Rapid, Membrane-Initiated Actions of 1,25 Dihydroxyvitamin D: What Are They and What Do They Mean?\(^1,2\)

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**ABSTRACT** Vitamin D is a conditionally required nutrient traditionally thought to influence physiology as the metabolite 1,25-dihydroxyvitamin D \([1,25(\text{OH})_2 \text{D}]\) by binding to the vitamin D receptor (VDR) and stimulating the transcription of genes through direct VDR-DNA interactions. However, over the past 15 y research has demonstrated that 1,25(OH)\(_2\)D, as well as other steroid hormones, can rapidly stimulate ion fluxes and activate protein kinases by transcription-independent mechanisms. This review summarizes recent research on the rapid actions of 1,25(OH)\(_2\)D and identifies questions that remain to be answered in this area. J. Nutr. 134: 3215–3218, 2004.

**KEY WORDS:** kinase • ion flux • vitamin D

Vitamin D has been viewed as a prohormone produced in the skin that regulates calcium metabolism after metabolic conversion in the liver and kidney to its hormonally active form, 1,25-dihydroxyvitamin D \([1,25(\text{OH})_2 \text{D}]\).\(^4\) When 1,25(OH)\(_2\)D interacts with the vitamin D receptor (VDR) it induces heterodimerization of VDR with the retinoid X receptor (RXR), DNA binding of the heterodimer to vitamin D response elements, and recruitment of various coactivators leading to the enhanced transcription of genes whose protein products control calcium homeostasis \(e.g.,\) transient receptor potential vanilloid-type family member 6; \(\text{TRPV6}\); osteocalcin; 25 hydroxyvitamin D; 24 hydroxylase (\(\text{CYP24A1}\)) (1). However, this model is being expanded by a variety of observations. First, vitamin D insufficiency has been observed in various subpopulations, which suggests that vitamin D is a required nutrient for some individuals \(e.g.,\) dark-skinned individuals, the elderly, and people who receive limited sunlight exposure (2). Second, the inverse association of vitamin D status with the occurrence of a wide variety of chronic diseases \(e.g.,\) cancer, diabetes demonstrates that controlling calcium metabolism represents only a portion of the biological actions of vitamin D (2). Third, the identification of extrarenal 1α hydroxylases suggests that 1,25(OH)\(_2\)D may act as an autocrine or paracrine signal in addition to its traditional endocrine role (3). Finally, rapid, transcription-independent events have been observed in response to physiologic levels of hormone. This suggests that a more complex mechanism mediates vitamin D action than that represented in the traditional ligand-activated transcriptional model.

Regarding this last point, there is now compelling evidence for the existence of 1,25(OH)\(_2\)D-inducible signal transduction pathways within various cell types. The history of this field has been previously reviewed in other reports and interested readers are encouraged to refer to those reviews (4,5) and to early reports of historical interest (6–9). However, a brief summary of this work shows that 1,25(OH)\(_2\)D rapidly \(\text{(within seconds and minutes)}\) stimulates events normally associated with the activation of membrane receptors for growth factors and peptide hormones. These include: 1) phospholipase C (PLC) and phospholipase D activity; 2) phosphoinositol turnover leading to the generation of the second messengers inositol 1,3,4-triphosphate (\(\text{IP}_3\)) and 1,2-diacylglycerol (\(\text{DAG}\)); 3) intracellular calcium by increasing calcium uptake and the release of intracellular calcium stores; 4) adenylylate cyclase activity to increase cAMP levels and stimulate protein kinase A (PKA) activity; 5) calcium-dependent protein kinase C (PKC) isoform activity \((\alpha, \beta, \delta)\) and cellular redistribution; and 6) Jun activated kinase and extracellular response activated kinase (ERK) mitogen activated protein kinase (MAPK) family activation (4,5,10). The effect of 1,25(OH)\(_2\)D treatment on signal transduction pathways may depend on adequate vitamin D status; rapid 1,25(OH)\(_2\)D-induced changes in phosphoinositol turnover, PKC translocation, and changes in intracellular calcium do not occur in colonocytes from vitamin D-deficient, hypocalcemic rats (11). Rapid activation of signal transduction pathways has also been observed for the other steroid hormones, e.g., estrogen, androgen, glucocorticoids (12). This suggests that viewing vitamin D \(\text{(and other steroid hormones)}\) biology through the prism of the traditional, nuclear receptor-mediated transcriptional responses is limited.

In this short review, I summarize some of the recent work that sheds light on the mechanism of how the rapid actions of 1,25(OH)\(_2\)D are initiated at the plasma membrane. I try to put these responses into a physiologic context, and I identify the questions that need to be answered by future research.

**Initiation of Rapid Actions at the Plasma Membrane.** One debate that has occupied scientists within this area is how the rapid signaling process is initiated. Is there a unique receptor for the 1,25(OH)\(_2\)D at the plasma membrane that functions similarly to the growth factor and peptide hormone receptors (i.e., membrane spanning with inherent kinase activity)? Or is rapid signaling a unique role for the traditional VDR? The idea that there might be a unique membrane receptor for 1,25(OH)\(_2\)D...
In addition to localization of the MARRS protein at the plasma membrane due to the presence of a myristoylation sequence in the protein, sequence analysis shows that membrane association is more likely localization limited to the plasma or basolateral membrane. Se-osteoblasts), and activation of PKC (16,17). Surprisingly, this phosphate (in the chick enterocyte), intracellular calcium flux (in osteoblasts), and activation of PKC (16,17). Surprisingly, this protein has a role in 1,25(OH)2D-stimulated uptake of phosphate (in the chick enterocyte), intracellular calcium flux (in osteoblasts), and activation of PKC (16,17). Surprisingly, this protein is not a traditional membrane spanning receptor nor is its localization limited to the plasma or basolateral membrane. Sequence analysis shows that membrane association is more likely due to the presence of a myristoylation sequence in the protein. In addition to localization of the MARRS protein at the plasma membrane, it is also found at the endoplasmic reticulum, and 1,25(OH)2D treatment induces a redistribution of the MARRS protein from these sites to the nucleus (18). While this doesn’t fit our classic description of membrane receptors that mediate signal transduction pathways, data from Schwartz et al. (19) indicate that MARRS has a VDR-independent action on PKC activation in matrix vesicles of growth zone chondrocytes. In these nucleus-free vesicles involved in cartilage calcification, Schwartz et al. found that 1,25(OH)2D activates PKC α by activating PLC β1 and β3 through the G-protein Gq. These matrix vesicles did not contain the traditional VDR and the effect of 1,25(OH)2D was inhibited by an antibody against the MARRS protein. Similarly, the rapid activation of PLC and PKC by 1,25(OH)2D that is normally observed in growth zone chondrocytes was not reduced in cells from VDR null mice (20). While this demonstrates that at least some of the plasma membrane initiated actions of 1,25(OH)2D are mediated through MARRS, it is not yet clear whether MARRS directly interacts with G-proteins or other mediators of signal transduction pathways.

**Evidence for the Traditional VDR.** Even as evidence on the importance of the MARRS protein has accumulated, there is growing direct evidence that the traditional VDR may also have a unique, nontranscriptional role in mediating plasma membrane initiated signaling. The most critical evidence supporting this model is that several groups have demonstrated that 1,25(OH)2D-induced rapid actions are lost in osteoblasts from VDR knock-out mice (21) or fibroblasts from patients with type II rickets (9,22). In addition, Huhtakangas et al. (23) recently used biochemical methods and immunodetection to identify VDR within caveoli in a variety of cell types (e.g., intestine, kidney, lung, leukemia cells, and osteoblast-like cells). While others had previously observed a 1,25(OH)2D-induced translocation of VDR to the plasma membrane of skeletal muscle cells (24), this was the first report of VDR associated with the lipid raft-rich areas of the plasma membrane where the caveoli protein caveolin is known to interact with the nonreceptor tyrosine kinase Src, the G-protein Gα subunits, and the central kinase h-Ras (25). Thus VDR is in close proximity to essential components of the signal transduction system.

The existence of VDR at the plasma membrane and the activation of ion fluxes and kinase responses following 1,25(OH)2D treatment suggest that the VDR will interact with G-proteins and nonreceptor tyrosine kinases that are proximal mediators of signal transduction pathways. However, at this time there is no direct evidence for the interaction of VDR with G-proteins. In contrast, Buitrago et al. (26) have shown that activation of the nonreceptor tyrosine kinase Src coincides with a 1,25(OH)2D-induced interaction between Src kinase and VDR in chick muscle cells. This is consistent with an earlier report in human keratinocytes showing 1,25(OH)2D-induced Src kinase activation, stimulation of interactions between Src- and the signaling adapter protein Shc that lead to phosphorylation of Shc, and formation of a complex including the Shc adapter, VDR, a second signaling adapter protein (Grb2), and the Ras kinase activator mSos in cells grown in high calcium medium (27,28). Activation of Src and the formation of these complexes are known to be proximal steps that can lead to the activation of the MAPK ERK1/2 (29) and phosphatidylinositol 3 kinase (30).

The interaction between Src and VDR is likely mediated through phosphorylation of an essential tyrosine residue between amino acids 160 and 174 on the chick VDR that permits interaction with the SH2 domains in Src (a putative tyrosine phosphorylation site at position 147 is conserved in both human and mouse VDR but phosphorylation of that site has not yet been confirmed). A recent study by Barletta et al. (31) shows that in addition to Src-estrogen receptor (ER) interactions through the SH2 domain of Src, ERα uses a protein called modulator of nongenomic activity of the estrogen receptor (MNAR) as a docking protein to the SH3 domain of Src. Since MNAR binds to ERα through the same LXXLL amino acid motif that the p160, p300, and mediator family of transcriptional coactivators use to bind steroid hormone receptors (including VDR), this may be a common protein used by all steroid hormone receptors during membrane-initiated signaling. This model and a summary of the downstream events initiated by rapid vitamin D signaling are presented in Figure 1.

**What Physiologic Role Do Membrane-Initiated Actions of 1,25(OH)2D Serve?** Although the phenomenon of rapid, membrane initiated 1,25(OH)2D signaling is becoming better understood, the question of what physiologic role the rapid actions serve is less clear. As I have already mentioned, the clearest case for a physiologic role of rapid vitamin D signaling comes from chondrocyte biology where matrix vesicle mineralization and function can be modulated by MARRS-dependent stimulation of calcium fluxes and PKC activity (32). Norman’s group (33) has also made the case that a rapid, 1,25(OH)2D-induced transcellular flux of calcium across the intestine that they have termed “transcalcitachia” is another physiologic manifestation of rapid vitamin D action. However, rapid fluxes of 1,25(OH)2D have not yet been observed associated with meal feeding (when calcium would be present in the intestine and available for absorption) so their observations are currently hard to reconcile with other aspects of calcium absorption physiology.

Two other processes are strong candidates for regulation by rapid, membrane initiated signaling. The first is cell proliferation. Bettouin et al. (34) recently reported that in the intestinal cell line Caco-2, VDR is associated with the catalytic subunit of the protein phosphatases PP1c and PP2Ac and that ligand binding induces the activity of these phosphatases. This activation results in the phosphorylation and inactivation of p70S6 kinase, an enzyme that is crucial for the G1-S transition in the cell cycle. This represents an early step in the growth inhibitory actions of
vitamin D that is likely followed by VDR-mediated transcrip-
tional activation of genes like the cyclin-dependent kinase-in-
hibitor p21 (35) and the insulin-like growth factor 1 antagonist,
IGF-binding protein 3 (36). Another possible role for rapid 1,25(OH)2 D signaling may be
to optimize the genomic actions of the hormone that are medi-
ated through the VDR. For example, pharmacologic suppression
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Major Remaining Questions. Although the area of mem-
brane-initiated vitamin D action has advanced dramatically in
the past 15 y, there are still a number of important questions and
issues that need to be resolved. First and foremost, the membrane
initiated signaling system needs to be more extensively charac-
terized in a wider variety of cell types and species. The current
model exists only by combining pieces of data from multiple
species and multiple cell types. The most complete data currently
comes from chick muscle cells and from rat chondrocytes.
Because several groups have reported opposing actions of 1,25(OH)2
D on endpoints like MAPK activation [e.g., activation in human
Caco-2 (10)], inhibition in tumor-derived mouse endothelial cells
(49)], understanding cell type, cell stage, and species differences
may be critical to a complete understanding of the role of
1,25(OH)2 D signaling in health. Beyond simple characterization
efforts, we also need to better understand whether rapid vitamin
D actions influence unique physiologic processes (e.g., as sug-
gested by data from chondrocytes) or whether the traditional
VDR is a critical component of both the rapid and the transcrip-
tional actions of 1,25(OH)2 D. The data from VDR null mice
demonstrate that the VDR is critical for intestinal calcium ab-
sorption and hair follicle development (50,51). However, the
possibility that rapid vitamin D actions modify these and other
processes has not yet been excluded. In a similar vein, what role
do rapid vitamin D actions have when the current evidence
suggests that serum 1,25(OH)2 D levels are stable and adapt over
the course of hours and days rather than minutes and seconds
(52)? Have we not looked at this issue in fine enough detail or
could local production of 1,25(OH)2 D and paracrine/autocrine
signaling (which may not be observed systemically) be a critical
component of rapid vitamin D signaling? These and other ques-
tions will continue to drive this field for the foreseeable future.

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