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Membrane-mediated Actions of 1,25-Dihydroxy Vitamin D₃: A Review of the Roles of Phospholipase A₂ Activating Protein and Ca²⁺/Calmodulin-dependent Protein Kinase II

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Abstract

The secosteroid 1 α ,25-dihydroxy vitamin D₃ [1 α ,25(OH)₂D₃] acts on cells via classical steroid hormone receptor-mediated gene transcription and by initiating rapid membrane-mediated signaling pathways. In its membrane-initiated pathway, after 1 α ,25(OH)₂D₃ interacts with protein disulfide isomerase, family A, member 3 (Pdia3) in caveolae, phospholipase A₂ (PLA₂) and protein kinase C (PKC) are activated. Recent efforts to determine the signaling proteins involved in the 1 α ,25(OH)₂D₃ signal from Pdia3 to PLA₂ have indicated that phospholipase A₂ activating protein (PLAA) and Ca²⁺/calmodulin-dependent kinase II (CaMKII) are required. PLAA is located in caveolae, where it interacts with Pdia3 and caveolin-1 (Cav-1) to initiate rapid signaling via CaMKII, activating PLA₂, leading to activation of protein kinase C (PKC) and PKC-dependent responses.

Keywords

1 α ,25(OH)₂D₃; PDIA3; PLA₂; PLAA; Protein Kinase C; Ca²⁺/calmodulin-dependent protein kinase II

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1. Introduction

The vitamin D metabolite $1\alpha,25$ -dihydroxy vitamin D3 [$1\alpha,25(\text{OH})_2\text{D}_3$] is known to play an important role in controlling extracellular Ca^{2+} homeostasis, regulating development of the cartilaginous growth plate and modulating bone growth and metabolism (1–5). $1\alpha,25(\text{OH})_2\text{D}_3$ regulates musculoskeletal cells via two different mechanisms: classical steroid hormone receptor dependent gene transcription, and rapid membrane-initiated signaling (6–8). $1\alpha,25(\text{OH})_2\text{D}_3$ selectively interacts with two identified receptors, the nuclear vitamin D receptor (VDR) and membrane-associated protein disulfide isomerase, family A, member 3 (Pdia3), to modulate cell functions via membrane-associated signaling pathways (9).

We have used cartilage and bone cell models to understand how Pdia3 mediates rapid responses to $1\alpha,25(\text{OH})_2\text{D}_3$. Growth zone chondrocytes (GC) isolated from the rat costochondral growth plate and MC3T3-E1 mouse osteoblasts respond to $1\alpha,25(\text{OH})_2\text{D}_3$ with a rapid increase in activities of phospholipase A₂ (PLA₂), phospholipase C β (PLC β) and protein kinase C α (PKC α) and PGE₂ release (10,11).

Pdia3 is present in caveolae and caveolae are required for the membrane functions of $1\alpha,25(\text{OH})_2\text{D}_3$ (12). The caveolae plasma membrane microdomains are characterized by presence of caveolin-1 (Cav-1), caveolin-2 (Cav-2), and caveolin-3 (Cav-3). GC chondrocytes isolated from Cav-1 knockout (Cav-1^{-/-}) mice and Cav-1 silenced osteoblasts are not capable of initiating the rapid PKC activation response to $1\alpha,25(\text{OH})_2\text{D}_3$ (12).

Figure 1 summarizes our findings demonstrating the critical role Pdia3 and the caveolae microenvironment in mediating the effects of $1\alpha,25(\text{OH})_2\text{D}_3$ on PKC. Rapid actions of $1\alpha,25(\text{OH})_2\text{D}_3$ in MC3T3-E1 cells can be blocked by antibodies to Pdia3 (data not shown), or by silencing either Pdia3 or Cav-1. However, rapid activation of PKC is seen in VDR-silenced MC3T3-E1 cells, indicating that the VDR is not required.

We have reported that PLA₂ activating protein (PLAA) stimulates PLA₂ activation. Moreover, the effects of PLAA mimic the effects of $1\alpha,25(\text{OH})_2\text{D}_3$ treatment on GC chondrocytes and MC3T3-E1 osteoblasts, suggesting that PLAA acts upstream of PLA₂ in $1\alpha,25(\text{OH})_2\text{D}_3$ membrane-mediated signaling (11,13). Recent studies also demonstrated that activation of Ca^{2+} /calmodulin-dependent kinase II (CaMKII) is required for the actions of PLA₂ (14). Collectively, these data indicate that PLAA and CaMKII are required for $1\alpha,25(\text{OH})_2\text{D}_3$ rapid membrane-mediated signaling but they don't clarify the relationship between PLAA and CaMKII. This review will focus on the role of PLAA in mediating $1\alpha,25(\text{OH})_2\text{D}_3$ -stimulated Pdia3-dependent events at the plasma membrane. It will also demonstrate the importance of CaMKII in mediating rapid activation of PLA₂ in response to $1\alpha,25(\text{OH})_2\text{D}_3$. Finally, this review will provide crucial evidence that $1\alpha,25(\text{OH})_2\text{D}_3$ stimulates PLA₂ via PLAA and CaMKII, a process initiated by Pdia3/PLAA interaction, which further triggers CaMKII-dependent PLA₂ activation.

2. Role of PLAA

There is now a considerable body of evidence indicating that PLA₂ plays an important role in $1\alpha,25(\text{OH})_2\text{D}_3$ -activated membrane-mediated signaling (13,15–18). Melittin and

mastasparan are stimulators of PLA₂ that have exhibited similar effects to those of 1 α , 25(OH)₂D₃ on PLC β and PKC α activation (19). In contrast, AACOCF₃, OEPC and quinacrine, which inhibit PLA₂ activity, antagonize the stimulatory effects of 1 α , 25(OH)₂D₃, placing activation of PLA₂ upstream of PLC β and PKC α (11,19). Despite accumulating experimental evidence indicating the activation of PLA₂ as one of the early events in 1 α , 25(OH)₂D₃-activated membrane-mediated signaling, the signaling molecules linking Pdia3 to PLA₂ remained unidentified.

Human PLAA was reported to have a region of 38% homology with melittin (20), raising the possibility that it was responsible for activation of PLA₂ in response to 1 α , 25(OH)₂D₃. To test this hypothesis, we first determined that GC chondrocytes express mRNAs for PLAA (13). The presence of PLAA mRNA in cells sensitive to 1 α , 25(OH)₂D₃ was demonstrated in the growth zone of rat growth plates by in situ hybridization (13). We subsequently showed that PLAA interacts with Pdia3 receptor complex (21).

To elucidate the role of PLAA in rapid actions of 1 α , 25(OH)₂D₃, we studied the effects of Plaa silencing in osteoblastic MC3T3-E1 cells. Silencing Plaa led to a significant reduction in CaMKII and PLA₂ activity in response to 1 α , 25(OH)₂D₃ treatment (21) (Fig. 2). PLA₂ activation generates arachidonic acid (AA), which is processed further to PGE₂ (19,22). As expected, ShPlaa MC3T3-E1 osteoblasts failed to rapidly release PGE₂ in response to 1 α , 25(OH)₂D₃ (21). AA can also stimulate PKC α activity directly (23). Additionally, G α q and lysophospholipid activate phosphatidylinositol-specific PLC β , producing diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3) (19,24). The binding of DAG to PKC α induces its recruitment to the plasma membrane (25). IP3-activated Ca²⁺ release from the endoplasmic reticulum is required for PKC α activation. Similar to our previous findings, shPlaa osteoblasts also failed to activate PKC in response to 1 α , 25(OH)₂D₃ (21). In agreement with results of studies using ShPlaa cells, PLAA-antibody blocked the actions of PLAA protein and consequently suppressed 1 α , 25(OH)₂D₃-stimulated PKC activation in GC and MC3T3-E1 cells (21).

Figure 2 summarizes our findings with respect to the role of PLAA in the rapid response of osteoblasts to 1 α , 25(OH)₂D₃ treatment. Silencing Plaa in MC3T3-E1 cells prevents activation of CaMKII, PLA₂ and PKC, as well as release of PGE₂. Moreover, our biochemical studies confirmed the subcellular localization of PLAA and its protein interacting partners. PLAA and Pdia3 receptor were present in plasma membranes and caveolae of GC cells and MC3T3-E1 cells (21). Immunoprecipitation studies showed that PLAA's interaction with Cav-1 did not change with 1 α , 25(OH)₂D₃ treatment; however, Pdia3 interacted with PLAA only after 1 α , 25(OH)₂D₃ treatment (21). This finding supports the hypothesis that PLAA is a crucial mediator of 1 α , 25(OH)₂D₃ signaling, which aids in transducing the signal from Pdia3 to PLA₂.

3. Role of CaMKII

Although we demonstrated that the rapid response to 1 α , 25(OH)₂D₃ requires the presence of PLAA (21), the mechanism underlying how PLAA mediated the signal from Pdia3 to PLA₂ remained unanswered. 1 α , 25(OH)₂D₃ mediates its actions via a cascade of Ca²⁺-dependent

pathways in which Ca^{2+} is released from intracellular stores or basal lateral membrane Ca^{2+} channels (6,26–28). The rise in intracellular Ca^{2+} is associated with activation of Ca^{2+} sensitive kinases including PKC, the ERK1/2 family of mitogen activated protein kinases (MAPK), and CaMKII. Recently, CaMKII was identified as one of the regulators of PLA_2 activity (14). The CaMKII inhibitor, KN-93 and CaMKII antisense oligonucleotide suppressed norepinephrine-stimulated PLA_2 activation and arachidonic acid release in vascular smooth muscle cells. This led us to hypothesize that CaMKII is a likely candidate for mediating the $1\alpha,25(\text{OH})_2\text{D}_3$ signal from PLAA to PLA_2 .

To investigate the role of CaMKII in rapid actions of $1\alpha,25(\text{OH})_2\text{D}_3$, we studied the effects of silencing Camk2a, the gene encoding CaMKII isoform α in osteoblastic MC3T3-E1 cells (29). Silenced Camk2a (ShCamk2a) MC3T3-E1 cells failed to rapidly activate CaMKII in response to $1\alpha,25(\text{OH})_2\text{D}_3$ (29). In contrast, silencing Camk2b, which encodes the CaMKII isoform β did not significantly reduce CaMKII activity in response to $1\alpha,25(\text{OH})_2\text{D}_3$ (data not shown). Collectively, these findings suggest that CaMKII mediates $1\alpha,25(\text{OH})_2\text{D}_3$ signal in an isoform-specific manner. Silencing Camk2a significantly reduced PLA_2 and PKC activities and PGE_2 release in response to $1\alpha,25(\text{OH})_2\text{D}_3$. In agreement with these results, the CaMKII inhibitors myristoylated calmodulin kinase IINtide (mer-CaMKIINtide) and KN-93 abrogated $1\alpha,25(\text{OH})_2\text{D}_3$ -stimulated PLA_2 and PKC activities and PGE_2 release (data not shown) (29–31).

The experiments summarized in Figure 3 demonstrate the important role of CaMKII in the signaling pathway initiated by $1\alpha,25(\text{OH})_2\text{D}_3$. Silencing Camk2a prevents activation of PLA_2 and PKC, as well as the release of PGE_2 . These experiments also implicate CaMKII in mediated the signal from PLAA to PLA_2 , as depicted in Figure 4 and discussed below.

4. Overview of $1\alpha,25(\text{OH})_2\text{D}_3$ Membrane-mediated Pathway

The studies reviewed here show the roles of PLAA and CaMKII in initiating rapid actions of $1\alpha,25(\text{OH})_2\text{D}_3$ at the plasma membrane (Figure 4). The binding of $1\alpha,25(\text{OH})_2\text{D}_3$ to the Pdia3 receptor stimulates the interaction between PLAA and Pdia3 protein complexes in caveolae microdomains, leading to activation of CaMKII. Subsequently, PLA_2 is activated initiating rapid release of AA. AA mediates its effects activating PKC directly, or it is further metabolized by cyclooxygenase I to producing PGE_2 , which binds its EP1 receptor resulting in downstream activation of PKC.

5. CONCLUSIONS

$1\alpha,25(\text{OH})_2\text{D}_3$ membrane-activated responses are mediated by PLAA, which is present in caveolae where it physically interacts with Pdia3 to initiate rapid responses. CaMKII isoform α is required for mediating the rapid actions of $1\alpha,25(\text{OH})_2\text{D}_3$ from PLAA to PLA_2 . Both PLAA and CaMKII play crucial roles in mediating the Ca^{2+} -dependent actions of $1\alpha,25(\text{OH})_2\text{D}_3$ suggesting their possible fundamental role in mediating other Ca^{2+} -dependent pathways. This review has focused on rapid activation of the PKC signaling pathway, which did not require VDR. However, VDR is involved in other rapid actions of the secosteroid, particularly with respect to Ca^{++} transport across the cell membrane (32).

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Highlights

- Silencing Camk2a inhibits $1\alpha,25(\text{OH})_2\text{D}_3$ -stimulated PLA2 and PKC activation.
- Silencing Plaa inhibits $1\alpha,25(\text{OH})_2\text{D}_3$ -stimulated CaMKII, PLA2 and PKC activation.
- $1\alpha,25(\text{OH})_2\text{D}_3$ membrane signaling acts via rapid activation of PLAA.
- PLAA activates CaMKII, PLA2 and PKC, arachidonic acid release and PGE2 production.

Figure 1.

Effects of Pdia3, Vdr and Cav-1 silencing on 1α,25(OH)₂D₃-stimulated PKC activation in MC3T3-E1 osteoblasts. The data show treatment vs. vehicle ratios. **p*<0.05, vs. vehicle; \$*p*<0.05, vs. 1α,25(OH)₂D₃ treated WT.

Figure 2.

Effects of Plaa silencing on CaMKII, PLA₂ and PKC activation, and PGE₂ release in MC3T3-E1 osteoblast in response to 1 α ,25(OH)₂D₃. The data show treatment vs. vehicle ratios. The dashed line represents the vehicle control (dashed line=1). **p*<0.05, treatment vs. control; #*p*<0.05, WT vs. ShPlaa.

Figure 3.

Effects of Camk2a silencing on CaMKII, PLA₂ and PKC activation, and PGE₂ release in MC3T3-E1 osteoblast cells. The data show treatment vs. vehicle ratios. The dashed line represents the vehicle control (dashed line=1). * $p<0.05$, treatment vs. control; # $p<0.05$, WT vs. ShCamk2a.

Figure 4.
Overview of $1\alpha,25(\text{OH})_2\text{D}_3$ membrane-mediated pathway.