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CALCIUM HANDLING BY THE MAMMALIAN KIDNEY

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Summary

The mammalian kidney plays a crucial role in the Ca^{2+} homeostasis of the body. To maintain a net Ca^{2+} balance, more than 98% of the filtered load of Ca^{2+} must be reabsorbed along the nephron. There are two potential pathways through which net Ca^{2+} reabsorption can occur. First, a paracellular and passive route that predominates in the proximal tubules and thick ascending limb of Henle's loop. Second, a transcellular, active transport that characterises Ca^{2+} reabsorption in the distal nephron. Transcellular Ca^{2+} transport involves passive influx across the luminal membrane, diffusion through the cytosol and active extrusion across the peritubular membrane. The rate of active Ca^{2+} reabsorption is controlled by the calciotropic hormones, i.e. parathyroid hormone, calcitonin and 1,25-dihydroxyvitamin D_3. The application of new techniques in renal physiology has greatly increased our knowledge of the renal handling of Ca^{2+} and allowed the examination of Ca^{2+} transport processes at the cellular and subcellular level. This review focuses primarily on the mechanisms and regulation of transcellular Ca^{2+} transport. The distal nephron consists of at least four discrete segments and the contribution of each segment to active transcellular Ca^{2+} is discussed in detail.

Introduction

In mammals, overall Ca^{2+} homeostasis is maintained by the concerted action of the intestine, bone and kidney. Given a daily dietary calcium intake of 1000mg, net intestinal absorption amounts to about 200mg, with the remaining 800mg being excreted in the faeces. To maintain a net balance, the kidney must excrete approximately 200mg, amounting to less than 2% of the filtered calcium load (Costanzo and Windhager, 1992). Thus, it is evident that the kidney is a crucial organ for the regulation of the calcium balance. As a result, a considerable amount of renal research has focused on the handling of calcium. Our present knowledge of renal calcium handling is mainly based on micropuncture and *in vitro* microperfusion techniques with which the segmental localisation of Ca^{2+} transport, its hormonal dependence and the relationship between Na^+ and Ca^{2+} reabsorption have been investigated. Although these techniques have been extremely useful, their limitations precluded further advances. The development of new techniques, such as single-cell fluorescent video microscopy, molecular biological approaches, patch-clamp and cell culture applications, has greatly furthered our insight into the mechanisms and regulation of renal Ca^{2+} transport. This review focuses on the

Key words: calcium reabsorption, cortical collecting duct, dorsal convoluted tubule, connecting tubule, calcibindin-D_{28K}.

regulation of active transcellular Ca^{2+} reabsorption in the distal nephron. Within this framework, the general handling of Ca^{2+} by the mammalian kidney will be briefly summarized and subsequently the physiological relevance of recent studies on isolated perfused tubules and isolated and cultured renal cells will be discussed in detail.

Mechanisms of Ca^{2+} transport

Following its filtration across the glomerular capillaries, Ca^{2+} can be reabsorbed along the nephron by two potential pathways (Fig. 1). One is a paracellular and passive movement in response to an electrical or chemical gradient and the other is a transcellular active transport route, which consists of passive influx across the luminal membrane, diffusion through the cytosol and active extrusion across the opposing basolateral membrane (van Os, 1987). Although Ca^{2+} influx proceeds *via* a steep electrochemical gradient, the molecular nature of this Ca^{2+} entry pathway is still undetermined. Conceivably, a carrier-mediated process or Ca^{2+} channels may be present to allow permeation of Ca^{2+} through the bilayer forming the plasma membrane. Once in the cell, Ca^{2+} diffuses to the opposing basolateral membrane, while intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is maintained within a narrow range to allow normal cell functioning. This is achieved by controlling the compartmentalisation of Ca^{2+} in intracellular organelles and by the formation of complexes with vitamin-D_3-dependent Ca^{2+}-binding proteins, such as calbindin-D_{28K}. Theoretical analysis and experimental evidence have confirmed the need for facilitated diffusion of Ca^{2+} through the cytosol.

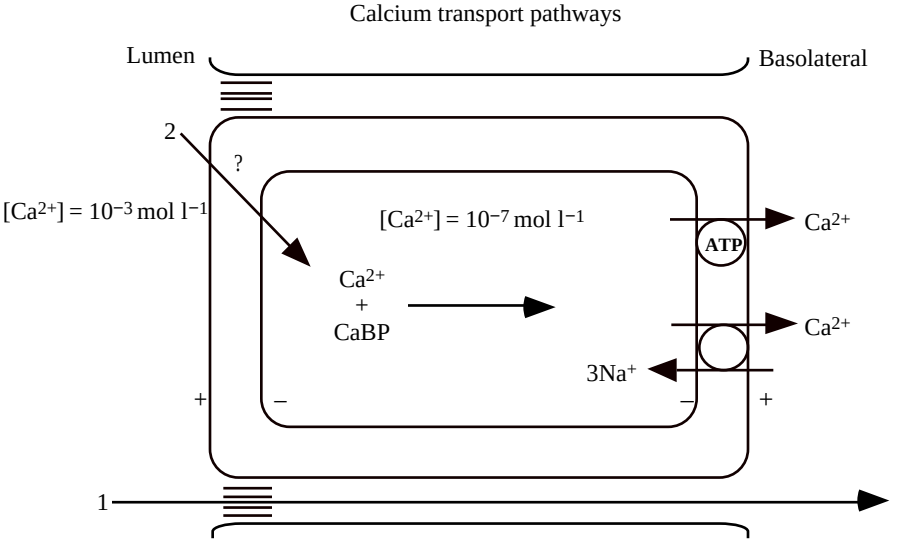

Fig. 1. Pathways of Ca^{2+} reabsorption in the nephron. The paracellular route (1) driven by electrical and chemical gradients and transcellular Ca^{2+} transport (2), which consists of passive influx across the luminal membrane, binding to calbindin-D_{28K} and subsequent diffusion through the cytosol with active extrusion at the peritubular membrane. CaBP, a calcium-binding protein such as calbindin-D_{28K}.

Presumably, calbindin-D_{28K} increases the diffusional flux of Ca^{2+} by increasing brush-border entry of Ca^{2+}, through decreasing $[Ca^{2+}]_i$ near the brush border, through increasing cytosolic flow by acting as a diffusional carrier and through increasing efflux by feeding Ca^{2+} to the basolateral extrusion mechanisms (Feher *et al.* 1992). In this scheme, the diffusion of Ca^{2+} through the cytosol is the rate-limiting step in transcellular Ca^{2+} movement. In the basolateral membrane, two Ca^{2+} transporters, Ca^{2+}-ATPase and a Na^+/Ca^{2+} exchange mechanism, have been shown to be involved in the efflux of Ca^{2+} from the cell.

Ca^{2+} reabsorption along the nephron

Micropuncture studies have shown that approximately 55% of the filtered load of Ca^{2+} is reabsorbed along the proximal convoluted tubule and an additional 10% is reabsorbed in the proximal straight tubule (Suki, 1979; Suki and Rouse, 1992; Costanzo and Windhager, 1992). Passive driving forces are the major determinants of Ca^{2+} transport in these nephron segments. Hence, Ca^{2+} reabsorption is a consequence of Na^+ and fluid reabsorption and is therefore obligatorily coupled to Na^+ transport (Suki and Rouse, 1992). Controversy exists as to whether some residual Ca^{2+} transport occurs *via* an active, transcellular pathway (Ng *et al.* 1984; Suki and Rouse, 1992). Ca^{2+} reabsorption in the loop of Henle has been attributed to the thick ascending limb, since the thin limbs have been shown to be relatively impermeable to Ca^{2+} (Rouse *et al.* 1980). It is generally accepted that about 20% of filtered Ca^{2+} is reabsorbed in the thick ascending loop of Henle (Suki, 1979; Costanzo and Windhager, 1992). The lumen-positive transepithelial potential difference generated by Cl^- reabsorption provides a driving force for passive Ca^{2+} reabsorption through the paracellular pathway. Other studies suggested that there may be an additional, possibly active, Ca^{2+} transport mechanism (Imai, 1978; Suki *et al.* 1980; Friedman, 1988). In the medullary segment, calcitriol enhances Ca^{2+} reabsorption, while in the cortical segment parathyroid hormone (PTH) exerts a stimulatory effect. In the distal convoluted and connecting tubule, Ca^{2+} reabsorption amounts to about 10% of the filtered load (Costanzo and Windhager, 1992). Both segments are a major regulatory site for Ca^{2+} excretion in the urine. Transport appears to be active, as it can proceed against an electrochemical gradient. Here, hormones such as PTH and calcitriol stimulate Ca^{2+} reabsorption (Costanzo and Windhager, 1992). In the distal convoluted tubule, Ca^{2+} reabsorption parallels Na^+ reabsorption, but thiazide diuretics dissociate Na^+ and Ca^{2+} reabsorption by inhibiting Na^+ transport and stimulating Ca^{2+} reabsorption (Costanzo and Windhager, 1978). Finally, the contribution of the collecting duct to overall Ca^{2+} reabsorption is small. Although little is known about Ca^{2+} transport in this segment, it is probably active (Sharegi and Stoner, 1981; Bourdeau and Hellstrom-Stein, 1982). The segmental Ca^{2+} reabsorption is summarized in Fig. 2.

Transcellular Ca^{2+} reabsorption in the distal nephron

The distal tubule is the main site of active transcellular Ca^{2+} transport and determines the fine tuning of Ca^{2+} excretion to the urine. Hitherto, it has been difficult to identify the segments or cell types responsible. This is due both to the heterogeneous nature of this part

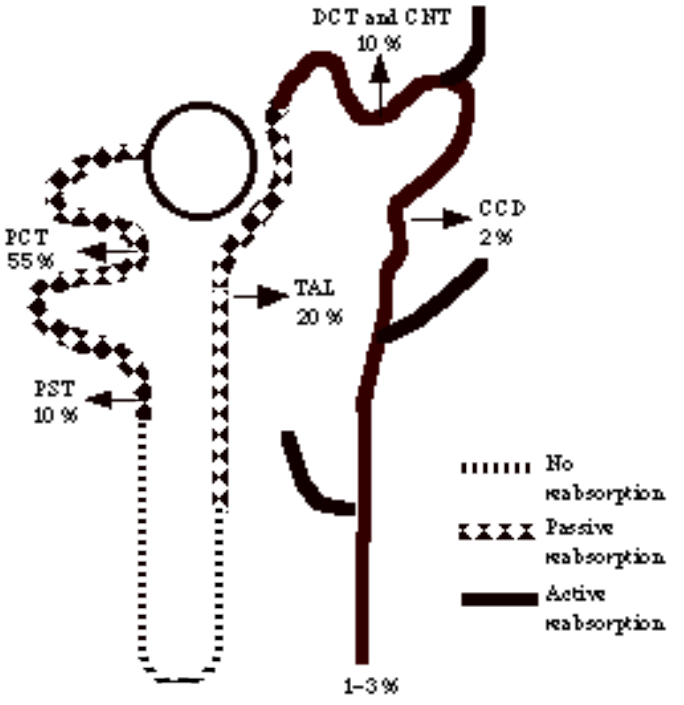

Fig. 2. Segmental Ca^{2+} reabsorption. The contribution of each segment to overall Ca^{2+} reabsorption is depicted as a percentage of the total. CCD, cortical collecting duct; CNT, connecting tubule; DCT, distal convoluted tubule; PCT, proximal convoluted tubule; PST, proximal straight tubule; TAL, thick ascending limb of Henle's loop.

of the nephron, in which numerous cell types can be recognized, and to considerable species differences. Identification of the cell types responsible has been greatly expedited by immunohistological localisation of the calcium-binding protein, calbindin-D_{28K} which, as mentioned above, facilitates transcellular Ca^{2+} movement. In mammals, such as rat, rabbit, pig, monkey and human, calbindin-D_{28K} is present in all the cells of the distal convoluted tubule and in most cells of the connecting tubule (Taylor *et al.* 1982; Schreiner *et al.* 1983; Bindels *et al.* 1991*b*). In rabbit, cells of the cortical collecting tubule also contain calbindin-D_{28K}, although less frequently (Taylor *et al.* 1982; R. J. M. Bindels, unpublished observation); in the rat, the presence of calbindin-D_{28K} is restricted to the transition between the connecting tubule and the cortical collecting duct (Taylor *et al.* 1982; Bindels *et al.* 1991*b*) (Fig. 3). Apparently, the relative contribution of the individual distal segments to active Ca^{2+} reabsorption differs significantly between rat and rabbit. In rabbit, the connecting tubule seems to be the most important site of hormone-regulated active Ca^{2+} reabsorption, whereas in the rat the distal convoluted tubule is more dominant (Morel, 1981; Imai, 1989; Bourdeau and Lau, 1990). Furthermore, the distal nephron of the rat lacks clear-cut segmentation with sharp transitions (Morel, 1981). Conceivably, hormone responsiveness may be determined by this heterogeneity in cell and site.

Recently, several groups have presented detailed information on the mechanism and

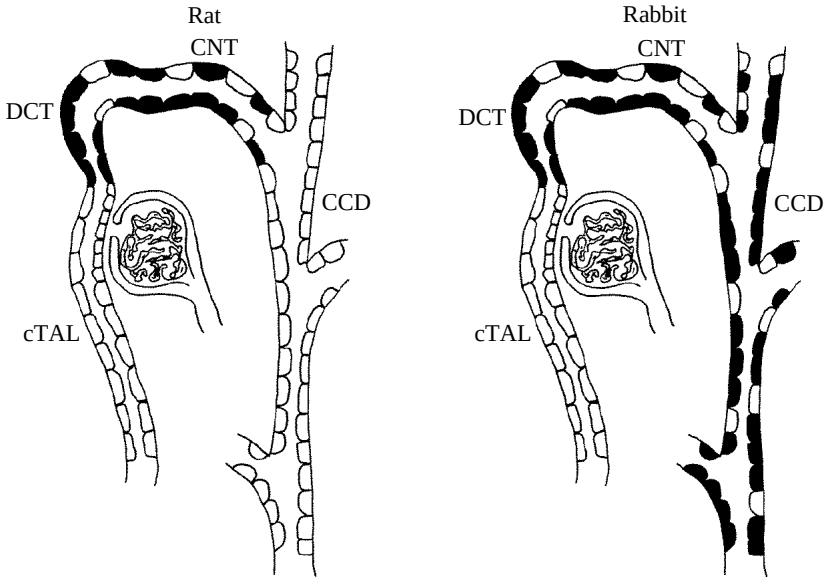

Fig. 3. Schematic representation of calbindin-D_{28K} distribution (dark cells) along the nephron from the cortex of rat (left) and rabbit (right) kidney. CCD, cortical collecting duct; CNT, connecting tubule; cTAL, cortical thick ascending limb of Henle's loop; DCT, distal convoluted tubule. Modified after Taylor *et al.* (1982) and Bindels *et al.* (1991*b*).

regulation of transcellular Ca^{2+} transport in the distal nephron. The involvement of all distal segments in calcium reabsorption is now well documented, although the cellular mechanisms remain to be clarified. Integration of this information into a single model of cellular Ca^{2+} transport is not possible, mainly because of the complexity of the various transport steps and incomplete information on the individual segments. Therefore, the characteristics of active Ca^{2+} transport in the distal segments, such as the thick ascending limb of Henle's loop, the distal convoluted tubule, the connecting tubule and the cortical collecting duct, will be discussed individually. Finally, at the end of each paragraph a cellular model is presented summarizing the various Ca^{2+} transporters that appear to participate in the transcellular movement of Ca^{2+} in that particular segment.

Thick ascending limb of Henle's loop

A considerable fraction of filtered Ca^{2+} is reabsorbed in the thick ascending limb of Henle's loop. Several groups have investigated the mechanisms of Ca^{2+} transport using micropuncture techniques and microperfused tubules in both rabbit and mouse, but the results are equivocal. Passive reabsorption is suggested by the transepithelial voltage-dependence and the furosemide-sensitivity of Ca^{2+} fluxes (Bourdeau and Burg, 1979; Wittner *et al.* 1993). Hormonal stimulation of Ca^{2+} transport occurs through hormone-mediated transepithelial voltage alterations (Wittner *et al.* 1993). Other studies suggest that there is an additional active Ca^{2+} reabsorption, which can be stimulated by PTH and calcitonin (Costanzo and Windhager, 1978; Suki *et al.* 1980; Friedman, 1988). It is

conceivable that under resting conditions passive Ca^{2+} reabsorption prevails and that after hormonal stimulation an additional active cellular pathway is activated (Friedman and Gesek, 1993). This controversy has not been resolved, but the conspicuous absence of calbindin-D_{28K} from this nephron segment strongly suggests an insignificant contribution of active, transcellular Ca^{2+} transport to the overall Ca^{2+} reabsorption.

Examination of the cellular mechanism of Ca^{2+} transport in the cortical thick ascending limb of Henle's loop has not provided additional evidence for the presence of active Ca^{2+} transport (Fig. 4). In cultured mouse cortical thick ascending limb of Henle's loop and distal convoluted cells, Bacskai and Friedman (1990) demonstrated a PTH-activated dihydropyridine-sensitive Ca^{2+} entry pathway. Subsequent studies by the same authors, performed with immortalized cells of similar origin, extended their previous results. PTH primarily increased Cl^- conductance and the subsequent membrane hyperpolarisation enhanced Ca^{2+} entry through putative Ca^{2+} channels (Gesek and Friedman, 1992). The physiological significance of these data with respect to Ca^{2+} reabsorption is not clear for two reasons. First, a mixture of distal nephron segments, i.e. the thick ascending limb of Henle's loop and the distal convoluted tubules, was originally used and it is, therefore, difficult to assign these findings to a specific segment. Second, localisation of the Ca^{2+} entry pathway or correlation with transcellular Ca^{2+} fluxes was not possible, since the observations were not made on polarized confluent monolayers. Their findings could also be interpreted as a basolateral Ca^{2+} entry playing a role in signal transduction of PTH. In this respect, it is interesting that a pH-sensitive Ca^{2+} influx pathway in the basolateral membrane of the rabbit cortical thick ascending limb of Henle's loop has recently been demonstrated (Hanaoka *et al.* 1993).

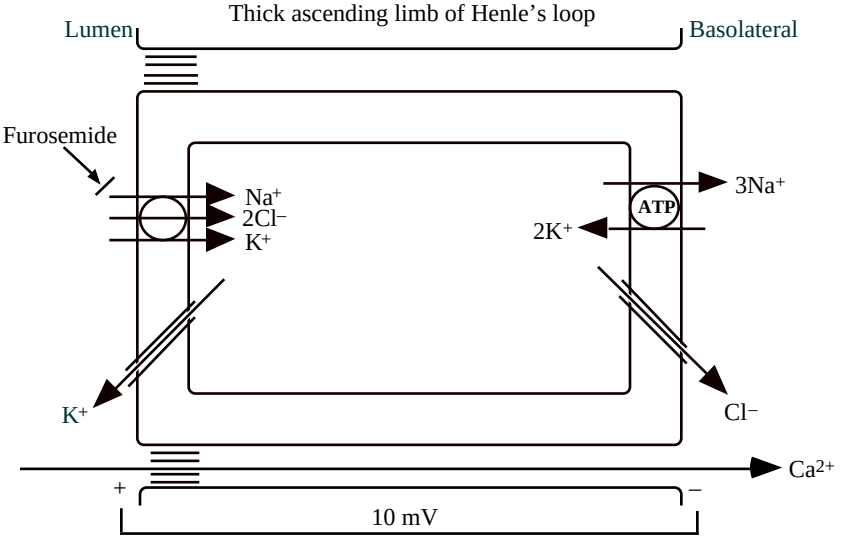

Fig. 4. Model of Ca^{2+} reabsorption in the thick ascending limb of Henle's loop. The lumen-positive transepithelial potential difference generated by furosemide-sensitive Cl^- reabsorption provides a driving force for passive Ca^{2+} reabsorption through a paracellular pathway.

There is also no indication of what kind of basolateral Ca^{2+} extrusion mechanism is present in this segment. No functional evidence could be obtained for the presence of a basolateral Na^+/Ca^{2+} exchanger in rabbit thick ascending limb of Henle's loop (Hanoaka *et al.* 1993). Furthermore, immunohistological and molecular biological studies failed to demonstrate the presence of a Na^+/Ca^{2+} exchanger in the thick ascending limb of Henle's loop (Reilly *et al.* 1992; Reilly and Shugrue, 1992; Yu *et al.* 1992*b*). Finally, only a low activity of a Ca^{2+}/Mg^{2+}-ATPase was detected in this segment, albeit sufficient activity to account for active Ca^{2+} transport (Doucet and Katz, 1982).

Distal convoluted tubule

In the distal convoluted tubule, Ca^{2+} reabsorption proceeds against both chemical and electrical gradients and is, therefore, by definition active in nature. The paracellular permeability for Ca^{2+} is very low (Costanzo and Windhager, 1992). Depending on the species, hormones such as PTH and calcitonin stimulate Ca^{2+} transport (Shareghi and Stoner, 1981; Imai, 1989; Shimizu *et al.* 1990). The presence of vitamin-D_3-dependent calbindin-D_{28K} suggests that 1,25-dihydroxyvitamin D_3 (1,25$[OH]_2D_3$) exerts a stimulatory action in this segment (Taylor *et al.* 1982; Bindels *et al.* 1991*b*), but at present there is no experimental evidence for such action. In general, only a few investigators have addressed the role of the distal convoluted tubule in the regulation of Ca^{2+} reabsorption and this reflects the technical difficulties involved in the identification, micropuncture and microperfusion of this nephron segment (Suki, 1979). Consequently, our knowledge of the mechanism of cellular Ca^{2+} transport in this segment is still incomplete (Fig. 5).

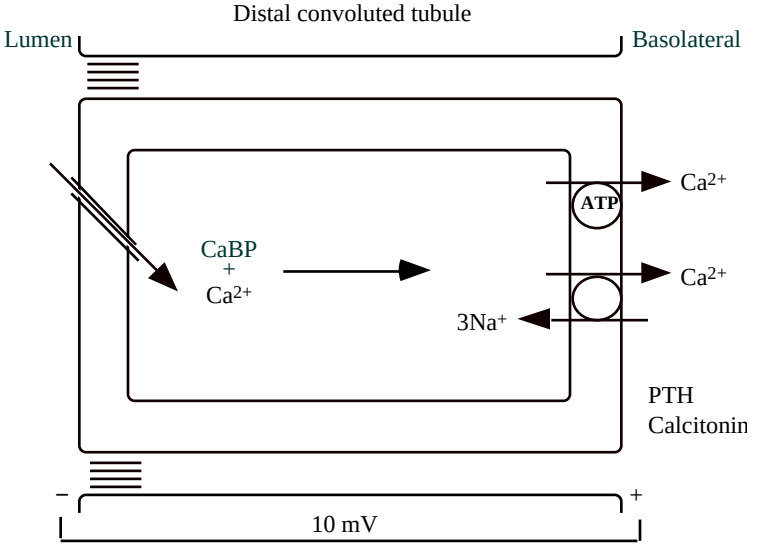

Fig. 5. Model of transcellular Ca^{2+} transport in the distal convoluted tubule. For an explanation, see the text. CaBP, a calcium-binding protein such as calbindin-D_{28K}; PTH, parathyroid hormone.

Recently, Poncet *et al.* (1992) characterised a Ca^{2+}-permeable channel in the apical membrane of primary cultures of the rabbit distal convoluted tubule. This is the first study using patch-clamp analysis to identify a Ca^{2+} channel in the distal nephron. The apical Ca^{2+} channel had a conductance of approximately 8pS and was blocked by La^{3+} and by high concentrations of verapamil and nifedipine. This channel could well be the apical entry pathway in transepithelial calcium transport. Interestingly, Yu *et al.* (1992*a*) recently identified an mRNA encoding partly for funnel web spider toxin-sensitive P-type Ca^{2+} channel that was exclusively expressed in the distal convoluted tubule of the rat.

The presence of both a Ca^{2+}-ATPase and a Na^+/Ca^{2+} exchange contributing to Ca^{2+} efflux across the basolateral membrane has been well documented. Epitopes for a plasmalemma Ca^{2+}-ATPase were exclusively present in the basolateral membrane of this segment in human and rat, as demonstrated with immunocytochemistry using a monoclonal antibody against the human erythrocyte Ca^{2+}/Mg^{2+}-ATPase (Borke *et al.* 1987, 1989). The immunoreactivity co-localised perfectly with the presence of calbindin-D_{28K} in the distal nephron of the rat, which suggests that, in addition to the distal convoluted tubule, the connecting tubule also expresses a Ca^{2+}-ATPase immunologically similar to that of human erythrocyte membranes. This idea is confirmed by the presence of numerous intercalated cells in the immunopositive segment. Another approach was used by Magocsi *et al.* (1992), who studied the localisation of mRNAs coding for isoenzymes of rat plasma membrane Ca^{2+}-ATPase using polymerase chain reaction (PCR) analysis. mRNA for one isoenzyme, rPMCA2, was detected in microdissected proximal tubules, cortical thick ascending limb of Henle's loop, distal convoluted tubules and perhaps also in cortical collecting ducts. The remaining tubule segments were not examined. The presence of this isoenzyme in most cortical tubules suggests a house-keeping function for rPMCA2 rather than a specific role in active transcellular Ca^{2+} transport. The molecular cloning of the cardiac Na^+/Ca^{2+} exchanger enabled other investigators to identify a similar antiporter in the mammalian kidney (Reilly and Shugrue, 1992; Yu *et al.* 1992*b*). PCR analysis performed on microdissected rat tubules revealed that the Na^+/Ca^{2+} exchanger is primarily expressed in the distal convoluted tubule (Yu *et al.* 1992*b*). Confusingly, in rabbit kidney, specific staining for immunoreactive Na^+/Ca^{2+} exchanger was found only along the basolateral membrane of the connecting tubules compared with the distal convoluted tubules in the rat (Reilly *et al.* 1992). Presumably, expression of the Na^+/Ca^{2+} exchanger in the distal nephron varies between different species. Finally, the relative contributions of the Na^+/Ca^{2+} exchanger and Ca^{2+}-ATPase to active Ca^{2+} reabsorption remain to be determined.

Connecting tubule

This tubule segment, which connects the distal convoluted tubule with the initial portion of the cortical collecting duct, is a heterogeneous epithelium consisting of at least two different cell types: connecting tubule cells and intercalated cells (Madsen *et al.* 1988). Only the former contain the vitamin-D_3-dependent calbindin-D_{28K} (Bindels *et al.* 1991*b*). Ca^{2+} reabsorption in this segment has been extensively studied by a diversity of technical approaches applied to both isolated perfused tubules and cultured cells. As in

the distal convoluted tubule, Ca^{2+} reabsorption is active because it proceeds against a steep electrochemical gradient. In isolated perfused connecting tubules, it has been demonstrated that PTH stimulates Ca^{2+} reabsorption through the activation of protein kinase A (Shareghi and Stoner, 1981; Imai, 1989; Shimizu *et al.* 1990). These findings have been confirmed in primary cultures of the cortical collecting system (Bindels *et al.* 1991*a*), as shown in Fig. 6. This culture system has been established in our laboratory from a mixture of cells from connecting tubules and cortical collecting ducts isolated by immunodissection from rabbit kidney. When cultured on permeable filters, the cells exhibited several functions of the original epithelium (Bindels *et al.* 1991*a*). Net basal Ca^{2+} reabsorption across the monolayers ranged between 15 and 20pmolmin^{-1}mm^{-2}. This value agrees with published values obtained in isolated perfused tubules (Costanzo and Windhager, 1978; Shimizu *et al.* 1990). In addition to protein kinase A, activation of protein kinase C plays a regulatory role in transcellular Ca^{2+} reabsorption. The phorbol ester 12-*o*-tetradecanoylphorbol 13-acetate (TPA) inhibited Ca^{2+} reabsorption and increased membrane-associated protein kinase C activity in primary cultures of rabbit cortical collecting system (Bindels *et al.* 1993) (Fig. 7). Interestingly, several kidney hormones, such as PTH, bradykinin, calcitriol and vasopressin, have been shown to activate protein-kinase-C-dependent signalling pathways (Shayman and Morrison, 1985; Hruska *et al.* 1987; Burnatowska-Hledin and Spielman, 1989). Therefore, further studies are needed to delineate the importance of this regulatory pathway. The final target for protein kinase C action has not yet been identified, but it could well be the apical Ca^{2+} channel, the basolateral Ca^{2+}-ATPase or the Na^+/Ca^{2+} exchanger. The phorbol ester treatment also abolished the transepithelial potential and doubled the transepithelial resistance because it also inhibited transepithelial Na^+ transport. Therefore, possible effects of protein kinase C activation on junctional permeability can be excluded. In the same primary culture system, we have demonstrated for the first time that 1,25$[OH]_2D_3$

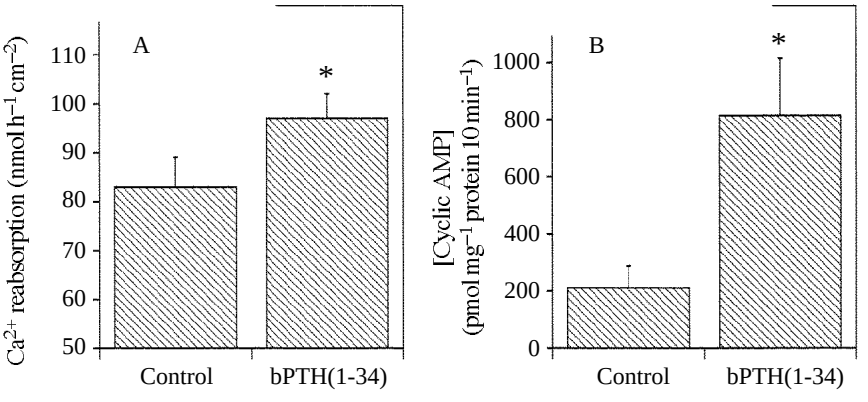

Fig. 6. Effect of bPTH(1-34) on transcellular Ca^{2+} transport (A) and cytosolic cyclic AMP concentration (B) in rabbit cortical collecting system in primary culture. bPTH(1-34) was applied only to the basolateral compartment at a concentration of 10^{-7} mol l^{-1}. Values are mean + S.E. (N=5). * denotes a significantly different value from the control (P<0.05). Modified after Bindels *et al.* (1991*a*).

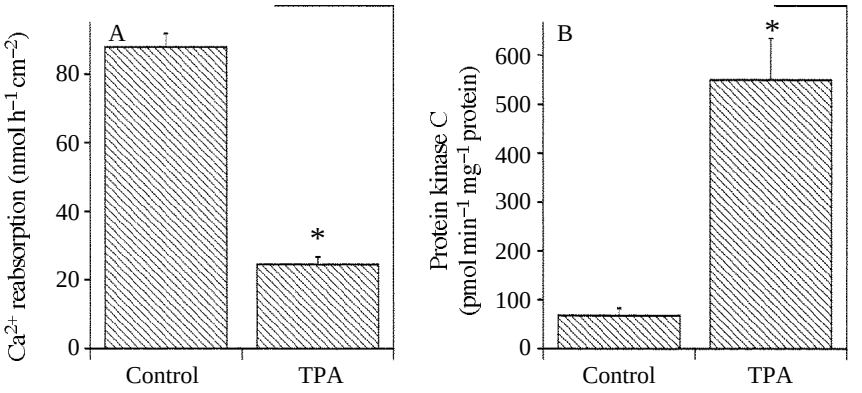

Fig. 7. Effect of the protein kinase C activator, 12-*o*-tetradecanoylphorbol 13-acetate (TPA) on transcellular Ca^{2+} transport (A) and membrane-associated protein kinase C activity (B) in rabbit cortical collecting system in primary culture. TPA was added to both compartments at a concentration of 10^{-7} mol l^{-1}. Values are mean + S.E. (N=5). * denotes a significantly different value from the control (P<0.05). Modified after Bindels *et al.* (1993).

stimulates transcellular Ca^{2+} reabsorption and concomitantly increases cytosolic calbindin-D_{28K} content (Bindels *et al.* 1991*a*) (Fig. 8). Maximal stimulation occurred between 48 and 72h after exposure to calcitriol, suggesting that the mechanism of stimulation is analogous to that of classical steroid hormones, which implies that 1,25$[OH]_2D_3$ binds first to a specific cytosolic receptor protein and then moves to the nucleus, where transcriptional events are initiated, leading to *de novo* synthesis of calbindin-D_{28K}. Calcium-binding proteins such as calbindin-D_{28K} have been shown to accelerate diffusion of Ca^{2+} through the cytosolic compartment (Feher, 1983; Feher *et al.*

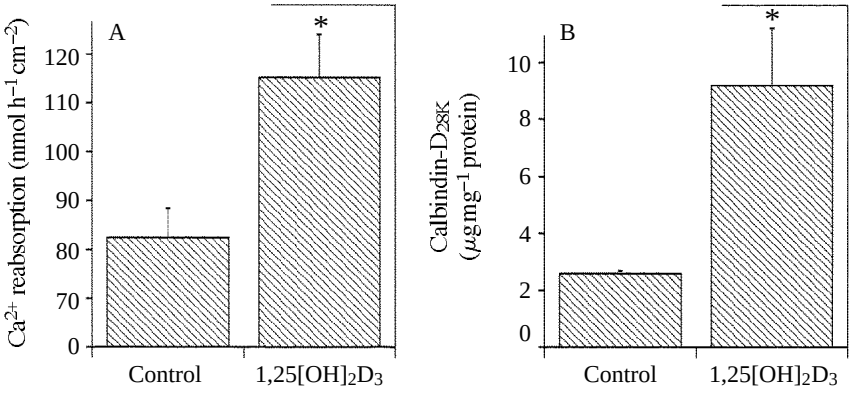

Fig. 8. Effect of 1,25-dihydroxyvitamin D_3 1,25$[OH]_2D_3$ on transcellular Ca^{2+} transport (A) and cytosolic calbindin-D_{28K} concentration (B) in rabbit cortical collecting system in primary culture. Preincubations with 1,25$[OH]_2D_3$ were performed for 48h (10^{-7} mol l^{-1}, basolateral side). Values are mean + S.E. (N=5). * denotes a significantly different value from the control (P<0.05). Modified after Bindels *et al.* (1991*a*).

1992). In addition, another genomic response of $1,25[OH]_2D_3$ was recently demonstrated in the intestine (Wasserman *et al.* 1992; Cai *et al.* 1993), where it was shown that $1,25[OH]_2D_3$ increased the net synthesis of the basolateral Ca^{2+} pump through an increase in Ca^{2+} pump gene expression (Cai *et al.* 1993). This intriguing finding has not yet been observed in the kidney.

At the cellular level, there are indications that several Ca^{2+} transporters participate in the transcellular movement of Ca^{2+}, as summarized in Fig. 9. Until now the molecular nature of the luminal Ca^{2+} entry pathway in the connecting tubule has been unknown. However, some properties of Ca^{2+} influx have been revealed. In isolated perfused rabbit connecting tubules, an apical Ca^{2+} influx has been suggested by the dependency of $[Ca^{2+}]_i$ on luminal Ca^{2+} (Bourdeau and Lau, 1992). In the same preparation, PTH stimulates Ca^{2+} influx across the luminal membrane, probably *via* activation of a cyclic-AMP-dependent protein kinase (Bourdeau and Lau, 1989). Preliminary studies performed on primary cultures of rabbit cortical collecting system demonstrated that Ca^{2+} influx was strongly inhibited by luminal acidification (Bindels *et al.* 1992*b*). The mechanism of inhibition resided at the external surface of the apical membrane. Our results are, therefore, at variance with the operation of an apical Ca^{2+}/H^+ exchanger, as suggested by Bourdeau and Lau (1992). These authors noticed that acute changes in luminal and cellular pH slightly influenced $[Ca^{2+}]_i$ in isolated perfused connecting tubules. Their findings are also consistent with an effect of pH on other Ca^{2+} channels.

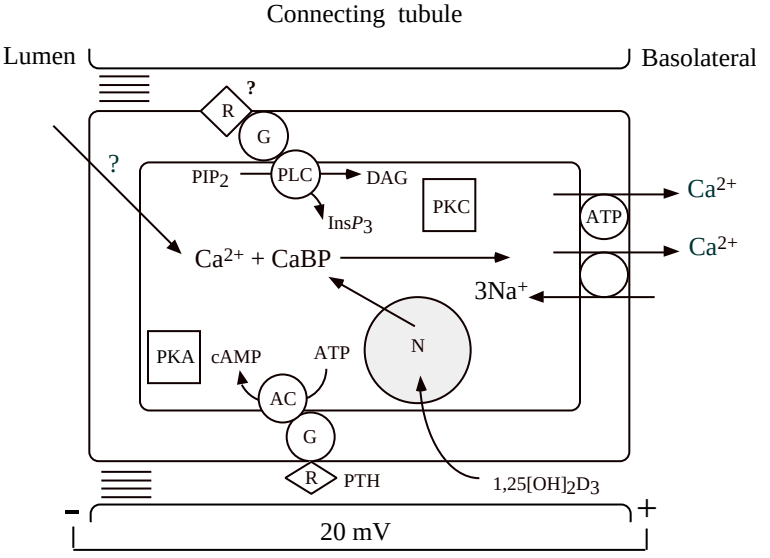

Fig. 9. Model of transcellular Ca^{2+} transport in the connecting tubule. For an explanation, see the text. Ca^{2+} transport is regulated through pathways dependent on protein kinases A and C. AC, adenylate cyclase; CaBP, a calcium-binding protein such as calbindin-D_{28K}; DAG, diacylglycerol; G, G-protein; InsP_3, inositol trisphosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PTH, parathyroid hormone; R, receptor; cAMP, cyclic AMP; N, nucleus.

From our studies, the involvement of Ca^{2+} channels resembling the voltage-dependent types seems unlikely, since administration of Ca^{2+} entry blockers, such as methoxyverapamil, diltiazem, flunarizine and felodipine, had no effect on Ca^{2+} transport (Bindels *et al.* 1992*a*,*b*). Taken together, these data are consistent with a pH-sensitive Ca^{2+} channel regulated by a cyclic-AMP-dependent kinase, but they do not prove the existence of such a channel. Therefore, more work is needed to define the nature of this putative channel.

Our observation that the calcitriol-induced increase in transcellular Ca^{2+} transport is accompanied by a substantial increase in cytosolic calbindin-D_{28K} confirms the idea that calbindin-D_{28K} facilitates diffusion of Ca^{2+} through the cytosol (Bindels *et al.* 1991*a*). These findings are in line with studies performed in the mammalian intestine, where 1,25$[OH]_2D_3$ also stimulates Ca^{2+} absorption and increases the cellular content of calbindin-D_{9K} (Gross and Kumar, 1990).

Finally, Ca^{2+} must be extruded at the basolateral membrane against an electrochemical gradient. Two transport mechanisms exist in this membrane. One is a high-affinity Ca^{2+}-ATPase, whose activity is highest in the connecting tubule (Doucet and Katz, 1982). The presence of the Na^+/Ca^{2+} antiporter exclusively in the basolateral membrane of the rabbit connecting tubule has been demonstrated by cytochemistry (Reilly *et al.* 1992). Several investigators evaluated the role of this extrusion mechanism in the regulation of $[Ca^{2+}]_i$ by measuring $[Ca^{2+}]_i$ during selective manipulation of extracellular $[Na^+]$ (Taniguchi *et al.* 1989; Bourdeau and Lau, 1990). For instance, it has been shown that, in isolated perfused tubules, $[Ca^{2+}]_i$ rose upon removal of Na^+ from the bathing medium and that this increase was virtually abolished in the absence of luminal Na^+ or basolateral Ca^{2+} (Bourdeau and Lau, 1990). In addition to involvement in regulating $[Ca^{2+}]_i$, this antiporter may participate in transcellular Ca^{2+} transport. Shimizu *et al.* (1990) demonstrated a decrease in Ca^{2+} reabsorption on removal of bath Na^+ or following treatment with ouabain. In primary cultures of the cortical collecting system, a dose-dependent relationship between basolateral $[Na^+]$ and transcellular Ca^{2+} transport was found and a Hill coefficient of around 2 pointed to at least two binding sites for Na^+ on the Na^+/Ca^{2+} exchanger (Bindels *et al.* 1992*a*). Ouabain inhibited Ca^{2+} transport to the same extent as did Na^+-free solutions. Depolarisation of the basolateral membrane, by raising basolateral $[K^+]$, also decreased transcellular Ca^{2+} transport, which suggests that the Ca^{2+} extrusion is electrogenic. Bourdeau and Lau (1990) calculated that the exchanger is quiescent under control physiological conditions because the net driving force for Na^+ and Ca^{2+} movements are balanced. Consequently, Na^+/Ca^{2+} exchange does not contribute to Ca^{2+} extrusion under physiological conditions. This is not in agreement with our observations, since reduction in basolateral $[Na^+]$ from 150 to 100mmol l^{-1} did not reduce Ca^{2+} transport across the cultured collecting tubules. However, lowering $[Na^+]$ to 50mmol l^{-1} significantly reduced Ca^{2+} transport. Taken together, these results suggest that connecting tubules exhibit a basolateral Na^+/Ca^{2+} exchanger which contributes to transcellular Ca^{2+} transport and is involved in regulating $[Ca^{2+}]_i$. In primary cultures of the rabbit cortical collecting system, a residual Ca^{2+} absorption of approximately 40 % was always observed, regardless of the mode of inhibition. The partial inhibition caused by removal of basolateral Na^+ and treatment with ouabain suggests that a Ca^{2+} extrusion

mechanism other than Na^+/Ca^{2+} exchange participates in Ca^{2+} reabsorption. This residual Ca^{2+} absorption probably reflects the activity of a basolateral Ca^{2+}-ATPase. Unfortunately, no specific inhibitors are available to evaluate the role of this transporter directly.

Collecting duct

The contribution of this terminal segment to overall Ca^{2+} reabsorption is small and the nature of the transport is controversial. Some studies reported an insignificant net Ca^{2+} flux that was voltage-dependent (Shareghi and Stoner, 1981; Bourdeau and Hellstrom-Stein, 1982). At variance with these results are those of Shimizu *et al.* (1990), who found a net Ca^{2+} reabsorption which, owing to the lumen-negative voltage in the cortical collecting duct, must proceed *via* a transcellular pathway. In line with this active component is the presence of calbindin-D_{28K} in the cortical portion of this segment in the rabbit. The cellular mechanisms of Ca^{2+} reabsorption await further investigations.

Cellular Ca^{2+} traffic and Ca^{2+} signalling

Renal cells involved in transcellular Ca^{2+} transport are continuously challenged by a substantial Ca^{2+} traffic through the cytosol while simultaneously maintaining low levels of $[Ca^{2+}]_i$. Calcium-binding protein, which is exclusively present in these renal cells, could fulfil this paradoxical function. In cultured cells of the rabbit cortical collecting system, the average calbindin-D_{28K} concentration amounts to at least $70\,\mu mol\,l^{-1}$ (Bindels *et al.* 1991*a*). According to Feher (1983), the calbindin-D_{28K} concentration in the cytosol should reach values of $50\,\mu mol\,l^{-1}$ or higher to facilitate cytosolic Ca^{2+} diffusion significantly. Consequently, calbindin-D_{28K} concentration in the cultured cells is high enough to play a significant role in cytosolic Ca^{2+} movement. The cellular calbindin-D_{28K} content is regulated by $1,25[OH]_2D_3$ (Kumar, 1990). It is, therefore, conceivable that the stimulation of Ca^{2+} reabsorption by this hormone, as demonstrated in the cultured cortical collecting system cells, is due to the increased calbindin-D_{28K} content. Calbindin-D_{28K} also acts as a Ca^{2+} buffer: during changes in transcellular Ca^{2+} transport rates, $[Ca^{2+}]_i$ remains buffered between 100 and $200 nmol\,l^{-1}$. Stimulated levels of Ca^{2+} transport were never accompanied by an elevation in $[Ca^{2+}]_i$ (R. J. M. Bindels, unpublished observation). However, in the same cell preparation, oscillations in $[Ca^{2+}]_i$ could be provoked irrespective of the calbindin-D_{28K} concentration (Koster *et al.* 1993). Similar Ca^{2+} oscillations were observed in intercalated cells, but here calbindin-D_{28K} is totally absent (Fig. 10). These findings suggest that, at physiological $[Ca^{2+}]_i$, calbindin-D_{28K} is saturated with Ca^{2+}, which is in line with an affinity of calbindin-D_{28K} for Ca^{2+} in the nanomolar range, as suggested by Feher (1983). Consequently, Ca^{2+} signalling regulating transport of other ions can occur during and independently of transcellular Ca^{2+} movement. For example, Na^+ reabsorption and K^+ secretion in the distal nephron are partly regulated by the activity of $[Ca^{2+}]_i$-dependent Na^+ and K^+ channels, respectively (Hébert *et al.* 1991; Schlatter *et al.* 1993). Besides these initial data, further

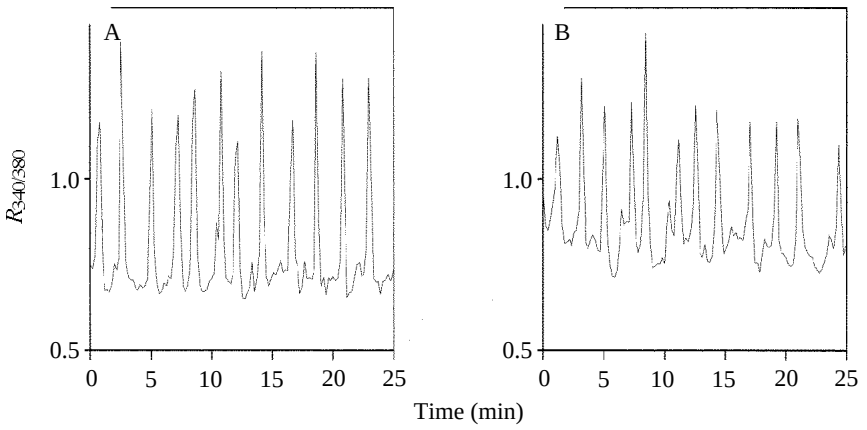

Fig. 10. $[Ca^{2+}]_i$ oscillations induced in extracellular Na^+-free medium measured simultaneously in a connecting tubule (A) and an intercalated (B) cell from the rabbit renal cortical collecting system in primary culture using digital imaging. $R_{340/380}$ represents the Fura-2 340nm/380nm excitation fluorescence emission ratio: a measure of $[Ca^{2+}]_i$. Intercalated cells were positively identified by peanut lectin staining. Representative traces of four experiments are shown (A,B). Modified after Koster *et al.* (1993).

experiments are needed to unravel the complex relationship between transcellular Ca^{2+} transport and $[Ca^{2+}]_i$ homeostasis.

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