Vitamin D Metabolism and Mechanisms of Calcium Transport

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ABSTRACT

Vitamin D$_3$ undergoes sequential hydroxylations in the liver and kidney to form 1,25-dihydroxyvitamin D$_3$, the biologically active form of the vitamin. 1,25-dihydroxyvitamin D$_3$ is metabolized by several processes in various target tissues that decrease the biological activity of the sterol. In addition, 1,25-dihydroxyvitamin D$_3$ is excreted in the bile as polar metabolites, such as glucuronides and, possibly sulfates and neutral polar steroids. These compounds undergo an enterohepatic recirculation in both man and experimental animals. 1,25-dihydroxyvitamin D$_3$ increases the absorption of calcium in the intestine and the reabsorption of calcium in the kidney. It induces the synthesis of several proteins, the most notable of which is calcium binding protein that is thought to play a role in the absorption of calcium. The vitamin D-dependent calcium binding proteins and the calcium-magnesium ATPase calcium pump are co-localized in several tissues that play a role in the absorption of calcium.

Key Words: Vitamin D; 1,25-dihydroxyvitamin D$_3$; calcium binding protein; calcium pump

This article reviews various aspects of epithelial calcium transport with particular emphasis on the vitamin D-endocrine system and the manner in which the hormonal form of vitamin D$_3$, 1,25-dihydroxyvitamin D$_3$, regulates the transport of calcium across cells. It also examines the role of the vitamin D-dependent calcium binding proteins in the movement of calcium across such cells and their possible relationship to calcium-pumping ATPases that are important in the movement of calcium from within the cell to the extracellular fluid.

THE PHYSIOLOGY OF VITAMIN D

Vitamin D Metabolism

For the purposes of this review, the term vitamin D will refer to both vitamin D$_3$ and vitamin D$_2$ (the structures of some vitamin D metabolites are shown in Figure 1). The two forms are metabolized in a similar fashion in mammalian but not in avian species; in birds, vitamin D$_2$ is considerably less active than vitamin D$_3$. Vitamin D, in association with parathyroid hormone, plays a vital role in the control of calcium and phosphorus homeostasis (1–7). In the absence of vitamin D, intestinal calcium and phosphorus absorption from the intestine diminish (8–13); as a result serum calcium decreases and neuromuscular irritability results with attendant complications. Bone mineralization is impaired because of a lack of calcium and phosphorus in extracellular fluid (14–24), growth is retarded, and muscular tone is diminished (6). In addition, recent information suggests that the vitamin via its active metabolite, plays an important role in the differentiation of cells in culture (25). A deficiency of the vitamin results in rickets in the growing organism and osteomalacia in the adult.

Rickets was first described in a complete fashion by Glisson and Whistler (26). Mellanby demonstrated that rickets was caused by a dietary deficiency and conjectured that it was due to a deficiency of vitamin A (27). McCollum et al., however, showed that the anti-rachitic activity was due to a separate substance (28–30). Huldshinsky (31) and Chick et al. (32) demonstrated that exposure to sunlight could cure rickets in children. Steenbock and Hart (33), Goldblatt and Soames (34), and Steenbock and Black (35) established the importance of ultraviolet light in curing rachitic lesions. Steenbock and Black conclusively demonstrated the role of ultraviolet light in the generation of vitamin D (35). Askew and Windaus pioneered the synthesis of vitamins D$_2$ and D$_3$ (36–39); soon after, the fortification of foods led to the elimination of rickets as a disease in the Western hemisphere.

Vitamin D$_3$ is not an exogenous essential micro-nutrient in the strict sense. The vitamin can be synthesized in the skin; however, with decreasing sunlight exposure an increasing need for dietary sources of the vitamin has become apparent. Vitamin D$_3$ is formed from a precursor, 7-dehydrocholesterol, in the skin under the influence of ultraviolet light (39–43) (Figure 2). The formation of vitamin D$_3$ from 7-
vitamin 24,25-Dihydroxyvitamin D3

5,6-Trans-vitamin D3

Figure 1. The structures of some commonly used vitamin D metabolites and analogs.

Dehydrocholesterol occurs via an intermediate, pre-vitamin D3 that undergoes thermal isomerization to vitamin D3 at 37°. Vitamin D3 is transported from the skin, possibly in association with vitamin D-binding protein, a protein that belongs to the alpha-fetoprotein-albumin superfamily of proteins (41, 44, 45). Vitamin D3 is biologically inactive as it cannot increase the active transport of calcium or phosphorus or mediate any of the other activities of vitamin D3 in physiologic amounts unless it is metabolized to other, more polar compounds. Vitamin D3 can also be obtained from dietary sources. Vitamin D3 is derived from the plant sterol, ergosterol, and is available only from dietary sources.

In the liver, specifically in hepatic microsomes and mitochondria, vitamin D3 is converted to 25-hydroxyvitamin D3 (46–51) (Figure 2). The Km of the mitochondrial enzyme is considerably higher than the Km of the microsomal enzyme (49). Although it appears that the microsomal enzyme is regulated by 25-hydroxyvitamin D3 and other vitamin D metabolites, the mitochondrial enzyme is not. Consequently, 25-hydroxyvitamin D3 concentrations accurately reflect circulating concentrations of vitamin D3. The vitamin D3 25-hydroxylase enzyme is a cytochrome P-450-like enzyme that utilizes NADPH for the conversion of vitamin D3 to 25-hydroxyvitamin D3 (50). 25-hydroxyvitamin D3, in and of itself, is biologically inert in physiologic concentrations and is converted in the kidney to the active metabolite of vitamin D3, 1,25-dihydroxyvitamin D3 (52–56). During states of calcium or phosphorus demand, the predominant metabolite formed from 25-hydroxyvitamin D3 is 1,25-dihydroxyvitamin D3; conversely, during states of adequate calcium or phosphate balance, 25-hydroxyvitamin D3 is metabolized predominantly to another dihydroxylated metabolite, 24R,25-dihydroxyvitamin D3 (1–7) (Figure 2).

The 25-dihydroxyvitamin D3 1α-hydroxylase enzyme that converts 25-hydroxyvitamin D3 to 1,25-dihydroxyvitamin D3 is located predominantly in the proximal tubule of the kidney (58–60). It is a mixed function oxidase comprised of a ferredoxin and an iron sulfur protein that is linked to a cytochrome P-450 (1, 57, 61–68). This enzyme is regulated by several factors that work in concert to maintain normal concentrations of plasma calcium and phosphorus. This article will not review all the factors that regulate the activity of the 25-hydroxyvitamin D3 1α-hydroxylase enzyme. Suffice it to say that parathyroid hormone, the concentrations of inorganic phosphate in extracellular fluid and other ions such as calcium and, perhaps, hydrogen ion directly influence the activity of this enzyme. The reader is directed to reviews that deal exclusively with the regulation of the 25-hydroxyvitamin D3 1α-hydroxylase enzyme (6, 69 and references therein). Table 1 summarizes what is known about the regulation of the 25-hydroxyvitamin D3 1α-hydroxylase. It is important to realize that adaptations to a low calcium diet are brought about by changes in the concentration of parathyroid hormone that, in turn, influences the activity of the 25-hydroxyvitamin D3 1α-hydroxylase enzyme in the proximal tubule. In parathyroidectomized animals, the response of the 1α-hydroxylase to a low calcium diet is absent or greatly blunted. On the other hand, the adaptation to a low phosphate diet can occur in the absence of parathyroid glands, suggesting that inorganic phosphate either directly
TABLE 1. Factors altering serum 1,25(OH)₂D₃ levels or 25(OH)D₃ 1α-hydroxylase activity

<table>
<thead>
<tr>
<th>Factor</th>
<th>1,25(OH)₂D₃</th>
<th>Effect on 1α-Hydroxylase Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25(OH)D₃</td>
<td>Increase</td>
</tr>
<tr>
<td>Parathyroid hormone</td>
<td></td>
<td>+₀</td>
</tr>
<tr>
<td>Serum inorganic phosphorus</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>1,25(OH)₂D₃</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Calcium (direct)</td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>Calciton</td>
<td></td>
<td>+₀, -₀, 0⁺</td>
</tr>
<tr>
<td>Hydrogen ion</td>
<td></td>
<td>+₀, -₀</td>
</tr>
<tr>
<td>Sex steroids</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Prolactin</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Growth hormone</td>
<td></td>
<td>+₀, -₀, 0⁺</td>
</tr>
<tr>
<td>Glucocorticoids</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Thyroid hormone</td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>Pregnancy</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

* +₀, stimulation or increase.
* -₀, suppression or decrease.
* ? , effect not known.
* 0⁺, no effect.
* Effects may be secondary to changes in calcium, phosphorus, or parathyroid hormone.

or by some other means influences the activity of the 25-hydroxyvitamin D₃ 1α-hydroxylase.

Although considerable attention has been devoted to the factors regulating the synthesis of 1,25-dihydroxyvitamin D₃, less attention has been paid to factors that are responsible for its metabolism and the control of tissue concentrations of the hormone. 1,25-dihydroxyvitamin D₃ is catabolized in several different ways (70-98, Figure 3). These include:

1. conversion to a 23-carbon acid, calcitroic acid in the intestine and liver (74-77);
2. hydroxylation at C-24 to form 1,24,25-trihydroxyvitamin D₃ in many tissues including the kidney, intestine, cartilage, and cells in culture (78-86);
3. formation of 1,25-dihydroxyvitamin D₃ 23,26-lactone (87);
4. formation of 1,25,26-trihydroxyvitamin D₃ (88,89);
5. formation of 23-oxo 1,25-dihydroxyvitamin D₃ and 23-oxo 1,25,26-trihydroxyvitamin D₃ (90); and
6. excretion via the bile of various polar metabolites of the hormone such as glucuronides, sulfates, and neutral polar compounds (91-98).

In general, the various metabolic processes that result in alterations in the side chain of 1,25-dihydroxyvitamin D₃ are induced by the hormone itself, occur in target tissues of 1,25-dihydroxyvitamin D₃, result in metabolites with reduced bioactivity, and can be thought of as processes that prevent the toxicity and terminate the hormonal response. If one interferes with the biologic transformations of 1,25-dihydroxyvitamin D₃ that involve alterations of the side chain, by synthesizing a variety of compounds with alterations in the side chain that preclude further biological processing, one obtains compounds that, in some instances, have enhanced or prolonged biologic activity. We recently synthesized one such compound, 1,25-dihydroxyvitamin D₃-26,27-dimethyl vitamin D₃ (98). This metabolite has a more prolonged biologic activity than 1,25-dihydroxyvitamin D₃ itself.

Metabolic processes that result in the alteration of the side chain of 1,25-dihydroxyvitamin D₃ account for only a fraction of the metabolism of the hormone (perhaps 30% to 35%). Therefore, other mechanisms that are responsible for the metabolism of 1,25-dihydroxyvitamin D₃ must exist. One of these mechanisms involves the biliary excretion of 1,25-dihydroxyvitamin D₃ (Figure 4). When one administers ³H-1,25-dihydroxyvitamin D₃ to a bile duct-cannulated normal rat, there is a rapid excretion of radioactivity in bile (91). This excreted material is then reabsorbed in the duodenum and undergoes an enterohepatic recirculation. Such a phenomenon is also observed in humans, in whom there is rapid disappearance of radioactivity from the plasma pool that is followed within 30 min by the excretion of radioactivity into bile (72). There is also reabsorption of radioactivity in the duodenum, suggesting that an enterohepatic recirculation process is also operative.
in humans. Biliary metabolites of 1,25-dihydroxyvitamin D₃ are more polar than the hormone and are both neutral and acidic in nature; the acidic compounds have characteristics of glucuronides and sulfates of 1,25-dihydroxyvitamin D₃. We have isolated one such metabolite from bile and have shown that it is a monoglucuronide of the hormone with the glucuronide moiety on the 1 or the 3 hydroxyl group in the A ring (92). We have synthesized several glucuronides and glucosides of vitamin D₃ homologs, including 1,25-dihydroxyvitamin D₃ and have shown that they are biologically active after deconjugation into the sterol and the sugar moiety (94–96,97). These compounds are less active than 1,25-dihydroxyvitamin D₃ itself. In general, the biliary pathway for 1,25-dihydroxyvitamin D₃ can be thought of as an excretory route for the sterol; the metabolites are less active than 1,25-dihydroxyvitamin D₃; some of them may be reused after deconjugation in the intestine. Abnormalities in the excretion of 1,25-dihydroxyvitamin D₃ metabolites can be seen in patients with primary biliary cirrhosis in whom less radioactivity is excreted in bile and proportionately more in the urine (99). These individuals have low parathyroid hormone levels, perhaps as a result of the retention of the 1,25-dihydroxyvitamin D₃ and accompanying transient hypercalcemia that turns off the formation of 1,25-dihydroxyvitamin D₃. This may contribute to the bone disease seen in these individuals.

One can utilize information obtained from the rapid disappearance of 1,25-dihydroxyvitamin D₃ in order to assess the metabolic clearance rates and production rates of 1,25-dihydroxyvitamin D₃ in man (73,100). We have performed such measurements using both bolus injections of ³H-1,25-dihydroxyvitamin D₃ and constant infusion experiments. Similar experiments have also been performed with 24,25-dihydroxyvitamin D₃ (101). The results of these experiments are shown in Table 2.

1,25-DIHYDROXYVITAMIN D ACTION IN CALCIUM TRANSPORTING TISSUES

The transport of calcium across the enterocyte or the distal tubular cell requires energy and sodium ions (Figure 5). The entry of calcium into the enterocyte is down a concentration and electrical gradient; although it is facilitated by vitamin D, it does not require the expenditure of energy (102,103). The energy requiring step is at the basolateral membrane where calcium is pumped out of the cell against a concentration and electrical gradient, most likely by a calcium pumping ATPase. Calcium-sodium exchangers may also play a role in this process. Within a cell, calcium is sequestered by various organelles and is bound to different calcium binding proteins, the major one of which is the vitamin D-dependent intestinal calcium binding protein.

1,25-dihydroxyvitamin D₃ brings about its effects in the intestine and, very likely, in the distal tubule, at least in part by a receptor-mediated mechanism that influences the expression of various genes and the activity of several enzymes and proteins (104–176). Receptors for the hormone have been characterized with respect to their physical characteristics, ligand-binding properties, and amino acid sequences (104–130). Protein synthesis is important in bringing about the actions of 1,25-dihydroxyvitamin D₃ in the intestine, although contrary views exist (131–135). The latter view conceives the role of 1,25-dihydroxyvitamin D₃ as one that predominantly enhances the diffusion of calcium ion into the cell across the luminal membrane; this movement is not dependent upon the synthesis of protein. Intestinal lipid metabolism, cyclic nucleotide levels, the activity and synthesis of proteins and enzymes, and the uptake of calcium by Golgi and endoplasmic reticulum are altered by 1,25-dihydroxyvitamin D₃ (136–142).

![Image](image-url)

**Figure 4.** The enterohepatic metabolism of 1,25-dihydroxyvitamin D₃.

**TABLE 2.** Metabolic clearance, production and excretion rates of 1,25-dihydroxyvitamin D₃ and 24,25-dihydroxyvitamin D₃ in normal man

<table>
<thead>
<tr>
<th></th>
<th>MCR (l/day)</th>
<th>PR (µg/day)</th>
<th>Billary Excretion at 6–8 h</th>
<th>Fecal Excretion at 6–7 days</th>
<th>Urinary Excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,25(OH)₂D₃</td>
<td>44.6 ± 5.7</td>
<td>~1.5</td>
<td>15.6 ± 1%</td>
<td>54 ± 6%</td>
<td>14 ± 2% (6-day)</td>
</tr>
<tr>
<td>24,25(OH)₂D₃</td>
<td>9.2 ± 1.5</td>
<td>26.4 ± 7.2</td>
<td>15.3 ± 1.3</td>
<td>48.8 ± 2.7</td>
<td>7.4 ± 1.8% (2-day)</td>
</tr>
</tbody>
</table>

Journal of the American Society of Nephrology
CALCIUM MOVEMENT ACROSS EPITHELIUM

This occurs in the following manner:
1. Movement of calcium from the luminal fluid into the cell (down an electrochemical gradient) occurs via calcium channels.
2. Calcium entering the cell is buffered or sequestered by various cellular proteins or organelles.
3. Finally, calcium is extruded at the basolateral membrane of the cell by a calcium pump (the membrane Ca\(^{2+}\)-ATPase) or by a calcium-sodium exchange process.
4. Paracellular pathways, though strictly not under the rubric of "transcellular pathways", also may be responsible for movement of significant amounts of calcium across tissues.

Figure 5. Calcium movement across epithelium.

VITAMIN D-DEPENDENT CALCIUM BINDING PROTEINS

Physiology and Structure of CaBPs

As noted earlier, in the intestine and in the distal tubule of the kidney, 1,25-dihydroxyvitamin D\(_3\) increases and sometimes decreases the synthesis of various proteins and alters the activity of various enzymes. In some instances, these changes are related to active intestinal calcium transport in vivo. The most notable of the proteins whose synthesis is altered by 1,25-dihydroxyvitamin D is the vitamin D-dependent CaBP (143–144); the appearance of the protein in the intestine is temporarily related to increases in calcium transport. Careful experiments have shown that CaBP is regulated by vitamin D in intestinal tissue and that it does not act merely as a calcium buffer (145). Recent work has shown that CaBP enhances the diffusion of calcium across the cellular compartment (163,164). The synthesis of vitamin D-dependent CaBPs appears to be under transcriptional and nontranscriptional controls (165–171).

There is considerable information available about the structure of CaBPs and their distribution in tissues (143–151,172,173). As noted in Table 3, CaBP exists in two forms. There is a ~9 kDa CaBP (~75 amino acids) present in the intestine and placenta of mammalian tissues; it is also found in mouse kidney (172,173). In addition, a ~28 kDa CaBP (~260 amino acids) is present in the intestine and other tissues of avian species, and in the brain and distal tubule of mammalian species (172,173). The amino acid sequences of porcine, murine and bovine intestinal CaBP are similar (148,150,174–176). The amino acid sequences of the 28-kDa CaBPs are also similar, regardless of whether these proteins are derived from chicken intestine, rat brain, or from human tissues (177–184). Data concerning the structure of these proteins and their genes demonstrates that they are related to other calcium binding proteins of the troponin-C superfamily of proteins (179–190).

Biophysical Properties of CaBPs

Recently several laboratories, including our own, have examined the biophysical properties of CaBPs in order to obtain information on the manner in which they bind calcium and to gain insights into how they might act as trigger proteins or facilitate the movement of calcium across cells. The 9-kDa bovine intestinal CaBP binds two moles of calcium, whereas the chick intestinal CaBP binds four moles of calcium per mole of protein (191,192). Both the 9-kDa and 28-kDa vitamin D induced CaBPs bind calcium within "E-F hand" structures. These are helix-loop-helix structures, initially described by Kretsinger and colleagues, that bind calcium in the loop region in association with oxygen containing amino acids (191–195). The 9-kDa CaBP has two E-F hand structures, whereas the 28-kDa CaBP has six (174,181–190,196–198).

We examined the mechanism of calcium and lanthanide binding of the 28-kDa CaBP in order to determine how this protein binds calcium (198). We demonstrated that the chick intestinal CaBP binds three moles of terbium (a lanthanide resembling calcium) with high affinity, another mole of terbium with relatively high affinity, and several other molecules of terbium with low affinity. Calcium binding occurs at two classes of sites; furthermore, the protein undergoes a conformational change upon binding calcium. Similar conformational changes occur with the binding of calcium to human kidney CaBP (199). We have confirmed, by NMR, that the conformation of the 28-kDa CaBP is controlled by calcium (200). Thus it appears that these proteins undergo a structural change upon binding calcium; in this respect they appear to resemble calmodulin, a known regulator of the plasma membrane calcium pump that moves calcium from within the cell into the extracellular fluid. CaBPs thus assist in moving calcium to the basolateral membrane of the cell where they may also activate plasma membrane calcium pumps.

RELATIONSHIPS BETWEEN CALCIUM BINDING PROTEIN AND INTESTINAL CALCIUM PUMPS

1,25-dihydroxyvitamin D\(_3\) increases calcium movement from within the cell to the extracellular fluid (201,202). This is due to an increase in the activity of a plasma membrane calcium pump found in the basolateral membrane of intestinal cells. Just how 1,25-dihydroxyvitamin D\(_3\) alters the activity of this pump is a matter of considerable interest. It is clear, based on some of the structural data of the calcium binding proteins and their biophysical behavior upon binding to calcium, that these proteins resemble calmodulin. As calmodulin is a major regulator of calcium pumps, several groups of investigators have attempted to determine whether vitamin D-dependent calcium binding proteins directly alter calcium
pump (calcium-magnesium ATPase) activity. Reports by Morgan et al. (203) and by Walters (204) show that vitamin D-dependent calcium binding proteins influence the activity of the plasma membrane calcium pump. Others, including ourselves, have been unable to show a direct effect of calcium binding protein upon calcium-magnesium ATPase activity (205–208). Nevertheless, the possibility that vitamin D-dependent calcium binding proteins influence calcium pumps directly or indirectly is an attractive hypothesis. We have taken the alternative route of trying to determine whether the vitamin D-dependent calcium binding proteins and the calcium pump co-localize in different cells that transport calcium.

Human calcium transporting tissues were examined to determine whether they contained a protein similar to the Ca\(^{2+}\)-Mg\(^{2+}\) adenosine triphosphatase (Ca\(^{2+}\)-Mg\(^{2+}\) ATPase) pump of the human erythrocyte membrane (209). Tissues were processed for immunoperoxidase staining using monoclonal antibodies against purified calcium pump. In human kidneys, specific staining was found only along the basolateral membrane of the distal convoluted tubules. Glomeruli and other segments of the nephron did not stain. To determine whether vitamin D-dependent 28-kDa calcium binding protein and Ca\(^{2+}\)-Mg\(^{2+}\) ATPase are present in the same cells of the human kidney, tissue was examined for immunoreactivity with antibodies.
directed against these proteins (210). A majority of the distal convoluted tubules contain epitopes to both of these proteins. All other portions of the nephron are negative for both proteins. Western blot analysis of kidney homogenates confirms the presence of calcium pump and 28-kDa CaBP. Taken together, these findings suggest a possible role for vitamin D-dependent 28kDa-CaBP and Ca\(^{2+}\)-Mg\(^{2+}\) ATPase in a calcium transport mechanism in the human kidney distal tubule. We have developed a new monoclonal antibody (5F10) directed against the human erythrocyte plasma membrane calcium pump that recognizes a protein of \(\approx\)140 kDa in rat kidney homogenates. Antibody 5F10 binds to an epitope in the basolateral membranes of rat kidney distal convoluted tubule principal cells. Polyclonal antibodies directed against chick intestinal 28-kDa vitamin D-dependent calcium binding protein (28-kDa CaBP) also bind epitopes in distal convoluted tubule cells, connecting tubules, and portions of collecting duct, but not intercalated cells. We have also found calcium-pump antigens in the rat duodenum, jejenum, and ileum (211). Calcium pump quantity appears to correlate with active calcium transport in different intestinal segments.

Immunohistochemistry with a calcium-pump antibody shows specific staining of the basal (fetal facing) surface of the human placental syncytiotrophoblast (212). In the rat placenta, immunohistochemistry demonstrates specific staining of the innermost (fetal facing) layer of the trophoblast and the basal surface of the endoderm of the intraplacental yolk sac. In Western blot of placental homogenates and membranes, the monoclonal antibody binds to a 140-kDa band, characteristic of calcium pumps in other tissues. In addition, calcium transport in basal membrane vesicles from human placenta is inhibited by polyclonal antibodies prepared against the erythrocyte calcium pump. The human placenta is known to contain a 9-kDa vitamin D-dependent CaBP. Thus calcium pump and CaBP appear to co-localize in the rat and human placenta.

Human osteoblast-like cells also have a calcium pump in the plasma membrane (213). Immunocytochemical staining of the osteoblast-like cells with a monoclonal antibody against human red cell Ca\(^{2+}\)-Mg\(^{2+}\) ATPase demonstrates the presence of an epitope of the Ca\(^{2+}\)-Mg\(^{2+}\) ATPase in the plasma membrane of these cells. Western blot analysis of osteoblast-like cell homogenates shows that the monoclonal antibody binds to a major band of Mw, 140,000. The presence in the osteoblast-like cells of a Ca\(^{2+}\)-Mg\(^{2+}\) ATPase similar to the human red cell calcium pump suggests that this enzyme may play a role in osteoblast intracellular calcium homeostasis.

Finally, we found a protein of \(\approx\)140 kDa in chroid plexus plasma membranes that forms a phosphorylated intermediate with characteristics of a plasma membrane calcium pump (214). A choroid plexus plasma membrane protein of this molecular weight also binds to a monoclonal antibody prepared against the human erythrocyte plasma membrane Ca\(^{2+}\)-Mg\(^{2+}\) ATPase calcium pump. The plasma membrane calcium pump is found primarily in the CSF-facing membranes of choroid plexus cells from rats, cats, and man. The localization of a plasma membrane calcium pump in the CSF-facing membranes of the choroid plexus suggests that the choroid plexus, by mechanisms including this pump, may regulate CSF calcium concentrations.

These observations show that calcium pump and CaBPs are present together in most calcium transporting cells, suggesting that they may be part of a calcium transporting system. 1,25-dihydroxyvitamin D\(_3\) thus influences calcium transport by altering calcium uptake at the luminal membrane of calcium transporting cells; the hormone induces the synthesis of CaBPs that enhance calcium diffusion across cells. Finally, 1,25-dihydroxyvitamin D\(_3\) increases plasma membrane calcium pump activity; this increase occurs either due to a direct effect of the hormone on the pump or to an effect of the vitamin D-induced CaBPs upon the pump. Further examination of the mechanism of 1,25-dihydroxyvitamin D\(_3\) induced calcium transport should reveal interesting information about how the cell moves calcium from the organ lumen through the cell into the extracellular fluid and blood.

REFERENCES

11. Nicolaysen R: Studies upon the mode of action of vitamin D. II. The influence of vitamin D on the faecal...

Journal of the American Society of Nephrology 37


151. Christakos S, Friedlander EJ, Frandsen BR, Norman AW: Studies on the mode of action of calciferol. XIII. Development of a radioimmunoassay for...


