cell line possesses specific properties of DCTs.} \)

\( \text{mpkDCT cells were derived from isolated DCTs, microdissected from the kidney of an adult transgenic mouse (SV-PK/} \)

---

### Table 1. Sequences of primers for RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Product (bp)</th>
<th>Annealing Temp (°C)</th>
<th>No. of Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRPV5</td>
<td>5′-CGTTGTTCTTATGAGGGTGAAC-3′</td>
<td>5′-GGTTGGAAGACACAGAGCTCTA-3′</td>
<td>168</td>
<td>50°</td>
<td>40</td>
</tr>
<tr>
<td>TRPV6</td>
<td>5′-ATGACGCTGATCATGGACACG-3′</td>
<td>5′-AGTTGTTCTCCTGATGTCTTTCCCAA-3′</td>
<td>80</td>
<td>50°</td>
<td>40</td>
</tr>
<tr>
<td>CaBP-D(_6)</td>
<td>5′-GCCGACGAGAGCTGCTTTGT-3′</td>
<td>5′-TGAACTCTTTCCCCACACATTTGCAT-3′</td>
<td>175</td>
<td>50°</td>
<td>40</td>
</tr>
<tr>
<td>CaBP-D(_{27})</td>
<td>5′-ACCAGCAGCGGAGGCAGGCA-3′</td>
<td>5′-TGACGGCTCTTTCGAGTGTTG-3′</td>
<td>87</td>
<td>50°</td>
<td>60</td>
</tr>
<tr>
<td>PMCA 1b</td>
<td>5′-CCGGACGTCTCAGTACTCCTGAGC-3′</td>
<td>5′-CAGCCGTGCTGTCAGGTCGAG-3′</td>
<td>109</td>
<td>50°</td>
<td>60</td>
</tr>
<tr>
<td>NCX1</td>
<td>5′-TCGGTTATCTGATGAGTCAGGACAC-3′</td>
<td>5′-TTTCTGATCATCCTGCTGATCTGATT-3′</td>
<td>142</td>
<td>50°</td>
<td>60</td>
</tr>
<tr>
<td>CaSR</td>
<td>5′-CTGGATCACTGCTGCTGCTGCTGATGATT-3′</td>
<td>5′-GCAAAATGACTGCTGTTATACACCA-3′</td>
<td>120</td>
<td>50°</td>
<td>40</td>
</tr>
<tr>
<td>NCC</td>
<td>5′-GTGGACTGCTGCTGCTGCTGAC-3′</td>
<td>5′-GTCGAGAAAATGACCGTGTG-3′</td>
<td>461</td>
<td>60°</td>
<td>40</td>
</tr>
<tr>
<td>AQP2</td>
<td>5′-GCCCAGGATGCGAGGCGGCTG-3′</td>
<td>5′-AACCATGGGACGGCGCGGCTGACG-3′</td>
<td>555</td>
<td>60°</td>
<td>40</td>
</tr>
<tr>
<td>PThr</td>
<td>5′-CCGGACGAGAGCTGCTTTGT-3′</td>
<td>5′-GGCGACCATAGAAGACAGAA-3′</td>
<td>531</td>
<td>54°</td>
<td>40</td>
</tr>
<tr>
<td>β-Actin</td>
<td>5′-AGGTCTGCTCTGCTGCTGCTGATGATT-3′</td>
<td>5′-GTATTGCGCTGCTGCTGCTGAC-3′</td>
<td>201</td>
<td>50°</td>
<td>40</td>
</tr>
</tbody>
</table>

PCR primers were purchased from Biologeo. Product size is in base pairs. CaBP-D\(_6\), and \(-\text{DCTs, calbindin } -\text{ DCTs, and } -\text{DCTs; PMCA 1b, plasma-membrane Ca}^{2+}-\text{ATPase 1b; NCX1, Na}^{+}/\text{Ca}^{2+}\) exchanger; CaSR, calcium-sensing receptor; NCC, Na\(^{+}/\text{Cl}^{-}\) cotransporter; AQP2, aquaporin-2; PThr, parathyroid hormone receptor.
TAG) (11, 32). To demonstrate the origin of nature of this microdissected DCT cell line, the expression of typical markers for DCT, CNT, and CD was determined using RT-PCR analysis. As shown in Fig. 1A, mpkDCT cells expressed NCC, which is a marker for DCT, but did not express aquaporin-2 (AQP2), known to be expressed in CNT and CD. To determine whether the mpkDCT cell line is suitable for the study of transcellular Ca\textsuperscript{2+} transport, the mRNA expression of key players involved in this process was investigated using RT-PCR. As shown in Fig. 1B, all the constituents of the transcellular Ca\textsuperscript{2+} transport process, including TRPV5, TRPV6, calbindin-D\textsubscript{9k}, calbindin-D\textsubscript{28k}, PMCA1b, NCX1, and CaSR, were expressed in the mpkDCT cell line.

**Transcellular Ca\textsuperscript{2+} 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 Ca\textsuperscript{2+} 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 cm\textsuperscript{2} (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 cm\textsuperscript{2} (n = 23). Net Ca\textsuperscript{2+} transport was calculated by subtracting basolateral-to-apical Ca\textsuperscript{2+} transport from apical-to-basolateral Ca\textsuperscript{2+} 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 Ca\textsuperscript{2+} transport across primary cultures of DCT cells was 0.4 ± 0.1 nmol·h\textsuperscript{-1}·cm\textsuperscript{-2} (Fig. 2A), whereas net Ca\textsuperscript{2+} transport across the mpkDCT cell line was significantly higher (2.4 ± 0.2 nmol·h\textsuperscript{-1}·cm\textsuperscript{-2}; Fig. 2B). The calculated net Ca\textsuperscript{2+} transport for the mpkDCT cell line was linear for up to 4 h after addition of \textsuperscript{45}Ca\textsuperscript{2+}. In subsequent experiments, net Ca\textsuperscript{2+} transport was calculated using the 60-min time point.

**Ca\textsuperscript{2+} transport across mpkDCT cells is RR sensitive.** To study RR-sensitive transcellular Ca\textsuperscript{2+} transport independent of voltage-operated Ca\textsuperscript{2+} 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 Ca\textsuperscript{2+} channels, and BaCl\textsubscript{2} (1 mM) was added to prevent changes in membrane potential. With the use of different concentrations of RR, the IC\textsubscript{50} of RR for the blockage of transcellular Ca\textsuperscript{2+} transport was determined. For mpkDCT cells, the IC\textsubscript{50} of RR on the Na\textsuperscript{+} current was 8.8 ± 0.3 μM (Fig. 3). In this study, the IC\textsubscript{50} of RR on the Na\textsuperscript{+} current of

---

**Fig. 1.** Characterization of murine distal convoluted tubule (mpkDCT) cells by RT-PCR. mpkDCT cells were grown on permeable membrane filter supports until confluence. A: detection of aquaporin-2 (AQP2) mRNA and thiazide-sensitive Na\textsuperscript{+}/Cl\textsuperscript{−} cotransporter (NCC) mRNA; B: detection of mRNA encoding for TRPV5, TRPV6, calbindin-D\textsubscript{9k}, calbindin-D\textsubscript{28k}, plasma membrane Ca\textsuperscript{2+} ATPase 1b (PMCA1b), Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger 1 (NCX1), Ca\textsuperscript{2+}-sensing receptor (CaSR). β-Actin was used as a control to demonstrate the integrity of the isolated RNA. As a negative control, a PCR (60 cycles) on β-actin was performed on a RT− sample. C: detection of mRNA encoding the parathyroid hormone receptor (PTHr) in mpkDCT cells.

**Fig. 2.** 45Ca\textsuperscript{2+} transport across cultured distal convoluted tubule (DCT) cell monolayers. Transcellular Ca\textsuperscript{2+} transport was measured at various time points between 15 and 180 min. Basolateral-to-apical transport was subtracted from apical-to-basolateral transport to determine net Ca\textsuperscript{2+} transport. □, basolateral-to-apical transport; ●, net transport. A: 45Ca\textsuperscript{2+} transport across primary cultures of DCT cells. B: 45Ca\textsuperscript{2+} transport across the mpkDCT cell line. Data are presented as means ± SE (n ≥ 6 per time point). *P < 0.05; apical-to-basolateral transport significantly different from basolateral-to-apical transport.
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 \[^{45}\text{Ca}^{2+}\] transport measured in the mpkDCT cells was inhibited by RR with an IC\(_{50}\) 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)\(_{2}\)D\(_{3}\) increased transcellular \[^{45}\text{Ca}^{2+}\] transport in mpkDCT cells. It has been shown that an increase of intracellular cAMP triggers stimulation of \[^{45}\text{Ca}^{2+}\] reabsorption in the kidney. Other studies showed that \[^{45}\text{Ca}^{2+}\] 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 \[^{45}\text{Ca}^{2+}\] transport was studied in the mouse mpkDCT cell line. Forskolin (10 μM) in combination with IBMX (100 μM) increased \[^{45}\text{Ca}^{2+}\] 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 \[^{45}\text{Ca}^{2+}\] 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)\(_{2}\)D\(_{3}\) (100 nM), bPTH (1–34) (100 nM), or dDAVP (10 nM) during the \[^{45}\text{Ca}^{2+}\] transport experiment. mpkDCT cells were incubated for 72 h with 1,25(OH)\(_{2}\)D\(_{3}\) (100 nM) to determine the long-term effects on \[^{45}\text{Ca}^{2+}\] transport. As shown in Fig. 6A, both dDAVP and long-term stimulation with 1,25(OH)\(_{2}\)D\(_{3}\) increased \[^{45}\text{Ca}^{2+}\] transport with 119 ± 7 and 70 ± 21%, respectively, compared with nontreated cells. 1,25(OH)\(_{2}\)D\(_{3}\)- and dDAVP-stimulated \[^{45}\text{Ca}^{2+}\] transport was significantly inhibited by RR (Fig. 6A). Because bPTH had no significant effect on transcellular \[^{45}\text{Ca}^{2+}\] 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 \[^{45}\text{Ca}^{2+}\] transport. Gesek and Friedman (17) showed a significant effect of PTH on \[^{45}\text{Ca}^{2+}\] 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 BaCl\(_{2}\), PTH did not stimulate transcellular \[^{45}\text{Ca}^{2+}\] transport in mpkDCT cells (Fig. 6B). Moreover, transcellular \[^{45}\text{Ca}^{2+}\] 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 \[^{45}\text{Ca}^{2+}\] transport in the DCT in vitro. This conclusion is based on the following observations: 1) mpkDCT cells express the key players necessary for transcellular \[^{45}\text{Ca}^{2+}\] transport: the epithelial \[^{45}\text{Ca}^{2+}\] channels TRPV5 and TRPV6, the intracellular \[^{45}\text{Ca}^{2+}\] translocators calbindin-D\(_{9k}\) and calbindin-D\(_{28k}\), and the basolateral \[^{45}\text{Ca}^{2+}\] extrusion proteins NCX1 and PMCA1b; 2) mpkDCT cells exhibit a significant net apical-to-basolateral \[^{45}\text{Ca}^{2+}\] transport; 3) RR completely inhibits net \[^{45}\text{Ca}^{2+}\] transport, sug-
suggesting a possible role for TRPV5 and/or TRPV6 in transcellular Ca\(^{2+}\) transport in mpkDCT cells; 4) stimulation of the cAMP/PKA pathway by forskolin in combination with IBMX increases net Ca\(^{2+}\) transport twofold; and 5) 1,25(OH)\(_2\)D\(_3\) and dDAVP increase Ca\(^{2+}\) 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.

Ca\(^{2+}\) reabsorption in the distal part of the nephron is stimulated by several hormones including PTH, 1,25(OH)\(_2\)D\(_3\), 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 Ca\(^{2+}\) reabsorption in the distal part of the nephron (14, 20). In the study of regulation of Ca\(^{2+}\) transport in confluent layers of mpkDCT cells, it was found that stimulation of PKC by PMA had no effect on transcellular Ca\(^{2+}\) transport, whereas a rise in cAMP caused by the addition of forskolin/IBMX significantly increased Ca\(^{2+}\) transport. Using RR, the increase in transcellular Ca\(^{2+}\) transport due to forskolin/IBMX stimulation could be completely inhibited, suggesting that the observed effect is due to RR-sensitive transcellular Ca\(^{2+}\) 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 Ca\(^{2+}\) 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 Ca\(^{2+}\) transport in CNT and CD. The mechanism by which cAMP activates Ca\(^{2+}\) 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 Ca\(^{2+}\) transport.

1,25(OH)\(_2\)D\(_3\) stimulated the Ca\(^{2+}\) transport by 70% across monolayers of mpkDCT cells grown on permeable filters. Previously, it was shown that 1,25(OH)\(_2\)D\(_3\) increases the mRNA expression level of both Ca\(^{2+}\)- and Na\(^{+}\)-transporting proteins, which is consistent with previous results (22).

Transcellular Ca\(^{2+}\) transport across mpkDCT cells increased by ~100% on stimulation with dDAVP, whereas PTH had no significant effect on transcellular Ca\(^{2+}\) transport, which is in line with previous studies in which PTH does not stimulate adenylyl cyclase in the DCT but does affect adenylyl cyclase activity in the cTAL, CNT, and CD of the nephron (31). Another study also reported that PTH does not stimulate Ca\(^{2+}\) 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 adenylyl 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 \(^{45}\)Ca\(^{2+}\) influx in

---

**Fig. 6. Effect of 1,25-dihydroxyvitamin D\(_3\), 1-deamino-8-D-arginine vasopressin (dDAVP), and parathyroid hormone (PTH) on Ca\(^{2+}\) transport across mpkDCT monolayers.**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Ca(^{2+}) Transport (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td></td>
</tr>
<tr>
<td>PTH</td>
<td></td>
</tr>
<tr>
<td>dDAVP</td>
<td></td>
</tr>
<tr>
<td>1,25(OH)(_2)D(_3)</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as means ± SE (n = 3 filters per condition). *P < 0.05 significantly different from control value.
a mixture of DCT and cTAL cells, which was abolished by nifedipine. The present study indicates that PTH has no significant effect on transcellular \( \text{Ca}^{2+} \) transport in mpkDCT cells in the absence of felodipine, verapamil, and \( \text{BaCl}_2 \), suggesting that the described dihydropyridine- and PTH-sensitive \( \text{Ca}^{2+} \) channels (17) are not present in the mpkDCT cells.

The aim of the present study was to develop a cell line that can be used in elucidating the molecular mechanism controlling transcellular \( \text{Ca}^{2+} \) 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 transcellular \( \text{Ca}^{2+} \) 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 \( \text{Ca}^{2+} \) channels are solely responsible for the transcellular \( \text{Ca}^{2+} \) transport process observed in mpkDCT cells.

In conclusion, the findings of the present study clearly demonstrate that the transmortalized mpkDCT cell line exhibits transcellular \( \text{Ca}^{2+} \) 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 transcellular \( \text{Ca}^{2+} \) reabsorption.

GRANTS

This study was financially supported by grants from the Dutch Organization of Scientific Research (Zon-Mw 902.18.298, Zon-Mw 016.006.001).

REFERENCES

32. Peng KC, Cluzeaud F, Bens M, Van Huyen JP, Wioda MA, Lacave R, and Vandewalle A. Tissue and cell distribution of the multidrug...