Calbindin-D$_{28K}$ facilitates cytosolic calcium diffusion without interfering with calcium signaling

H.P.G. KOSTER, A. HARTOG, C.H. VAN OS and R.J.M. BINDELS

Department of Cell Physiology, University of Nijmegen, Nijmegen, The Netherlands

Abstract — The role of calbindin-D$_{28K}$, in transcellular Ca$^{2+}$ transport and Ca$^{2+}$ signaling in rabbit cortical collecting system was investigated. Rabbit kidney connecting tubules and cortical collecting ducts, hereafter referred to as cortical collecting system, were isolated by immunodissection and cultured to confluence on permeable filters and glass coverslips. Calbindin-D$_{28K}$ was present in the cytosol of principal cells, but was absent from the intercalated cells. 1,25(OH)$_2$D$_3$ (48 h, 10$^{-7}$ M) significantly increased cellular calbindin-D$_{28K}$ levels (194 ± 15%) and stimulated transcellular Ca$^{2+}$ transport (41 ± 3%). This stimulatory effect could be fully mimicked by the endogenous Ca$^{2+}$ chelator, BAPTA (30 μM BAPTA/AM), which suggests that the presence of Ca$^{2+}$ chelators alone is sufficient to enhance transcellular Ca$^{2+}$ transport. Stimulation of Ca$^{2+}$ transport was not accompanied by a rise in [Ca$^{2+}$]. Isosmotic replacement of extracellular Na$^+$ ([Na$^+$]$_o$) for N-methylglucamine (NMG) generated oscillations in [Ca$^{2+}$] in individual cells of the monolayer. The functional parameters of these oscillations such as frequency of spiking, resting [Ca$^{2+}$], increase in [Ca$^{2+}$] and percentage of responding cells, were not affected by the level of calbindin-D$_{28K}$. In contrast, loading the cells with BAPTA abruptly stopped these [Ca$^{2+}$] oscillations. This suggests that the kinetics of Ca$^{2+}$ binding by calbindin-D$_{28K}$ are slow relative to the initiation of the [Ca$^{2+}$] rise, so that calbindin-D$_{28K}$, unlike BAPTA, is unable to reduce [Ca$^{2+}$] rapidly enough to prevent the initiation of Ca$^{2+}$-induced Ca$^{2+}$ release.
however, calbindin-D28K is present in a subpopulation of neurons scattered in most but not all areas of the central nervous system, where its presence is not influenced by the vitamin D3 status and where its function is unknown [1].

Free cytosolic Ca2+ ([Ca2+]i) is generally maintained at low resting values and, for example, increasing [Ca2+]i in the intestine results in decreased NaCl absorption in villi and increased secretion in crypts [10]. Also, in renal cells, [Ca2+]i is implicated in the regulation of transport processes. For example, Na+ reabsorption and K+ secretion in the distal nephron are partly regulated by activation of Ca2+-dependent Na4 and K5 channels [11, 12]. The cellular Ca2+ homeostasis in duodenum and distal nephrons is continuously challenged by large and variable rates of transcellular Ca transport, which is tuned to the need of the body. In addition, calbindin-D28K could, in theory, interfere with cellular Ca2+ signaling in view of its Ca2+ chelating properties.

In Ca2+ absorbing epithelial cells, the tuning of transcellular Ca2+ transport to cellular Ca2+ homeostasis is still poorly understood [13]. We have addressed this question by using a primary culture of renal connecting tubule and cortical collecting duct cells. These renal cells in culture retain the ability to transport Ca2+ transeellularly under control of PTH and 1,25(OH)2D3 [14]. In addition, [Ca2+]i oscillations can be provoked in these cells [15]. In the present study, the intracellular Ca2+ buffering capacity was manipulated by exposure to 1,25(OH)2D3 to increase calbindin-D28K content or by loading the cells with the Ca2+ ligand, BAPTA. Evidence is now provided that calbindin-D28K enhances diffusional flux of Ca2+ but does not interfere with Ca2+ signaling. In contrast, BAPTA is shown to enhance transcellular Ca2+ transport, but quenches [Ca2+]i oscillations completely.

Materials and methods

Isolation of rabbit kidney cortical collecting system cells

Rabbit kidney cortical collecting system cells were isolated from New Zealand white rabbits by immunodissection using monoclonal antibody R2G9 [14]. The cells were subsequently cultured in culture medium (equilibrated with 5% CO2-95% air at 37°C) on circular glass coverslips (diameter = 22 mm) or on 0.3 cm2 permeable filters (Costar, Badhoevedorp, The Netherlands) coated with rat tail collagen as described previously [14]. All experiments were performed on monolayers grown to confluency (4–7 days after seeding).

Fura–2 and BAPTA loading

Fura–2 was loaded into the cells during a 30 min incubation at 37°C in culture medium supplemented with 5 μM Fura–2 acetoxymethyl ester (Fura–2/AM), 0.4% (w/v) DMSO, 0.02% (w/v) Pluronic F127 and 4% (v/v) decomplemented fetal calf serum. Cells were loaded with the Ca2+ chelator BAPTA by exposure to incubation medium (at 37°C) containing 30 μM BAPTA/AM; 0.4% (w/v) DMSO; 0.02% (w/v) Pluronic F12 during the experiment.

Measurement of [Ca2+]i in single cells

After loading the cells with Fura–2, the coverslips were transferred to a thermostated ‘Leiden-Chamber’ [15] and mounted on an inverted Diaphot microscope (Nikon, Amsterdam, The Netherlands). The cells were washed by superfusion with incubation medium for 3 min (2 ml/min, 37°C) after which, under continued superfusion, the experiment was started. The MagiCal imaging system was used to measure [Ca2+]i (Joyce Loeble, UK). The Fura–2 loaded cells were alternatingly excited at 340 and 380 nm (bandwidth 10 nm) and images of the Fura–2 fluorescence of 30–40 cells emitted at 492 nm (bandwidth 30 nm) were captured (capture time 0.32 s; average of 8 frames) by a CCD camera at intervals of 7 s, using TARDIS software for digital analysis as described in detail by Neylon et al. [16]. In some experiments, the Newcastle Photometric System (NPS system) was used, in which Fura–2 fluorescence from single cells is measured by a photomultiplier as described previously [15]. [Ca2+]i was calculated according to the formula derived by Grynkiewicz et al. [17].
**Determination of transepithelial \( \text{Ca}^{2+} \) fluxes**

Filter cups were washed and bathed at 37°C in incubation medium. Previously, we determined that transepithelial \( \text{Ca}^{2+} \) absorption from a medium containing 1 mM \( \text{Ca}^{2+} \) was linear up to 3 h [18]. In the present study, \( \text{Ca}^{2+} \) absorption was established by removing duplicates of 25 μl apical fluid following an incubation of 90 min. The total \( \text{Ca}^{2+} \) concentration of the samples was assayed using a colorimetric test kit (Boehringer, Mannheim, Germany) and \( \text{Ca}^{2+} \) absorption was expressed in nmol h\(^{-1}\) cm\(^{-2}\).

**Identification of principal and intercalated cells by immunocytochemistry**

The primary cultures contain principal and intercalated cells [15]. In order to distinguish intercalated cells [19], monolayers loaded with Fura-2, were exposed to FITC-conjugated peanut lectin (5 μg/ml for 5 min) and, before starting [\( \text{Ca}^{2+} \)]\(_i\) measurements, the FITC-labelled monolayers were examined. Principal cells could be visualized after Fura-2 imaging by immunohistology using a polyclonal antiserum against chicken calbindin-D\(_{28K}\) as described previously [20]. As secondary antibody, an FITC-conjugated antirabbit Ig was used. One image of the resulting staining pattern was captured (excitation 490 nm, emission above 510 nm, MagiCal system) to match the presence of calbindin-D\(_{28K}\) or peanut lectin with [\( \text{Ca}^{2+} \)]\(_i\) oscillations. Loading of principal cells with Fura-2 appeared to be far better than of intercalated cells and when the CCD camera was used [\( \text{Ca}^{2+} \)]\(_i\) measurement in intercalated cells were unreliable. Therefore, in some experiments, a more sensitive photomultiplier (NPS system) was used to record fluorescence from Fura-2 loaded intercalated cells.

**Calbindin-D\(_{28K}\) assay**

An ELISA for calbindin-D\(_{28K}\) was performed as described previously [14]. Briefly a 96-well polystyrene plate was: (i) coated with 100 ng purified rabbit calbindin-D\(_{28K}\); (ii) blocked with 0.1% w/v BSA; (iii) 50 μl samples containing either cytosolic fractions of cultured collecting system cells or samples of purified rabbit calbindin-D\(_{28K}\) for a calibration curve were added, both followed by 50 μl rabbit polyclonal antiserum against chick calbindin-D\(_{28K}\) (diluted 1:750); (iv) peroxidase-conjugated goat anti-rabbit IgG (H and L) (diluted 1:500) was added and finally 0.5 mg/ml o-phenylenediamine and 0.1% w/v H\(_2\)O\(_2\) were used to develop the color. After each step, the ELISA plate was washed 4 times.

**Experimental procedures**

Culture medium: DME/F12 (1:1) (Gibco, Breda, The Netherlands) supplemented with 5% (v/v) de-complemented fetal calf serum; 50 μg/ml gentamicin; 10 μg/ml non-essential amino acids (Gibco); 5 μg/ml insulin; 5 μg/ml transferrin; 50 nM hydrocortisone; 70 ng/ml PGE\(_1\); 50 nM Na\(_2\)SeO\(_3\); 5 pM triiodothyronine. Incubation medium (in mM): 140 NaCl; 2 KCl; 1 K\(_2\)HPO\(_4\); 1 KH\(_2\)PO\(_4\); 1 MgCl\(_2\); 1 CaCl\(_2\); 5 glucose; 5 L-alanine; 10 HEPES/Tris, pH 7.40. Fura-2/AM, BAPTA/AM and Pluronic F127 were obtained from Molecular Probes Inc. (Eugene, OR, USA). 1,25(OH)\(_2\)D\(_3\) was kindly provided by Solvay-Duphar (Weesp, The Netherlands). All other chemicals were obtained from Sigma (St Louis, MO, USA).

**Fig. 1** Effect of removal of medium Na\(^+\) (Na\(^+\)\(_o\)) on [\( \text{Ca}^{2+} \)]\(_i\) in cultured cells from rabbit cortical collecting system. Na\(^+\)\(_o\) (NaCl) was iso-osmotically replaced with N-methylglucamine (NMGC1). [\( \text{Ca}^{2+} \)]\(_i\) was calculated from the Fura-2 340/380 nm excitation fluorescence emission ratio which was recorded with the fluorescence imaging MagiCal system. Representative trace from 6 experiments is shown, in which a total of 36 cells were analyzed.
Statistical analysis

In all experiments, data were assessed from at least 3 isolations. Analysis of variance was used to determine statistical differences between two independent groups [21].

Results

In a previous study, we described that in cells of cortical collecting system in primary culture, removal of extracellular Na⁺ induced oscillations of [Ca²⁺]ᵢ, which arise from phospholipase C activation in concert with Ca²⁺-induced Ca²⁺ release [15]. The present study extends our previous observations by employing a fluorescence imaging system equipped with a CCD camera which permits simultaneous analysis of the [Ca²⁺]ᵢ responses of several cells at the single cell level and estimation of cytosolic calbindin-D₂₈K content. Isosmotic replacement of medium Na⁺ (Na⁺₀) for N-methylglucamine (NMG) results in [Ca²⁺]ᵢ oscillations in primary cultures of rabbit cortical collecting system cell. The type of oscillations most frequently observed (~80% of the occurrences) was an increase in [Ca²⁺]ᵢ in an oscillatory fashion with Ca²⁺ returning to resting levels in between two spikes, as shown in Figure 1.

Characterization of [Ca²⁺]ᵢ oscillations in principal and intercalated cells

Since the primary culture of rabbit cortical collecting system is composed of two cell types, individual

Fig. 2 Na⁺₀-free induced [Ca²⁺]ᵢ oscillations in a principal (A,C) and an intercalated (B,D) cell of rabbit cortical collecting system in primary culture. [Ca²⁺]ᵢ was calculated from the Fura-2 340/380 nm excitation fluorescence emission ratio which was recorded with the NPS system. Monolayers were double stained to distinguish principal from intercalated cells. Principal cells were recognized by immunohistological staining with a polyclonal antibody against chicken calbindin-D₂₈K (A) and intercalated cells were identified by exposure to FITC-conjugated peanut lectin (B). Bars represent 10 μm. Representative data from 6 experiments are shown, in which a total of 36 cells were analyzed.
Table 1 Characterisation of Na⁺-free induced [Ca²⁺]ᵢ oscillations in principal and intercalated cells recorded with the NPS system. Resting and peak [Ca²⁺]ᵢ, oscillations frequency and percentage of cells showing oscillations when exposed to Na⁺ free medium. Values represent mean ± SE with n > 19.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Resting [Ca²⁺]ᵢ (nM)</th>
<th>Peak [Ca²⁺]ᵢ (nM)</th>
<th>Frequency (min⁻¹)</th>
<th>Oscillating cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercalated</td>
<td>116 ± 11</td>
<td>261 ± 30</td>
<td>0.59 ± 0.05</td>
<td>73 ± 8</td>
</tr>
<tr>
<td>Principal</td>
<td>123 ± 14</td>
<td>326 ± 28</td>
<td>0.64 ± 0.06</td>
<td>65 ± 13</td>
</tr>
</tbody>
</table>

cells were identified by immunocytochemistry, using peanut lectin to recognize intercalated cells and an antiserum against chicken calbindin-D₂₈K to recognize principal cells (Fig. 2A,B) [19]. The majority of cells (79 ± 4%, n = 400) were calbindin-D₂₈K positive and peanut lectin negative and, therefore, identified as principal cells, whereas a minority (18 ± 5%) were calbindin-D₂₈K negative and peanut lectin positive and classified as intercalated cells. In principal cells, calbindin-D₂₈K was evenly distributed throughout the cytosol.

Na⁺-free medium induced in both cell types oscillatory increases in [Ca²⁺]ᵢ as depicted in Figure 2. The characteristics of these oscillations, i.e. oscillatory frequency, resting and peak values of [Ca²⁺]ᵢ, together with the percentage of cells that exhibit [Ca²⁺]ᵢ oscillations, are shown in Table 1. There were no significant differences between these parameters among principal and intercalated cells (P < 0.05, n ≥ 19).

Effect of 1,25(OH)₂D₃ on Ca²⁺ transport, calbindin-D₂₈K content and Ca²⁺ signaling

The monolayers were incubated for 48 h with 10⁻⁷ M 1,25(OH)₂D₃ and subsequently transcellular Ca²⁺ transport, cellular calbindin-D₂₈K content and [Ca²⁺]ᵢ oscillations were examined. 1,25(OH)₂D₃ significantly increased transcellular Ca²⁺ transport by 41 ± 3% (Fig. 3) and calbindin-D₂₈K content from 0.69 ± 0.09 to 2.03 ± 0.31 μg.mg protein⁻¹ (P > 0.2, n = 4). However, the characteristics of [Ca²⁺]ᵢ oscillations were not significantly altered in principal cells cultured for 2 days in the presence of 1,25(OH)₂D₃ when compared with control cells

Table 2 Characterisation of Na⁺-free induced [Ca²⁺]ᵢ oscillations in principal cells exposed to 10⁻⁷ M 1,25(OH)₂D₃ for 48 h (or to vehicle) measured with the fluorescence imaging MagiCal system. Resting and peak [Ca²⁺]ᵢ, oscillations frequency and percentage of cells showing oscillations when exposed to Na⁺ free medium. Values represent mean ± SE with n ≥ 100.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Resting [Ca²⁺]ᵢ (nM)</th>
<th>Peak [Ca²⁺]ᵢ (nM)</th>
<th>Frequency (min⁻¹)</th>
<th>Oscillating cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>98 ± 6</td>
<td>201 ± 4</td>
<td>0.71 ± 0.02</td>
<td>79 ± 8</td>
</tr>
<tr>
<td>1,25(OH)₂D₃</td>
<td>108 ± 6</td>
<td>214 ± 4</td>
<td>0.73 ± 0.02</td>
<td>66 ± 6</td>
</tr>
</tbody>
</table>
Effect of BAPTA on Ca\(^{2+}\) transport and Ca\(^{2+}\) signaling

Loading cells of the cortical collecting system with the Ca\(^{2+}\) chelator BAPTA (30 \(\mu\)M BAPTA/AM) significantly \((P < 0.05, n = 4)\) enhanced transcellular Ca\(^{2+}\) transport by 28 ± 5\% (Fig. 3). Resting \([\text{Ca}^{2+}]_i\), however, was not influenced by BAPTA. \([\text{Ca}^{2+}]_i\) was 108 ± 3 and 98 ± 3, \((P > 0.2, n = 24)\) for control and BAPTA-loaded cells, respectively (Fig. 5A). On the contrary, when proximal tubule cells in primary culture, which lack calbindin-D\(_{28K}\), are loaded with BAPTA, \([\text{Ca}^{2+}]_i\) is lowered from

Fig. 4 Effect of calbindin-D\(_{28K}\) concentration on Na\(^+\)-free induced \([\text{Ca}^{2+}]_i\) oscillations in principal cells of rabbit cortical collecting system in primary culture. Principal cells were recognized by immunohistological staining with a polyclonal antibody against chicken calbindin-D\(_{28K}\) (A). \([\text{Ca}^{2+}]_i\) oscillations were analyzed in a cell with a relatively low (cell A, B) and a relatively high (cell *, C) level of calbindin-D\(_{28K}\), respectively. \([\text{Ca}^{2+}]_i\) was calculated from the Fura-2 340/380 nm excitation fluorescence emission ratio which was recorded with the fluorescence imaging MagiCal system. Bar represents 20 \(\mu\)m. Representative data from 4 experiments are shown, in which a total of 32 cells were analyzed.

To corroborate further the interaction between calbindin-D\(_{28K}\) and Ca\(^{2+}\) signaling, we compared within one single monolayer characteristics of \([\text{Ca}^{2+}]_i\) oscillations in principal cells expressing different levels of calbindin-D\(_{28K}\). In line with the above mentioned results, principal cells containing different concentrations of calbindin-D\(_{28K}\) exhibit identical \([\text{Ca}^{2+}]_i\) oscillations (Fig. 4, \(n = 32\)).

Fig. 5 Effect of the Ca\(^{2+}\) chelator BAPTA on resting \([\text{Ca}^{2+}]_i\) in principal cells of rabbit cortical collecting system in primary culture (A) and in cells of rabbit proximal tubules in primary culture (B). Monolayers were incubated in BAPTA/AM (3 \(\times\) 10\(^{-5}\) M). \([\text{Ca}^{2+}]_i\) was calculated from the Fura-2 340/380 nm excitation fluorescence emission ratio which was recorded with the fluorescence imaging MagiCal system. Representative traces from 4 experiments are shown, in which a total of 24 cells were analyzed.
Feher et al. developed a mathematical model which explains the role of calbindin-D9K in 1,25(OH)2D3-stimulated intestinal Ca2+ absorption [6,8]. In this model, calbindin enhances transcellular Ca2+ transport by: (i) stimulating apical entry of Ca2+ through releasing the negative feedback on the entrance step; (ii) increasing the rate of cytosolic transport by acting as a diffusional carrier; (iii) increasing Ca2+ efflux rate by feeding Ca2+ to the starved basolateral Ca2+-ATPase and Na+/Ca2+ exchanger. The present study provides experimental evidence for a stimulatory effect of Ca2+ ligands on transcellular Ca2+ transport in the cortical collecting system. We localized calbindin-D28K in the cytosol of principal cells, where concentrations reach 100 μM [14]. An increase in calbindin-D28K content was accompanied by an increase in the rate of transcellular Ca2+ transport. This stimulatory effect of calbindin-D28K could be fully mimicked by the Ca2+ chelator, BAPTA, which strongly suggests that the presence of diffusible Ca2+ chelators alone is sufficient to enhance transcellular Ca2+ transport. This implies that Ca2+ chelators influence Ca2+ fluxes at the entrance and exit step [6].

In the present study, stimulated rates of transcellular Ca2+ transport were not accompanied by an increase in [Ca2+]i. Furthermore, addition of BAPTA to the incubation medium interrupted [Ca2+]i oscillations within 1.9 ± 0.2 min (Fig. 6, n = 24).

Discussion

The present study demonstrates that increased levels of cytosolic Ca2+ ligands, as calbindin-D28K and BAPTA, stimulate active transcellular Ca2+ transport in the rabbit cortical collecting system. In addition, calbindin-D28K does not interfere with [Ca2+]i signaling, while BAPTA completely inhibits [Ca2+]i oscillations.

Table 3 Chelator forward and reverse rate constants for the Ca2+ chelator/Ca2+ binding (k_on and k_off), and dissociation constants are given for BAPTA and calbindin-D28K.

<table>
<thead>
<tr>
<th>Chelator</th>
<th>k_on (M⁻¹s⁻¹)</th>
<th>k_off (s⁻¹)</th>
<th>K_a (nM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAPTA</td>
<td>6.02 x 10⁸</td>
<td>96.7</td>
<td>100–500</td>
<td>[23]</td>
</tr>
<tr>
<td>Calbindin-D28K</td>
<td>2 x 10⁷</td>
<td>8.6</td>
<td>430</td>
<td>[6]</td>
</tr>
</tbody>
</table>

Fig. 6 Effect of the Ca2+ chelator BAPTA on Na⁺0-free induced [Ca2+]i oscillations in principal cells of rabbit cortical collecting system in primary culture. Na⁺0 (NaCl) was isosmotically replaced with N-methylglucamine (NMGCl) and monolayers were subsequently incubated in BAPTA/AM (3 x 10⁻⁵ M). [Ca2+]i was calculated from the Fura-2 340/380 nm excitation fluorescence emission ratio which was recorded with the fluorescence imaging MagiCal system. A representative trace from 4 experiments is shown, in which a total of 24 cells were analyzed.
buffer seems in conflict with the fact that \([\text{Ca}^{2+}]_i\) fluctuations are an essential step in regulatory pathways, since an increased \(\text{Ca}^{2+}\) buffering capacity most likely dampens the transient rise in \([\text{Ca}^{2+}]_i\) evoked by receptor activation. The present study, however, clearly demonstrates that calbindin-D28K does not interfere with \(\text{Ca}^{2+}\) signaling processes, since oscillations in \([\text{Ca}^{2+}]_i\) could be provoked irrespective of the absence or presence of calbindin-D28K in the cell. In contrast, loading the cells with BAPTA abruptly stopped the \([\text{Ca}^{2+}]_i\) oscillations. This remarkable difference between both calcium chelators must reside in the \(\text{Ca}^{2+}\) binding kinetics. The difference is not explained on the basis of \(\text{Ca}^{2+}\) affinities, since the \(K_d\)s of both \(\text{Ca}^{2+}\) ligands for \(\text{Ca}^{2+}\) are similar (see Table 3). It is theoretically possible that BAPTA reaches significantly higher cytosolic concentrations than calbindin-D28K. When hypocalpampal neurons were incubated for 30 min at 37°C with 30 \(\mu\text{M}\) BAPTA/AM the intracellular BAPTA concentration reached \(\sim300\ \mu\text{M}\) [22]. We observed that within 2 min after addition of 30 \(\mu\text{M}\) BAPTA/AM, the \([\text{Ca}^{2+}]_i\) oscillations stopped, which implies that the cytosolic concentration of BAPTA does not differ widely from the cytosolic calbindin-D28K concentration, which was estimated to be \(\sim100\ \mu\text{M}\) [14]. The most plausible explanation for the observed differences is that the \(k_{\text{on}}\) rate of \(\text{Ca}^{2+}\) binding to calbindin-D28K is too slow, so that calbindin-D28K, unlike BAPTA, is unable to reduce the upstroke of a \(\text{Ca}^{2+}\) spike rapidly enough to prevent the initiation of \(\text{Ca}^{2+}\)-induced \(\text{Ca}^{2+}\) release. The \(k_{\text{on}}\) rate is in fact more than one order of magnitude slower for calbindin-D28K than for BAPTA (Table 3). Indeed, calbindin has been reported to buffer \(\text{Ca}^{2+}\) sluggishly when compared to troponin and calmodulin [4]. A similar explanation accounted for differences between EGTA and BAPTA in attenuating \(\text{Ca}^{2+}\)-activated \(K^+\) currents in chromaffin cells and in reducing evoked neurotransmitter release at the squid giant synapse [23].

Until now, little is known about the role of calbindin-D28K in non-epithelial cells, such as Purkinje cells in the cerebellum, specific neurons in the brain and several endocrine cells [1-4]. The characteristics of calbindin-D28K outlined in the present study should also hold in these tissues. For example, calbindin-D28K will bind \(\text{Ca}^{2+}\) in the cytosol during intense neural activity and thus protects neurons from \(\text{Ca}^{2+}\) overload. After these \(\text{Ca}^{2+}\) pulses, calbindin-D28K will facilitate redistribution of \(\text{Ca}^{2+}\) within the cell which could mediate stimulation-evoked changes in neuronal cell shape or mediate memory effects in brain [4,24,25]. Roberts made a theoretical analysis of a mechanism by which millimolar concentrations of calbindin-D28K found in certain sensory receptors and neurons can influence \([\text{Ca}^{2+}]_i\) signaling [26]. He demonstrated that high levels of calbindin-D28K are necessary to serve as a mobile \(\text{Ca}^{2+}\) buffer that reduces and localizes changes in \([\text{Ca}^{2+}]_i\) by shuttling \(\text{Ca}^{2+}\) away from the \(\text{Ca}^{2+}\) channel arrays. Indeed, in rat sensory neurons, it has been shown that injection of high concentrations of calbindin-D28K into the cell has no effect on basal \([\text{Ca}^{2+}]_i\), but affects the kinetics of \([\text{Ca}^{2+}]_i\) increase [27]. Our findings in epithelial cells
show that with lower, i.e. submillimolar, levels of calbindin-D28K, there is no interference with Ca\(^{2+}\) signaling. Also, in a previous study by Muir et al. [28], a similar conclusion was reached. These investigators stably expressed calbindin-D28K in NIH3T3 cells and the presence of calbindin-D28K did not affect resting [Ca\(^{2+}\)]i nor did it change the increase in [Ca\(^{2+}\)]i which occurred in response to serum stimulation.

The findings of the present study can be summarized in a model shown in Figure 7. Transcellular Ca\(^{2+}\) movement involves the sequential transport of Ca\(^{2+}\) across the apical membrane, cytosol and basolateral membrane. The apical entry mechanism is still unidentified, but is postulated to be inhibited by high [Ca\(^{2+}\)]i adjacent to the apical membrane, referred to as a negative-feedback inhibition of Ca\(^{2+}\) entry [6]. Calbindin-D28K binds Ca\(^{2+}\) ions which enter the cytosol and facilitates cytosolic diffusion. Finally, calbindin-D28K increases the supply of Ca\(^{2+}\) to the Ca\(^{2+}\) pumps in the basolateral membrane [6]. During transcellular Ca\(^{2+}\) movement, [Ca\(^{2+}\)]i remains constant. Due to the slow binding kinetics of calbindin-D28K, Ca\(^{2+}\) signaling can occur independently of transcellular Ca\(^{2+}\) movement mediated by calbindin-D28K. The summarized properties of calbindins are compatible with substantial cytosolic Ca\(^{2+}\) diffusion and protection of the cell from being flooded with Ca\(^{2+}\) and guarantees an unaltered [Ca\(^{2+}\)]i signaling in epithelial cells involved in transcellular Ca\(^{2+}\) transport.

Acknowledgements

The authors thank Dr H. Raat for determining the effect of BAPTA on [Ca\(^{2+}\)]i of proximal tubules in primary culture. Mrs A. Hartog was supported by a grant from the Dutch Kidney Foundation (#91.1112).

References


Please send reprint requests to: Dr René J.M. Bindels, 162 Cell Physiology, University of Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands.

Received: 9 March 1995
Accepted: 9 June 1995