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Nongenomic Actions of the Steroid Hormone 1α,25-Dihydroxyvitamin D_3

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Abstract Recent studies indicate that the vitamin D hormone, 1α,25-dihydroxyvitamin D_3 exerts rapid effects (seconds to minutes) in a variety of cell types. These rapid nongenomic actions in osteoblasts include effects on membrane voltage-gated calcium channels, phospholipase C activity, and the sodium/hydrogen antiport. Since the rapid effects occur in osteoblasts that lack the nuclear vitamin D receptor, it is postulated that the nongenomic responses to the hormone reflect interaction with a separate, membrane localized signalling system. Preliminary studies demonstrate the presence of a receptor on the membranes of osteoblasts that lack the nuclear vitamin D receptor. This membrane receptor recognizes 1α,25-dihydroxyvitamin D_3 and its inaction 1β epimer, but not 25-hydroxyvitamin D_3. These rapid nongenomic actions generated by interaction with the membrane receptor modulate the effects of the hormone on gene transcription. Thus, the rapid nongenomic pathway may play a regulatory function in modulating the genomic pathways affected by 1α,25-dihydroxyvitamin D_3. © 1994 Wiley-Liss, Inc.

Key words: steroid hormone, vitamin D hormone, nongenomic actions, osteoblasts, membrane receptor

1α,25-Dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$) exerts its effects in cells by both genomic and non-genomic mechanisms. The genomic actions of the hormone are mediated by interaction with a nuclear receptor (VDR). The binding of 1α,25-$(OH)_2D_3$ to its receptor, and subsequent interaction of the hormone receptor complex with selected DNA sequences, was originally thought to be the mechanism explaining all of the hormone's actions [DeLuca et al., 1976]. However, since our initial observations that the hormone rapidly increases cellular calcium levels in hepatocytes [Baran et al., 1986] and HL60 cells [Desai et al., 1986], a variety of cell types have been shown to rapidly respond (seconds to minutes) to 1α,25-$(OH)_2D_3$ with increases in cellular calcium and pH, and alterations in phospholipid metabolism. The rapidity of these responses was the first evidence to suggest that not all actions of 1α,25-$(OH)_2D_3$ are mediated by genome activation.

The nongenomic actions of 1α,25-$(OH)_2D_3$ have been most clearly delineated in osteoblasts and osteoblast-like cells where 1α,25-$(OH)_2D_3$-induced increments in cellular calcium occur in rat osteosarcoma cells lacking the VDR [Baran et al., 1991]. In addition to altering cellular calcium in osteoblasts, the hormone increases nuclear calcium [Sorensen et al., 1993a], cellular calcium uptake [Kim et al., 1987; Lieberherr, 1987; Oshima et al., 1987; Caffrey et al., 1989], phospholipase C activity [Civitelli et al., 1990; Grosse et al., 1993], and intracellular pH [Jenis et al., 1993]. These nongenomic effects of 1α, 25-$(OH)_2D_3$ have a functional significance in osteoblasts. The rapid actions modulate the hormone-induced increments in osteocalcin and osteopontin mRNA steady-state levels [Jenis et al., 1993] and in osteocalcin gene transcription [Baran et al., 1992].

Since 1α,25-$(OH)_2D_3$ has been shown to have very rapid effects on membrane phospholipids and membrane calcium channels, it follows that the hormone may interact with a membrane signalling system. Both 1α,25-$(OH)_2D_3$ and its 1β epimer, which appears to be a specific antagonist of the rapid effects of the hormone [Baran et al., 1991, 1992; Norman et al., 1993], are able to displace the binding of ^3H-1α,25-$(OH)_2D_3$ to the membrane receptor, while 25-hydroxyvitamin D_3 cannot [Baran et al., in press]. This membrane binding activity may be involved in the

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receptor signalling system that mediates the rapid effects of vitamin D.

Focusing on osteoblasts, this article will review the nongenomic actions of the hormone, their physiologic significance, and the initial studies that characterize a membrane receptor for 1α,25-$(OH)_2D_3$.

NONGENOMIC EFFECTS OF 1α,25-$(OH)_2D_3$ ON OSTEOBLASTS

Early studies demonstrated that 1α,25-$(OH)_2D_3$ stimulated ^{45}Ca accumulation in rat osteoblast-like cells [Kim et al., 1987]. Treatment of the cells for times as short as 15 min with 1α,25$(OH)_2D_3$, 2 pM, increased ^{45}Ca accumulation by 10–20%. The development of fluorescent Ca^{+2} indicators made possible the more precise quantitation of changes in intracellular calcium. The first generation fluorescent Ca^{+2} indicator Quin 2AM was used to assess vitamin D-induced changes in cellular calcium in mouse osteoblasts [Lieberherr 1987; Oshima et al., 1987]. 1α,25-$(OH)_2D_3$ 10–100 pm increased intracellular calcium by 50%, an effect that was dependent upon the presence of extracellular calcium. Calcium channel blockers also inhibited the vitamin D effects on intracellular calcium suggesting that membrane voltage-gated calcium channels were involved in the process. These voltage-gated channels, modulated within 1 min by 1α,25-$(OH)_2D_3$, were subsequently confirmed by electrophysiologic studies [Caffrey et al., 1989]. The accumulated studies suggested that 1α,25-$(OH)_2D_3$ altered calcium channel function in osteoblasts. In some, but not all, studies 24,25-dihydroxyvitamin D was without effect demonstrating the specificity of the rapid actions. Since the calcium ion is important in intracellular processes, these studies raised the potential for a physiologic role of the rapid actions of 1α,25-$(OH)_2D_3$. Moreover, these studies pointed to an interaction of the hormone with a plasma membrane signalling system.

The rapid effects of 1α,25-$(OH)_2D_3$ are not limited to interaction with the plasma membrane. The hormone rapidly increased nuclear calcium levels in both intact cells and isolated nuclei suggesting that rapid nongenomic activation of nuclear calcium may also play a role in the regulation of osteoblastic activity [Sorensen et al., 1993a]. Using digital microscopy changes in cytosolic and nuclear region fluorescence were observed in single osteoblast-like cells within 3 min of exposure to 1α,25$(OH)_2D_3$. Within 5 min, the hormone increased cytosolic fluorescence by 29% and nuclear fluorescence by 30%. The direct effects of 1α,25-$(OH)_2D_3$ on the nuclear envelope were confirmed by observations that the hormone increased calcium levels in isolated nuclei.

Numerous hormones have been shown to rapidly increase intracellular calcium by a process involving phospholipid metabolism. 1α,25-$(OH)_2D_3$ was also shown to rapidly increase phospholipase C activity resulting in the generation of inositol triphosphate (IP_3), a recognized mediator of hormone-induced cellular calcium increments [Civitelli et al., 1990; Grosse et al., 1993].

As in the plasma membrane, 1α,25-$(OH)_2D_3$ treatment of isolated nuclei also increased IP_3 levels in the nuclear envelope [Sorensen et al., 1993b]. The phospholipid environment is thought to be important in the regulation of nuclear function. Phospholipid phosphorylation may be related to signals to the genetic apparatus and/or interaction with the genetic apparatus in the nuclear matrix [Capitani et al., 1989; Payrastre et al., 1992]. The nuclear phospholipids change as cells grow and differentiate suggesting that the phospholipids play a role in these processes [York et al., 1994]. The vitamin D-induced changes in nuclear phospholipid metabolism and the resultant changes in nuclear calcium movement may play a role in the hormone's physiologic effects.

Although the rapidity of these vitamin D actions indicated direct interaction with a membrane signalling system, it was unclear whether the VDR was necessary for the hormone to exert its rapid effects. Utilizing a rat osteoblast-like cell line that lacked the VDR, it was demonstrated that 1α,25-$(OH)_2D_3$ 0.2 nM was able to increase cellular calcium within 5 minutes [Baran et al., 1991]. We showed that these cells lack the mRNA for the VDR. In addition, the functional absence of VDR in these cells was demonstrated by the lack of VDR in the nuclear extracts that was capable of binding to the vitamin D response element of the osteocalcin gene. Moreover, the 1β epimer of 1α,25-$(OH)_2D_3$ which does not interact with the VDR [Holick et al., 1980] inhibited the rapid effects of the hormone in osteoblast cells that possess and lack the VDR. This suggested that the epimer binds to but does not activate the signalling system mediating the rapid actions of 1α,25-$(OH)_2D_3$ and that this signalling system was independent of the VDR. The presence of a distinct plasma

membrane associated $1\alpha,25\text{-}(OH)_2D_3$ receptor was confirmed using vitamin D_3 analogs [Farach-Carson et al., 1991]. A structural hierarchy of vitamin D_3 analogs was demonstrated with regard to their efficacy as transducers of the genomic and nongenomic pathways [Norman et al., 1993a,b]. Like the 1β epimer, these analogs preferentially bind to either the VDR or the membrane receptor eliciting either genomic or nongenomic actions.

MEMBRANE BINDING OF $1\alpha,25\text{-}(OH)_2D_3$

$1\alpha,25\text{-}(OH)_2D_3$ has very rapid effects on membrane physiologic processes suggesting that the hormone may interact with a membrane signalling system. The presence of membrane receptors for other steroid hormones has been reported. Aldosterone acts rapidly to stimulate membrane sodium transport in human mononuclear white cells [Wehling et al., 1991]. Iodinated aldosterone binds to the membrane of these cells with an apparent dissociation constant (K_D) of 10^{-10} M [Wehling et al., 1992]. Utilizing a membrane preparation derived from the rat osteoblast-like cell line lacking the VDR, we have demonstrated that $1\alpha,25\text{-}(OH)_2D_3$ and its 1β epimer displace $^3H\text{-}1\alpha,25(OH)_2D_3$ binding from the membranes, while 25-hydroxyvitamin D_3 does not (Table I). The apparent K_D for $1\alpha,25(OH)_2D_3$ determined by displacement of $^3H\text{-}1\alpha,25(OH)_2D_3$, 15 Ci/mmole, yielded a value of 8×10^{-7} M [Baran et al., 1994]. The calculated apparent K_D for the 1β epimer was 5×10^{-7} M. These apparent dissociation constants are considerably lower than the binding of hormone to the VDR, 10^{-10} M. The K_D is also lower than the concentration of $1\alpha,25(OH)_2D_3$ needed to elicit rapid actions in biologic systems. The hormone has been shown to alter cellular calcium in the ROS 24/1 cells at concentrations between 0.2 and 10 nM. These differences in the K_D of vitamin D binding to membranes, in contrast to that of aldosterone, may reflect the low specific activity of the vitamin D ligands or the difficulties in dealing with fat soluble ligands. Because vitamin D is fat soluble and there is fat in the membrane preparation, the free monomeric concentration of the vitamin D analogs may be much lower than that assumed for K_D calculation. This would lead to a much lower calculated affinity. Nevertheless, the observations that the 1β epimer, but not 25-hydroxyvitamin D_3 can displace $1\alpha,25(OH)_2D_3$ binding to membranes coupled with the previous findings

TABLE I. Percent Displacement of $^3H\text{-}1\alpha,25(OH)_2D_3$ Binding From the Membranes of ROS 24/1 Cells by Vitamin D Compounds

	Concentration (nM)		
	7.25	72.5	725
$1,25(OH)_2D_3$	19%	25%	49%
$1\beta,25\text{-}(OH)_2D_3$	27%	38%	ND
$25\text{-}OH\text{-}D_3$	ND	0	0

ND, not determined.

that the 1β epimer is a specific inhibitor of the rapid actions of $1\alpha,25(OH)_2D_3$ suggests that this binding activity may be involved in the receptor signalling system that mediates the rapid nongenomic effects of vitamin D.

PHYSIOLOGIC ROLE OF THE NONGENOMIC EFFECTS

To begin to define the physiologic role of the nongenomic actions of $1\alpha,25(OH)_2D_3$, we have employed the 1β epimer which inhibits the rapid effects of the hormone but does not alter binding of the $1\alpha,25(OH)_2D_3$-VDR complex to the *VDRE* of the osteocalcin gene. The epimer inhibited $1\alpha,25(OH)_2D_3$-induced increases in osteocalcin gene transcription and steady-state levels [Baran et al., 1992]. The biochemical changes resulting from the rapid nongenomic actions of $1\alpha,25(OH)_2D_3$ may modify subtle structural and/or functional properties of the nuclear vitamin-receptor DNA complex, or may affect other protein DNA interactions that support osteocalcin gene transcription. Although one of the rapid actions of $1\alpha,25(OH)_2D_3$ is to increase cellular calcium, the increases in cellular calcium per se do not appear to be the sole signal for modulation of gene expression [Jenis et al., 1993]. Substitution of extracellular sodium with choline prevents the $1\alpha,25(OH)_2D_3$-induced increases in intracellular pH and osteocalcin and osteopontin mRNA levels. These effects on gene expression occur despite the fact that cellular calcium rapidly increases in the sodium-free medium. Thus, although the genomic effects of $1\alpha,25(OH)_2D_3$ appear to be modulated by the rapid nongenomic actions, it does not appear that the rapid increases in cellular calcium are the sole regulators of these processes.

SUMMARY

The vitamin D hormone 1α,25-dihydroxyvitamin D_3 has been shown to act on cellular processes

through genomic and nongenomic pathways. The latter effects, which appear to be mediated by interaction with a receptor on the membrane surface, include rapid changes in cellular calcium and pH, and alterations in phospholipase C activity. These rapid effects modulate the hormone's effect on gene transcription and steady-state level in osteoblasts. Thus, it is hypothesized that the rapid nongenomic pathway may serve regulatory function in part controlling the hormone's effects on genomic pathways in osteoblasts.

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REFERENCES

Baran DT, Milne ML (1986): 1,25-Dihydroxyvitamin D increase hepatocyte cytosolic calcium levels. J Clin Invest 77:1622–1626.

Baran DT, Sorensen AM, Shalhoub V, Owen T, Oberdorf A, Stein G, Lian J (1991): 1α,25-dihydroxyvitamin D_3 rapidly increases cytosolic calcium in clonal rat osteosarcoma cells lacking the vitamin D receptor. J Bone Mineral Res 6:1269–1275.

Baran DT, Sorensen AM, Shalhoub V, Owen T, Stein GS, Lian JB (1992): The rapid nongenomic actions of 1α,25-dihydroxyvitamin D_3 modulate the hormone-induced increments in osteocalcin gene transcription in osteoblast-like cells. J Cell Biochem 50:124–129.

Baran DT, Ray R, Sorensen AM, Honeyman T, Holick MF, Baran DT (in press). Binding characteristics of a membrane receptor that recognizes 1α,25-dihydroxyvitamin D_3 and its epimer, 1β,25-dihydroxyvitamin D_3. J Cell Biochem.

Caffrey JM, Farach-Carson MC (1989): Vitamin D_3 metabolites modulate dihydropyridine-sensitive calcium currents in clonal rat osteosarcoma cells. J Biol Chem 264:20265–20274.

Capitani S, Bertagnolo Y, Mazzoni M, Santi P, Previati M, Antonucci A, Manzoli FA (1989): Lipid phosphorylation in isolated rat liver nuclei: Synthesis of polyphosphoinositides at subnuclear level. FEBS Lett 254:194–198.

Civitelli R, Kim YS, Gunsten SL, Fugimori A, Huskey M, Avioli LV, Hruska KA (1990): Nongenomic activation of the calcium message system by vitamin D metabolites in osteoblast-like cells. Endocrinology 127:2253–2262.

DeLuca HF, Schnoes HK (1976): Metabolism in mechanism of action of vitamin D_3. Annu Rev Biochem 45:631–666.

Desai SS, Appel MC, Baran DT (1986): Differential effects of 1,25-dihydroxyvitamin D_3 on cytosolic calcium in two human cell lines. J Bone Mineral Res 1:497–501.

Farach-Carson MC, Sergeev I, Norman AW (1991): Nongenomic actions of 1,25-dihydroxyvitamin D_3 in rat osteosarcoma cells: Structure-function studies using ligand analogs. Endocrinology 129:1876–1884.

Grosse B, Bourdeau A, Lieberherr M (1993): Oscillations in inositol 1,4,5,-triphosphate and diacylglycerol induced by vitamin D_3 metabolites in confluent mouse osteoblasts. J Bone Mineral Res 8:1059–1069.

Holick SA, Holick MF, MacLaughlin JA (1980): Chemical synthesis of [1β-^{3}H] 1α,25-dihydroxyvitamin D_3 and [1α-^{3}H] 1β,25-dihydroxyvitamin D_3: Biological activity of 1β, 25-dihydroxyvitamin D_3. Biochem Biophys Res Commun 97:1031–1037.

Jenis LG, Lian JB, Stein GS, Baran DT (1993): 1α,25-Dihydroxyvitamin D_3-induced changes in intracellular pH in osteoblast-like cells modulate gene expression. J Cell Biochem 53:234–239.

Kim YS, Birge SJ, Avioli LV, Miller R (1987): Early manifestations of vitamin D effects in rat osteogenic sarcoma cells. Calcif Tissue Int 41:223–227.

Lieberherr M (1987): Effects of vitamin D_3 metabolites on cytosolic free calcium in confluent mouse osteoblasts. J Biol Chem 262:13168–13173.

Norman AW, Okamura WH, Farach-Carson MC, Allewaert K, Branisteanu D, Nemers I, Muralidharan KR, Bouillon R (1993a): Structure function studies of 1,25-dihydroxyvitamin D_3 and the vitamin D endocrine system. J Biol Chem 268:13811–13819.

Norman AW, Bouillon R, Farach-Carson MC, Bishop JE, Zhou L-X, Nemere I, Zhao J, Muralidharan KR, Okamura WH (1993b): Demonstration that 1β,25-dihydroxyvitamin D_3 is an antagonist of the nongenomic but not genomic biological responses and biological profile of the three A-ring diastereomers of 1α,25-dihydroxyvitamin D_3. J Biol Chem 268:20022–20030.

Oshima J, Watanabe M, Hirosumi J, Orimo H (1987): 1α,25-$(OH)_2D_3$ increases cytosolic Ca^{++} concentration of osteoblastic cells, clone MC3T3-E1. Biochem Biophys Res Commun 145:956–960.

Payrastre B, Nievers M, Boonstra J, Breton M, Verkleij AJ, Van Bergen en Henegouwen PMP (1992): A differential location of phosphoinositide kinases, diacylglycerol Kinase, and phospholipase C in the nuclear matrix. J Biol Chem 267:5078–5084.

Sorensen AM, Bowman D, Baran DT (1993a): 1α,25-dihydroxyvitamin D_3 rapidly increases nuclear calcium levels in rat osteosarcoma cell. J Cell Biochem 52:237–242.

Sorensen AM, Baran DT (1993b): 1α,25-dihydroxyvitamin D_3 rapidly alters phospholipid metabolism in the nuclear envelope of osteoblasts. J Bone Miner Res 8:S127.

Wehling M, Kamayr J, Theisen K (1991): Rapid effects of mineralocorticoids on sodium-proton exchanges: Genomic or nongenomic pathway. Am J Physiol 260:E719–726.

Wehling M, Christ M, Theisen K (1992): Membrane receptors for aldosterone: A novel pathway for mineralocorticoid action. Am J Physiol 263:E974–979.

York JD, Majerus PW (1994): Nuclear phosphatidylinositols decrease during S-phase of the cell cycle in HeLa cells. J Biol Chem 269:7847–7850.