Regulation of Intestinal Calcium Transport

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calcium channels, vesicular transport, facilitated diffusion, $1,25(OH)_2D_3$, parathyroid hormone

Abstract

Calcium is an essential ion in all organisms and participates in a variety of structural and functional roles. Calcium (re)absorption occurs in epithelia, including the intestine, kidney, mammary glands, placenta, and gills of fish. Its transport is regulated by a complex array of processes that are mediated by hormonal, developmental, and physiological factors involving the gastrointestinal tract, bone, kidney, and the parathyroids. Here we review the calcium transport mechanisms—paracellular, which is energy independent, and transcellular, which is energy dependent—primarily focusing on the intestine. We provide a new perspective on the facilitated diffusion and vesicular transport models to account for the emerging concepts on transcellular calcium transport. Finally, we discuss how $1,25(OH)_2D_3$ and parathyroid hormone regulate calcium transport.

Contents

INTRODUCTION

Calcium is an essential ion in all organisms and participates in a variety of structural and functional roles ranging from the formation and maintenance of the skeleton to the temporal and spatial regulation of neuronal function and inhibition of proliferation of cancer cells. Intracellular calcium, particularly the cytosolic free calcium, is an important second messenger and cofactor for proteins and enzymes regulating key cellular processes such as neurotransmission, motility, hormonal secretion, and cellular proliferation. Extracellular calcium, on the other hand, is an integral part of the mineral phase of the bone, serves as a cofactor for adhesion molecules, clotting factors, and other proteins, and regulates neuronal excitability. The suitability of calcium in such diverse roles can be attributed to its physical properties (24).

Extracellular concentrations of unbound calcium are around 1 μM, whereas the intracellular free concentration under resting conditions is in the range of 0.1 μM, creating a steep gradient between intra- and extracellular concentrations. Such extreme differences between the two concentrations put severe constraints and demands on plasma membrane transport proteins to safeguard the integrity of the intracellular milieu. Moreover, intracellular calcium can undergo large, rapid changes due to either influx through the cell membrane or release from intracellular stores (9), whereas extracellular calcium remains relatively constant under normal circumstances (66). In this review, we focus on calcium transport across the intestine, including hormonal regulation by $1,25(OH)_2D_3$ and parathyroid hormone (PTH), with occasional pertinent references to work done in kidney.

$1,25(OH)_2D_3$: 1,25-dihydroxyvitamin D_3

PATHWAYS OF CALCIUM TRANSPORT

The two distinct processes involved in the absorption of Ca^{2+} across epithelia are paracellular and transcellular transport. The first one allows direct exchange of calcium ions between two compartments; the latter involves transport across at least two plasma membranes (54). Absorption of dietary Ca^{2+} through the transcellular pathway occurs predominantly in the duodenum, is to a large extent regulated by $1,25(OH)_2D_3$, and requires energy. The paracellular route is a concentration-dependent diffusion of the ion that takes place throughout the length of the intestine (15) and does not depend on energy. When Ca^{2+} intake is low (<20 mM), transcellular transport in the duodenum accounts for approximately 80% of the absorbed Ca^{2+}, but its contribution to overall Ca^{2+} transport is minor when Ca^{2+} intake is high (50 mM), primarily because of the short transit time (3 min in the duodenum) and downregulation of $1,25(OH)_2D_3$-dependent Ca^{2+} channels and intestinal calbindin (15). The relative contribution of the para- and transcellular transport mechanisms to the overall Ca^{2+} transport is given in **Table 1**.

Paracellular Calcium Transport

Polarized epithelial cells form intercellular tight junctions at specialized apical membrane domains. Diffusion of small molecules and ions past the tight junctions and into the extracellular space is known as paracellular transport. It has been suggested that tight junctions

in epithelia manifest the same biophysical properties, such as ion and size selectivity, concentration-dependent ion permeability, pH sensitivity, and competition between permeable molecules (21, 127).

Tight junction permeability is constantly regulated under various physiological conditions (21, 52, 99) and modulated by cytokines, growth factors, bacterial toxins, hormones, protein kinase (PK) C, serine threonine kinase, and as yet unknown factors (8, 48, 53, 140, 146). Another important factor determining Ca^{2+} transport is the intestinal sojourn time, which is highest in the ileum (15), and where, under normocalcemic diets, the highest proportion of Ca^{2+} is absorbed (76). It should be noted that ileal cells contain no calbindins (15). Because intestinal sojourn time itself is a function of the length of the intestine, the overall contribution of paracellular Ca^{2+} transport in this organ may also vary among species. Although paracellular Ca^{2+} transport is the major route in the intestine with high-calcium diets, its importance has not been thoroughly investigated. Earlier findings suggest that it may be of vital significance in many disease states, such as familial hypomagnesemia (118), hypertension (145), and tumorigenesis, which may well be regulated by claudin (116)—a protein regulating tight junction integrity and function (146).

Transcellular Calcium Transport

There are three possible cellular routes for calcium transport. The first to be conceived was diffusion through the cytoplasm, the second mechanism is vesicular transport along microtubules, and the third could be tunneling through the endoplasmic reticulum. Transcellular Ca^{2+} transport is generally envisaged as a three-step process consisting of passive entry of Ca^{2+} across the apical membrane, the transcellular movement of Ca^{2+} from the point of entry to the basolateral membrane (BLM), and its extrusion from the BLM into the circulatory system. Most of these models were formulated and evaluated as a function of $1,25(OH)_2D_3$-stimulated calcium transport, and thus are presented in that context.

Table 1 Relative (%) contribution of para- and transcellular pathways to calcium transport in absorptive epithelia[a]

Absorptive region	Paracellular	Transcellular
A. Kidney		
Proximal distal tubule	90	10
Distal convoluted tubule	0	100
Thin ascending loop	50	50
B. Intestine		
Duodenum		
Low-Ca diet	20	80
High-Ca diet	>90	<10
Ileum[b]	100	0
Jejunum	80	20
Large intestine[c]	?	?
Colon[c]	?	?

[a]References: 13, 16, 45, 58, 92, 98, 117, 131.
[b]In addition, some reports indicate transcellular Ca^{2+} transport in the ileum (46, 64), including human (136).
[c]Although exact proportion not determined, transport occurs primarily via paracellular pathway in the large intestine, whereas a majority of Ca^{2+} transport in the colon occurs via transcellular pathway.

Facilitated diffusion.

Entry at the apical membrane. Calcium is postulated to enter the epithelial cells via selective Ca^{2+} channels at the luminal membrane under the influence of a steep, inwardly directed electrochemical gradient. Although there was some evidence indicating the existence of such channels in mediating Ca^{2+} entry in absorptive epithelia (59, 148), the molecular identity of such channels remained obscure for a long time. Moreover, apical administration of a variety of Ca^{2+} channel agonists failed to affect Ca^{2+} absorption (38). The classic approach for identification by purification and amino acid sequencing was hindered by the lack of a rich source of channel protein. Success was finally achieved with functional expression cloning using a rabbit primary connecting tubule/cortical collecting duct cDNA library in *Xenopus laevis* oocytes (54) for identification of a single transcript encoding for a novel epithelial Ca^{2+} channel,

TRPV: transient receptor potential of the vanilloid type

PKC: protein kinase C

ECaC, later renamed transient receptor potential of the vanilloid type (TRPV), receptor 5 (TRPV5) (78, 107), which is now accepted as exhibiting the defining properties of a Ca^{2+} transporter. Functional expression techniques successfully identified the Ca^{2+} transporter 1 (CaT1, ECaC2, or, more recently, TRPV6) from rat intestine, which shares about 80% amino acid sequence homology with TRPV5.

Epithelial Ca^{2+} channels. The proteins TRPV5 and TRPV6 form a distinct subfamily of TRPs. These nonvoltage-gated cation channels vary significantly in their ion selectivity and mode of activation (31, 78) and fulfill certain physiological functions ranging from phototransduction and olfaction to Ca^{2+} transport in the epithelia (17, 54). The rabbit TRPV5 contains an open reading frame of 2190 nucleotides encoding a protein of 730 amino acids, whereas a 2995-base-pair TRPV6 cDNA has an open reading frame of 2181 nucleotides encoding 727 amino acid residues (54, 100). Both of them have a predicted molecular mass of 83 kDa and have six membrane-spanning domains. The TRPV6 has four ankyrin repeats (providing interactions with the cytoskeleton; 149), an amino-terminal hydrophilic segment (326 amino acids), and one potential pore region formed by a hydrophobic stretch between domains 5 and 6. It also has one putative protein kinase A (PKA) and five putative protein kinase C (PKC) phosphorylation sites in the cytoplasmic domains, suggesting that Ca^{2+} transport may be regulated by phosphorylation, and no motifs in the carboxy terminus. However, TRPV5 has only three ankyrin repeats and no PKA phosphorylation site in the cytoplasmic domain, but it has three PKC and two PKA sites in its carboxy terminus. One N-glycosylation site is predicted in the first extracellular loop of TRPV5 (54). The two channels share a varying degree of sequence identity from one species to another and from one organ system to the other. They have a tetrameric stoichiometry and can combine with each other to form heteromultimeric channels with novel properties (54). Genomic analysis reveals that the two channels originate from two genes juxtaposed on human chromosome 7q35 and mouse chromosome 6 (79). Extensive reviews on these proteins are presented in several other recent publications (40, 54, 95, 129).

The differences revealed after comparing the N- and C-termini of the two channels may account for their unique electrophysiological properties. The differences include faster initial inactivation in TRPV6 than in TRPV5, and the kinetic differences between Ca^{2+} and Ba^{2+} currents are more pronounced for TRPV6 than for TRPV5 (54).

Both TRPV5 and TRPV6 are subject to Ca^{2+}-dependent feedback inhibition (54), including Ca^{2+}-dependent binding of calmodulin to the carboxy terminus in TRPV6 (94). The EF-hands 3 and 4 of calmodulin appear to bind Ca^{2+} to positively affect TRPV6 activity (68). Although TRPV5 appears to be devoid of a calmodulin binding site, carboxyl-terminal truncations and mutations also modulate Ca^{2+}-dependent inactivation of the protein (54). Recently, Chang et al. (27) have reported that β-glucuronidase activates TRPV5.

Brunette et al. (20) demonstrated that ATP directly enhances channel activity, whereas a novel protein, 80K-H, functions as a Ca^{2+} sensor controlling TRPV5 activity (51). A specific interaction of 80K-H protein and TRPV5 occurs with colocalization of both proteins in the kidney and transcriptional regulation by dietary Ca^{2+} (51). In addition, age, dietary Ca^{2+}, and G proteins also appear to modulate Ca^{2+} channels (18, 20, 137). Negative regulators of TRPV5 and TRPV6 include, respectively, BSPRY and RGS2 (114, 135).

It has been established that acidification at the apical region inhibits transcellular Ca^{2+} transport, apparently through pH modulation of TRPV5 affinity for Ca^{2+} (54). Not much is known about pharmacological agents, except that ruthenium red and econazole (54) are both effective inhibitors of TRPV5 and TRPV6.

Some of the major characteristics of TRPV5 knockout mice include impaired Ca^{2+}

reabsorption, hypercalciurea, hypervitaminosis D, hyperparathyroidism, rickets, and intestinal hyperabsorption of Ca^{2+} (109). Increased urinary Ca^{2+} excretion appears to be the result of defective Ca^{2+} reabsorption within the initial region of the distal convoluted tubule, where TRPV5 is mostly localized (54, 109). At the molecular level, there were reduced mRNA levels of renal calbindin -D_{9K} and -D_{28K}, and Na^+/Ca^{2+} exchanger (NCX) in TRPV5 knockout mice (109). The TRPV6 knockout mice have defective Ca^{2+} absorption in the intestine, increased urinary excretion, decreased bone mineral density, reduced weight gain, and reduced fertility (10). Although TRPV6 knockout mice have normal Ca^{2+} and increased PTH levels, they fail to further increase serum PTH and $1,25(OH)_2D_3$ on a low-Ca^{2+} diet (10).

Downregulation of TRPV5 and TRPV6 is likely involved in the impaired Ca^{2+} (re)absorption during aging, and TRPV5(−/−) mice are likely to develop age-related hyperparathyroidism and osteoporosis at an earlier age than TRPV5(+/+) mice, suggesting an important role in overall Ca^{2+} homeostasis (54). The TRPV5(−/−) mice display reduced active Ca^{2+} reabsorption in the distal convoluted tubules of kidney despite elevated levels of $1,25(OH)_2D_3$ (54). Dysfunctional TRPV5 and TRPV6 may be associated with other multifunctional pathological disorders, such as stone disease and postmenopausal osteoporosis (95).

Several tissues have been shown to express TRPV5 and TRPV6. Although TRPV6 expression was confirmed in the intestine and kidney, TRPV5 was exclusively confined to kidney (97).

Ca^{2+} carriers. In addition to Ca^{2+} channels, there is some evidence that Ca^{2+} entry may be carrier-mediated (see 47 for review). One candidate is the H^+/Ca^{2+} exchange (70), which is the primary means of sequestering Ca^{2+} by mitochondria (70), though demonstrating its presence in the plasma membrane has remained elusive. Another is Na^+/H^+ (103, 115) exchange that catalyzes H^+/Ca^+ exchange in membrane vesicles in *Escherichia coli* strain EP432 (42) and has been demonstrated in rat distal convoluted tubules (50).

NCX: Na^+/Ca^{2+} exchanger

Transcellular movement. A large body of literature is available on the role of calbindin in $1,25(OH)_2D_3$-mediated Ca^{2+} transport across epithelia at the whole animal, cellular, and molecular level (4, 26, 28, 30, 32). However, its role is largely confined to either being a ferry or acting as a buffer (6, 15, 56), and both roles have remained controversial.

Calbindin D_{28K} was first found in the chick duodenum (142, 143). Later, $1,25(OH)_2D_3$-induced calbindin D_{9K} in mammalian duodenum was identified and characterized (44) and now has been reported in tissues such as brain, stomach, and prostate (29, 30). Although for many years there have been indications of calbindin not being involved in Ca^{2+} absorption (121, 123), these reports have largely been dismissed by those who feel that only the classical vitamin D receptor (VDR) is involved with $1,25(OH)_2D_3$-mediated Ca^{2+} and phosphate transport across epithelia (96, 104).

Recently, DeLuca and coworkers (2, 67) created a calbindin D_{9K}-null 129/OlaHsd mouse. This mouse was indistinguishable from the wild type in phenotype and in serum Ca^{2+} level regardless of age or gender (2). These mice were fully capable of absorbing Ca^{2+} from the intestine in response to $1,25(OH)_2D_3$, clearly proving that the protein is not needed for steroid-induced Ca^{2+} absorption. Moreover, these mice were also able to reproduce normally and had no impaired Ca^{2+} homeostasis (67). Earlier, Lee et al. (71) suggested that in humans, duodenal calbindin D_{9K} may not be involved in Ca^{2+} transport because of an increase in the protein with age even though blood Ca^{2+} levels decreased at the same time and no significant age-associated change in the VDR occurred. The "cytoplasmic ferry" model is cast into further doubt by the observation that the preponderance of calbindin D_{28K} in chicks is found in transport vesicles and is secreted during Ca^{2+} transport (85). Since lysosomes transport phosphate as well as calcium (see below), calbindin

PMCA: plasma membrane Ca^{2+}-ATPase

may serve to prevent precipitation of the ions in transport vesicles.

Extrusion at the basal lateral membrane. The efflux of Ca^{2+} at the serosal side of the cell occurs against a considerable electrochemical gradient, but is not considered a rate-limiting step (15). Two Ca^{2+} transporters have been identified at the BLM of absorptive epithelia to extrude Ca^{2+}: the plasma membrane Ca^{2+}-ATPase (PMCA) and NCX.

The PMCA is a P-type ATPase that acts through formation of an aspartyl phosphate intermediate (25). It is a high-affinity Ca^{2+} efflux pump present in virtually all eukaryotic cells and is responsible for the maintenance and resetting of the resting intracellular Ca^{2+} levels (12, 126). However, its activity is highest in epithelia that exhibit appreciable rates of transcellular Ca^{2+} transport (43), and the activity is localized to the BLM (5, 12, 25, 50, 70). The high-affinity Ca^{2+}-ATPase displays a mean affinity constant of 0.3×10^{-6} M, which is similar to that of other plasma membrane Ca^{2+}-ATPases (25). Four genes encode separate isoforms of the enzyme designated PMCA1–4 (124). The putative Ca^{2+}-binding domain lies on the cytoplasmic side of the pump molecule and spans the entire membrane (15). Differences among the isoforms are primarily at the 3′ end, which encodes a calmodulin-binding site and a consensus PKA phosphorylation site toward the carboxyl end. It is also suggested that PMCA 1 and 4 are housekeeping isoforms involved in the maintenance of cellular Ca^{2+} homeostasis (124). The isoform PMCA1b is abundantly expressed in the intestine and is postulated to be the one involved in extrusion mechanism in the intestinal Ca^{2+} absorption (5, 54). Calcium is expelled through a channel-like opening formed by the transmembrane elements, and phosphorylation is believed to bring about the necessary conformational change such that Ca^{2+} bound to the enzyme is propelled through the opening (15). The estimated Vm of the intestinal enzyme is reported to be in the range of 20 to 30 nM Ca/min per mg protein (15). This is generally adequate to extrude Ca^{2+} even at the highest rates of Ca^{2+} transport (119), and enzyme levels have been shown to increase after seco-steroid (143). However, plasma $1,25(OH)_2D_3$ was not found to be correlated with PMCA1 expression in humans (138).

Some evidence supports a role for NCX (12, 131), although controversy exists (47). Three genes for NCX, designated NCX1, NCX2, and NCX3, have been identified (12). Sequence similarities indicate ~70% homology. The exchanger has an amino-terminal signal sequence, two sets of multiple transmembrane α-helices near the ends of the protein, and a large intracellular loop (12). Splicing of RNA transcripts would generate further diversity in the exchanger system. Of the three isoforms, NCX1 is widely distributed among different mammalian tissues, including absorptive epithelia (62, 132), whereas NCX2 and NCX3 are confined primarily to brain and skeletal muscle (73, 93). It has been demonstrated that NCX1 is the primary extrusion mechanism in the distal tubular cells (11, 132), but its role in the enterocytes may be of minor importance only (54). Unfortunately, targeted deletion of NCX1 appears to be not a suitable model to study this system since it leads to death in utero (63, 110). **Figure 1** presents certain elements discussed above in schematic form.

Vesicular Transport Model

Entry at the apical membrane. In the vesicular transport model (reviewed in 69), Ca^{2+}-enriched vesicles can be formed by influx of Ca^{2+} through Ca^{2+} channels or transporters in the apical membrane. This rapid increase in Ca^{2+} concentration around the apical region can disrupt the actin filaments near the Ca^{2+} channels and initiate the formation of endocytic vesicles. Alternatively, influx through a Ca^{2+} channel may also promote the exocytic delivery of vesicles containing Ca^{2+} transporters (120), which are coupled to the formation of endocytic vesicles. Both TRPV5 and TRPV6 have been found to colocalize with Rab11a protein in vesicular structures underlying the

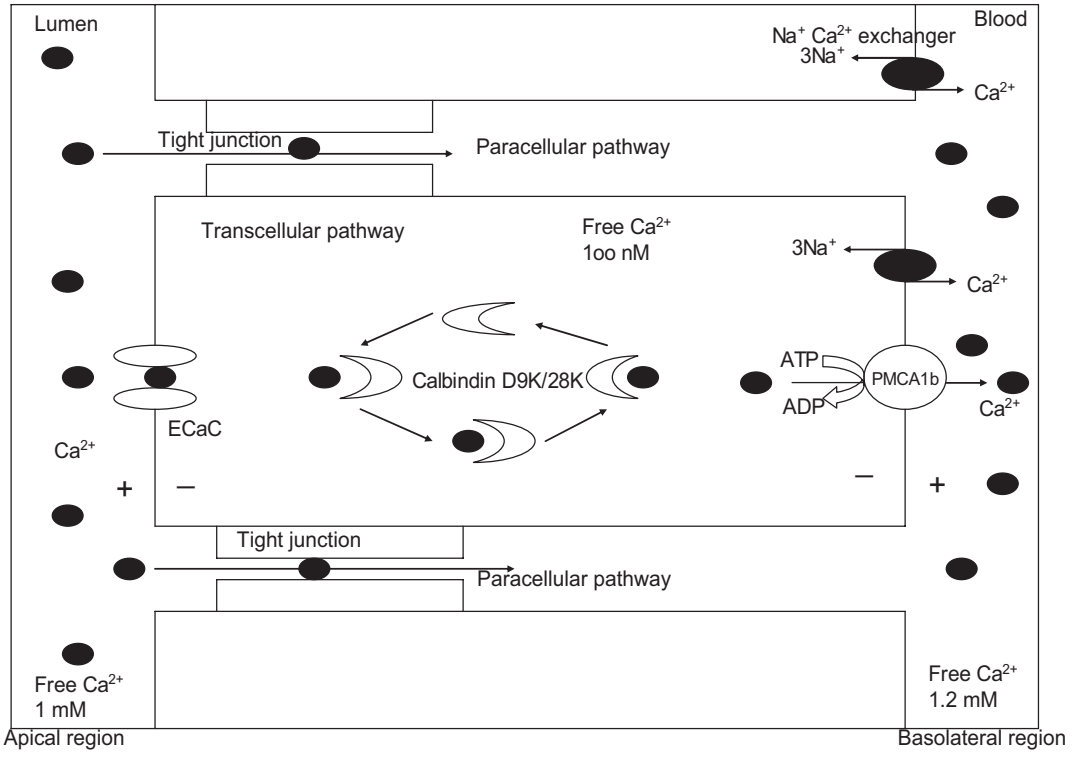


Figure 1

Molecular illustration of paracellular and transcellular Ca^{2+} transport pathways in epithelia (adapted and modified from 54). Paracellular transport is primarily a passive mechanism driven by Ca^{2+} concentration in the lumen and integrity of the tight junction. Transcellular transport is a three-step process: entry of Ca^{2+} into the cytoplasm at the apical region facilitated by epithelial Ca channels TRPV5 and TRPV6, movement across the cell, and extrusion at the basal lateral membrane.

apical plasma membranes of Ca^{2+}-transporting epithelial cells (134), lending further credence to the vesicular transport model of Ca^{2+} in the epithelia. A third possibility is direct filling of a vesicle by the channel. The newly formed vesicles are then transported vectorially by microtubules, and some fuse with lysosomes (86).

Transcellular movement. Electron microscopy of chick duodenum led Jande & Brewer (55) to propose the vesicular transport model for intestinal Ca^{2+} absorption induced by $1,25(OH)_2D_3$. Warner & Coleman (141), using x-ray probe analysis, found discrete localizations of Ca^{2+} under transport conditions rather than a diffuse cytoplasmic localization. These vesicles were subsequently identified as lysosomes (37). There was an increased Ca^{2+} concentration and lysosomal count in intestinal epithelial cells following treatment of rachitic animals with $1,25(OH)_2D_3$ (35, 36). These organelles also showed increased acid phosphatase, a lysosomal marker enzyme, and stained intensely with pyroantimonate, indicating high Ca^{2+} content (37). These vesicular/vacuolar/lysosomal structures are membrane bound, move laterally, and eventually coalesce with the lateral plasma membrane, causing exocytosis of their contents (37). Several other studies implicated the involvement of not only lysosomes in the transcellular Ca^{2+} transport in intestinal epithelia (84–91) but also of microsomes (113).

Nemere et al. (84) used biochemical methods to determine subcellular Ca^{2+} localization in vitamin D-deficient chicks dosed with vehicle

$1,25D_3$-MARRS receptor: $1,25(OH)_2D_3$-membrane-associated, rapid-response, steroid-binding receptor

or $1,25(OH)_2D_3$ in vivo and found the highest levels of ^{45}Ca in a fraction containing lysosomes, mitochondria, Golgi, and basal lateral membranes. Very little calcium was found to be cytoplasmic. Further resolution of the postnuclear fraction on Percoll gradients implicated lysosomes as the Ca^{2+} carriers in $1,25(OH)_2D_3$-stimulated transport. In a time course study (86), net ^{45}Ca transport into the blood exactly paralleled levels of the radionuclide in lysosomes with regard to onset, maximum, and decline. At 43 h after $1,25(OH)_2D_3$, both transport and lysosomal ^{45}Ca had decreased while calbindin levels remained elevated, thus bringing into question the role of calbindin D_{28K} as the most important carrier in transcellular Ca^{2+} transport.

Extrusion at the basal lateral membrane. In spite of the studies performed on Ca^{2+} extrusion proteins, it is possible that the ATPase and exchangers are involved in regulating signaling Ca^{2+} rather than transport Ca^{2+}. In the vesicular transport model, exocytosis delivers Ca^{2+} at the BLM. Indeed, net transport of Ca^{2+} is inhibited by chloroquine, a drug known to interfere with lysosomal function (84), and the facilitated diffusion model does not explain this. Calcium transport is also accompanied by secretion of cathepsin B, a lysosomal proteinase (88) and vesicular calbindin (85). Moreover, a very rapid signaling response to $1,25(OH)_2D_3$ is activation of a voltage-gated calcium channel on the basal lateral membrane (38, 39). Indeed, extracellular calcium at the BLM is required to initiate signaling for net calcium transport across the epithelium (38, 39), which further suggests that Ca^{2+} pumps turn the signal off. We have found instead that the PKC signaling pathway mediates exocytosis of transport calcium (81).

Tunneling through intracellular stores. The third and final model was demonstrated in pancreatic acinar cells (101; reviewed in 69) and may also constitute a possible route in epithelial cells. Ca^{2+} enters the cell through channels as in the preceding models and transport to the BLM occurs through passive diffusion in the endoplasmic reticulum. It involves active buffering of Ca^{2+} rather than the passive buffering that occurs in facilitated diffusion. Extrusion of Ca^{2+} to extracellular media occurs through Ca^{2+}-ATPases and Na^+/Ca^{2+} exchangers present in the BLM. Although this is at odds with microscopic observations (35–37, 141), it may be that the endoplasmic reticulum buds off transport vesicles filled with calcium. Indeed, a localization of calcium in endoplasmic reticulum was noted to occur within 1 min of initiating absorption, but not at later times (86).

Figure 2 (69) depicts the three distinct but potentially complementary models of transcellular Ca^{2+} transport discussed above. All three models are probably coordinated to meet the short- and longer-term needs of the animal.

ENDOCRINE REGULATION OF Ca^{2+} TRANSPORT

$1,25(OH)_2D_3$

The steroid hormone $1,25(OH)_2D_3$ is made by sequential hydroxylations of the parent metabolite in liver and kidney. Regulation of epithelial Ca^{2+} transport by $1,25(OH)_2D_3$ through genomic actions involving the classical VDR is widely recognized (14, 34, 57, 72, 96, 104, 139). Its nongenomic regulation through a separate membrane receptor—which has been identified as the $1,25D_3$-MARRS (membrane-associated, rapid response, steroid-binding) protein (82, 112) and that has been reviewed recently (60, 61)—is also accepted now. The TRPV proteins are primarily regulated by $1,25(OH)_2D_3$ (54, 97, 122, 138, 147). $1,25(OH)_2D_3$ had no effect on TRPV5/6 in mice with mutant, nonfunctioning VDR even though a putative response element (VDRE) was detected in TRPV5 (54). In $1,25(OH)_2D_3$-deficient rats, TRPV5 mRNA and protein levels of the kidney cortex were significantly decreased compared with replete controls (54). In Caco-2 cells, increased TRPV6 mRNA expression preceded by several hours the $1,25(OH)_2D_3$-mediated induction of

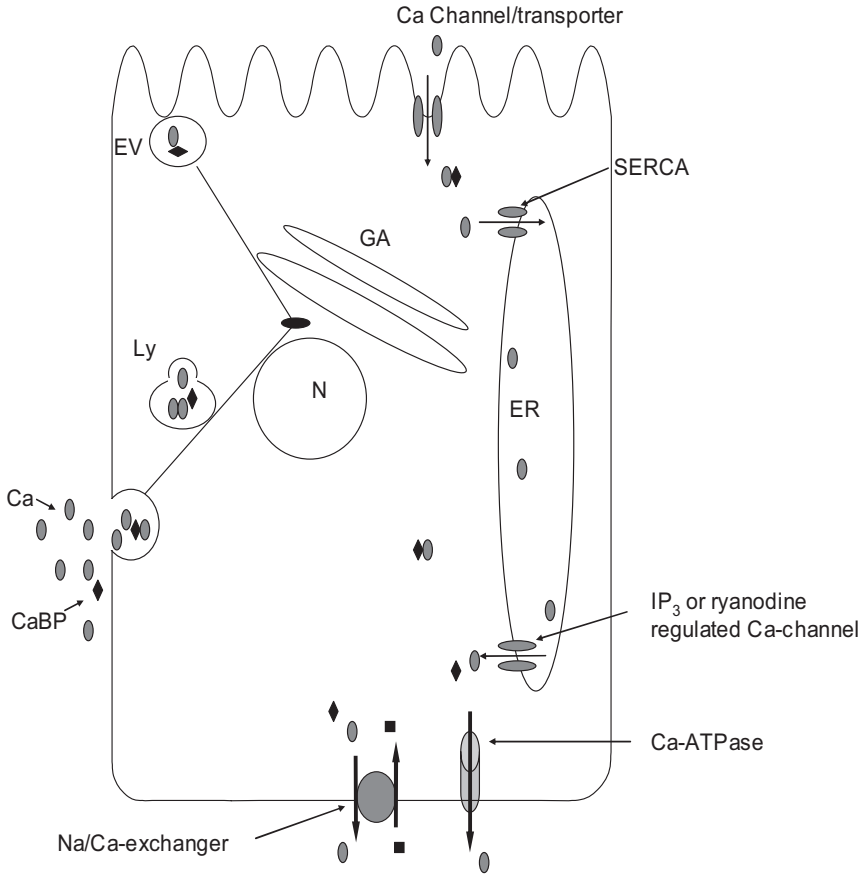


Figure 2

Schematic illustration of vesicular Ca^{2+} movement in epithelia (adapted from 69). Calcium uptake occurs through a specific transporter/channel protein and can be directly routed either to vesicles or to endoplasmic reticulum (tunneling). The endocytic vesicles fuse with lysosomes (a means to concentrate calcium). Both vesicles and lysosomes contain calbindin. Vectorial transport occurs along microtubules to the basal lateral membrane, where exocytosis completes the transport process. Cytoplasmic-signaling calcium (from hormone stimulation) is removed by exchangers and pumps.

calbindin D_{9K} (147). TRPV6 expression was $1,25(OH)_2D_3$ dependent in men but not in older women, where expression of both TRPV6 and VDR were reduced (137, 138). However, the promoter region of the TRPV6 gene lacks recognizable VDREs, which suggests that TRPV6 gene expression may be controlled by a novel $1,25(OH)_2D_3$-mediated mechanism (121). The possibility arises that the $1,25D_3$-MARRS receptor/PDIA3/ERp57 is involved in this mechanism, since it is translocated to the nucleus after binding ligand (89).

In intestinal cells from vitamin D-sufficient adult chickens or cultures of young chicks, $1,25(OH)_2D_3$ stimulates calcium uptake within minutes. We have discovered that agents that stimulate PKA activity promote the secretion of β-glucuronidase, an activator of TRPV5 (27). Treatment of isolated intestinal epithelial cells with β-glucuronidase in turn promotes calcium uptake. And transfection of cells with siRNA to either β-glucuronidase or TRPV6 abolish $1,25(OH)_2D_3$-enhanced calcium uptake (R.C. Khanal, manuscr. submitted). These

data suggest further that one of the primary defects in vitamin D-deficient animals is the absence of $1,25(OH)_2D_3$-stimulated adenylate cyclase activity (33, 81) rather than the absence of calbindin. This would explain why a response to steroid is measured in hours rather than minutes under vitamin D-deficiency conditions.

The endocrine effects of $1,25(OH)_2D_3$ on the BLM extrusion systems, NCX and PMCA, have been documented (47, 54, 144), but with conflicting results (54). For instance, $1,25(OH)_2D_3$ induced increased levels of mRNA for NCX in one such study (75), whereas in another there was no response (56). Both vitamin D and $1,25(OH)_2D_3$ have been found to increase PMCA mRNA and protein levels in intestine (22, 56). Van Abel et al. (132) and Van Cromphaut et al. (133) showed enhanced PMCA1b expression in intestine but not in kidney. These results suggested that the effect of the steroid on PMCA may be animal, organ, or tissue specific. The synthesis of the enzyme Ca^{2+}-ATPase has not been shown to be $1,25(OH)_2D_3$ dependent.

Parathyroid Hormone

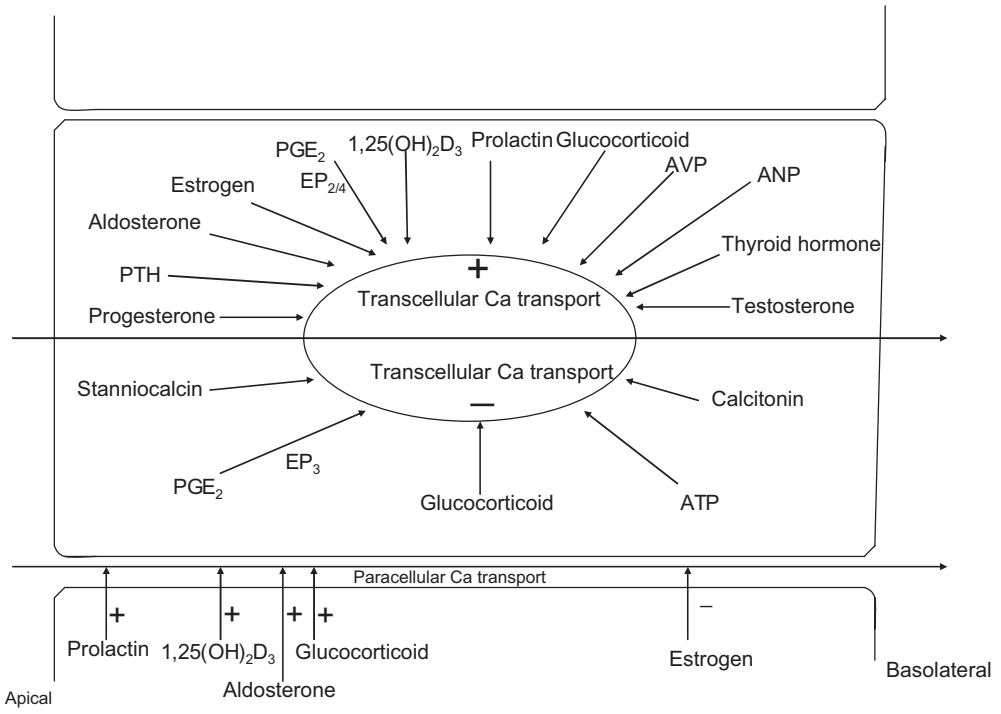
The parathyroid glands secrete parathyroid hormone (PTH) in response to low serum calcium detected by Ca^{2+}-sensing receptors (CaR; 18), utilizing a mechanism similar to those of G protein–coupled receptors (18, 21, 41, 49, 106, 111). This was later confirmed with the cloning of a G protein–coupled extracellular CaR from bovine parathyroid gland that encoded 1085 amino acids (1, 19). Abnormalities in PTH regulation have been found to be related to low expression of CaR (23). The detailed structure, function, occurrence, and many other patho-physiological aspects of CaR have been reviewed extensively (18, 108, 109).

Parathyroid Hormone Actions

Actions of PTH can be divided into classical and nonclassical. The classical action is based on the initial belief that the major biological activities of PTH are subserved by the 34 residues in its N-terminal domain and that the PTH residues located beyond position 34 are largely irrelevant (105, 130). Nonclassical actions are now known to require additional regions of the peptide hormone and include binding of intact PTH to rat and avian osteoclasts (6, 77, 128), actions unique to intact PTH (reviewed in 80).

Intestinal calcium absorption. We have previously summarized reports from many labs that intestinal epithelial cells contain functional PTH receptors and respond to the hormone with stimulated calcium transport in perfused duodenal loops and enhanced calcium uptake in isolated enterocytes (7, 83, 90, 91). Originally it was believed that PTH had only indirect effects on intestinal absorption since the models in use at the time were vitamin D-deficient animals. In retrospect, the absence of hormone-stimulated adenylate cyclase (33, 81) in vitamin D-deficient animals explains the lack of an effect by PTH.

In rat, the action of PTH was reportedly blocked by the Ca^{2+} channel antagonists verapamil and nitrendipine (see 83 and references therein). However, in chick intestine, voltage-regulated calcium channels are localized in the basal lateral membrane (38, 39)—and not the brush border—which suggests that the antagonists interfered with signaling rather than transport. The signal transduction pathway activated by PTH binding to its receptor includes PKA-mediated calcium uptake (125). Forskolin has been reported to stimulate calcium uptake in isolated enterocytes (102), and the antagonist RpcAMP has recently been demonstrated to block the actions of PTH in primary cultures of chick intestinal cells (129). Phorbol ester (an agonist of the PKC pathway) does not stimulate calcium uptake (R.C. Khanal, manuscr. submitted). Since these pathways have been conserved in both rats and chicks, they are most likely physiologically important. Indeed, the anabolic bone effects of small doses of PTH in humans (74) may very well be due



Schematic model for endocrine control of Ca transport in epithelial cells

Figure 3

An overview of endocrine effects on Ca^{2+} transport across epithelia. Some of the hormones have both positive and negative effects (glucocorticoid, thyroid hormone, and PGE_2), whereas some others have positive as well as no effects (testosterone). Varying effects of the same hormone probably depended on the cell (organ) system, physiological stage, and hormone concentration. See text for references.

in part to stimulated calcium absorption in the intestine.

$24,25(OH)_2D_3$

The metabolite $24,25(OH)_2D_3$, made under conditions of vitamin D sufficiency, is an endogenous inhibitor of both $1,25(OH)_2D_3$ and PTH-stimulated calcium transport. We have recently reviewed (60) the mechanism by which this occurs: $24,25(OH)_2D_3$ binds to the enzyme catalase and decreases its activity with concomitant increases in H_2O_2 levels. This in turn leads to inactivation of the $1,25D_3$-MARRS receptor (but not the VDR) as well as PKC. **Figure 3** indicates additional hormones that contribute to the endocrine regulation of intestinal calcium transport.

CONCLUSIONS

Many distinct proteins have been identified at the cell surface that may be involved in calcium transport across epithelia, although in most cases it is not known to what extent each contributes to the process. Notable exceptions are the recently identified TRPV channels/transporters. Basal lateral pumps are likely regulators of cytosolic calcium involved in signal transduction, but net calcium transport by facilitated diffusion now seems tenuous since calbindin-D_{9k} is not necessary for the process, and calbindin-D_{28k} is largely in membrane-delimited vesicles. And as noted above, facilitated diffusion does not explain the complete inhibition of $1,25(OH)_2D_3$-stimulated calcium transport by

chloroquine within 30 min of introduction to the lumen. However, many plasma membrane proteins are dynamically inserted and retrieved by vesicular trafficking. Therefore, some clarification of each protein's contribution to the absorption process may be made by investigating its relationship to vesicular transport calcium.

SUMMARY POINTS

1. TRPV channels are most likely to be responsible for calcium uptake into intestinal epithelium.
2. The PKA-signaling pathway, rather than calbindins, may be the rate-limiting step in initiating $1,25(OH)_2D_3$-stimulated calcium transport in vitamin D–deficient animals.
3. There is little evidence to support the facilitated diffusion model of transport in comparison with the vesicular carrier model.
4. Basal lateral membrane proteins that extrude calcium may be for the regulation of signal-transduction calcium rather than transport calcium.

FUTURE ISSUES

1. Tissue-specific knockouts of basal lateral calcium extrusion proteins are needed to determine their role in calcium transport.
2. Dissection is needed to determine how TRPV6 fills vesicular carriers.
3. The effect of siRNA knockdown to calbindin D_{28k} in cultured chick intestinal cells on calcium update should be evaluated.

DISCLOSURES

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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Errata

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