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1α,25-Dihydroxyvitamin D_3 Rapidly Alters Phospholipid Metabolism in the Nuclear Envelope of Osteoblasts

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Abstract 1α,25-Dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$) has been shown to increase cytosolic calcium and inositol triphosphate levels in rat osteosarcoma cells (ROS 17/2.8) and to increase nuclear calcium in these cells. To determine the mechanism(s) of 1α,25-$(OH)_2D_3$-induced changes in nuclear calcium, the effect of the hormone on phospholipid metabolism in isolated osteoblast nuclei was assessed. 1α,25$(OH)_2D_3$, 20 nM, increased inositol triphosphate levels in the nuclei after 5 min of treatment. The biologically inactive epimer, 1β,25-$(OH)_2D_3$, had no significant effect on inositol triphosphate levels. ATP, 1 mM, also increased inositol triphosphate levels in the isolated nuclei after 5 min. 1α,25-$(OH)_2D_3$, 20 nM, increased calcium in the isolated nuclei in the presence but not in the absence of extranuclear calcium within 5 min. Nuclear calcium was also increased within 5 min by ATP, 1 mM, and inositol triphosphate, 1 mM. The effect of ATP on nuclear calcium was not additive with 1α,25-$(OH)_2D_3$, suggesting that these two agents increase nuclear calcium in these osteoblast-like cells by similar mechanisms. In summary, 1α,25-$(OH)_2D_3$ and ATP rapidly increase inositol triphosphate levels in nuclei isolated from ROS 17/2.8 cells. The hormone, the nucleotide, and the inositol phospholipid increase nuclear calcium. Thus, the 1α,25-$(OH)_2D_3$ and ATP effects on nuclear calcium may be mediated by changes in phospholipid metabolism in the nuclei of these osteoblast-like cells.

Key words: nuclear calcium, inositol triphosphate, osteosarcoma cells, osteoblast nuclei, vitamin D

Recent studies suggest that 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$) exerts its effects in cells by genomic and nongenomic mechanisms. In osteoblasts, concentrations of 10 pM to 20 nM of the hormone have been shown to increase cellular calcium [Lieberherr, 1987], modulate calcium currents [Caffrey and Farach-Carson, 1989; Farach-Carson et al., 1991], and activate phospholipase C [Civitelli et al., 1990; Grosse et al., 1993] and the Na+/H+ antiport [Jenis et al., 1993] within seconds to minutes. The 1α,25-$(OH)_2D_3$-induced increments in cellular calcium occur in clonal rat osteosarcoma cells lacking the vitamin D receptor, indicating that these rapid effects are mediated through a signalling system that does not involve the classic nuclear vitamin D receptor [Baran et al., 1991]. Moreover, these nongenomic effects of 1α,25-$(OH)_2D_3$ have a functional significance in the osteoblast-like cells. The nongenomic 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].

The nuclear membrane contains calcium permeability barriers and transport systems that are hormonally sensitive [Waybill et al., 1991]. 1α,25-$(OH)_2D_3$ rapidly increases calcium levels in isolated hepatic nuclei [Baran et al., 1989]. In addition, the hormone rapidly and specifically increases nuclear calcium levels in osteoblast-like cells as well as in their isolated nuclei [Sorensen et al., 1993]. These increments in nuclear calcium are observed in nuclei isolated from osteoblast-like cells that lack the classic nuclear vitamin D receptor indicating that the classic receptor is not required for the rapid actions of 1α,25-$(OH)_2D_3$ on nuclear calcium [Sorensen et al., 1993].

ATP also increases calcium levels in rat osteoblast-like cells [Kumagai et al., 1989, 1991; Reimer and Dixon, 1992] and in nuclei isolated from hepatocytes [Baran et al., 1989; Nicotera et al., 1989]. Like 1α,25-$(OH)_2D_3$ [Civitelli et al., 1990; Grosse et al., 1993], the ATP-induced

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increase in cellular calcium in osteoblast-like cells is accompanied by the generation of inositol phosphates [Kumagai et al., 1991].

In this study, we show that 1α,25-$(OH)_2D_3$, ATP, and inositol triphosphate increase calcium levels in nuclei isolated from rat osteosarcoma cells (ROS 17/2.8). In addition, both the hormone and the nucleotide stimulate the generation of inositol triphosphate in the nuclear envelope. Since the effects of 1α,25-$(OH)_2D_3$ and ATP on nuclear calcium are not additive, the data suggest that both agents may alter nuclear calcium through a mechanism involving inositol phosphates.

MATERIALS AND METHODS

Cell Cultures

Osteoblast-like rat osteosarcoma cells, ROS 17/2.8 (generously supplied by Dr. G. Rodan, Merck Sharp Dohme, West Point, PA), were grown in culture medium consisting of DMEM: F12 (50:50) plus 10% fetal calf serum.

Cells were grown for 6–7 days and harvested for experiments by trypsinization with 0.25% trypsin and 0.002% EDTA and by sedimentation at 100*g* for 8 min. Cell numbers were assessed by counting an aliquot of cells in a hemocytometer, and viability was determined by trypan blue dye exclusion.

Isolation of Nuclei by Hypotonic Swelling

Rat osteosarcoma cells, ROS 17/2.8, were washed in an isotonic buffer consisting of 125 mM KCl, 30 mM Tris/HCl (pH 7.9), 5 mM $MgCl_2$, and 10 mM beta mercaptoethanol (BME). The cells were centrifuged and resuspended in 1 ml per 10^7 cells of ice cold hypotonic swelling buffer (10 mM KCl, 30 mM Tris/HCl [pH 7.9] 5 mM $MgCl_2$, and 10 mM BME). The cells were allowed to swell for 20 min on ice and then Dounce homogenized with a tight fitting pestle for 15 strokes. There was a 100% recovery of the nuclei originally present; 95–98% of these nuclei were intact viewed at 100-fold magnification [Baran et al., 1989; Sorensen et al., 1993].

Anion Exchange Chromatography of Water Soluble Inositol Phosphates

ROS 17/2.8 cells were exposed to ^{3}H-inositol (50 μM) (specific activity 23.4 Ci/mmole). Nuclei were isolated as described above. At 18 h, the incorporation of ^{3}H-inositol into the nuclear envelope had plateaued (Fig. 1).

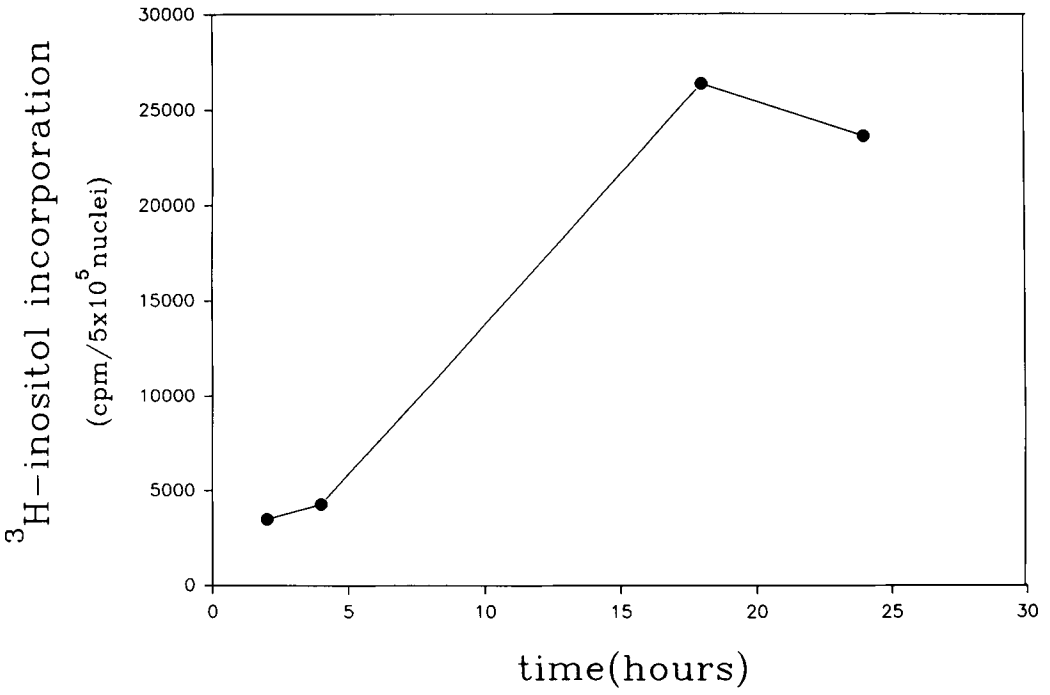

Fig. 1. Time course of the incorporation of ^{3}H-inositol into the nuclear envelope. Values represent the mean of 2 experiments.

Nuclei 1×10^7 per ml were suspended in a HEPES buffered salt solution containing 50 nM calcium and treated with LiCl 10 mM for 10 min. $1\alpha,25\text{-}(OH)_2D_3$, 20 nM, $1\beta,25\text{-}(OH)_2D_3$, 20 nM, or ATP, 1 mM, were added to the nuclei for 5 min. Nuclei were centrifuged at 50*g* for 1 min, and the supernatant was removed before 0.75 ml of $CHCl_3/CH_3OH/HCl$ (100:50:1) was added to 5×10^6 nuclei to quench the reaction. This solution was vortexed and kept at 3°C overnight.

Separation of phases. After 24 h, the solution was separated by centrifugation at 200*g* for 20 min and the supernatant was removed from the protein pellet. Three volumes of H_2O were added to the supernatant to separate the phases at 4°C. The upper, aqueous layer from each 0.5 ml of cell pellet, containing free inositol and inositol phosphates, was removed and neutralized to pH 7.0 before being applied to Dowex columns (Bio-Rad, Hercules, CA) in the formate form.

Analytic procedure. Water-soluble inositol phosphates were separated by anion-exchange chromatography on 0.8 ml Dowex 1×8 columns in the formate form [Baran et al., 1988]. Of the applied radioactivity, 80–85% was recovered. After the columns were equilibrated, two 5 ml fractions of H_2O eluted the free inositol; three 5 ml fractions of 5 mM sodium borate and 60 mM sodium formate eluted the glycerophosphorylinositol (GPI); three 5 ml fractions of 5 mM sodium borate and 180 mM sodium formate eluted the inositol phosphate (IP); two 5 ml fractions of 0.1 M formic acid and 0.4 M ammonium formate eluted the inositol bisphosphonate (IP_2); and three 5 ml fractions of 0.1 M formic acid and 1 M ammonium formate eluted the inositol triphosphate (IP_3).

Optiflour (5 ml; Packard, Meriden, CT) was added to each fraction collected and the radioactivity determined in a Beckman LS6000SC liquid scintillation spectrometer (Wakefield, MA).

Determination of Calcium Levels In Isolated Nuclei

Nuclei (10^7/ml) were loaded with 1 μM Fura 2 AM (Molecular Probes, Eugene, OR) for 60 min at 4°C [Nicotera et al., 1989]. Nuclei were washed, resuspended, and equilibrated at 37°C under an atmosphere of 95% O_2:5% CO_2 for 15 min [Sorensen et al., 1993]. Nuclear calcium was calculated using the 340/380 fluorescence ratio of Fura 2 loaded nuclei.

Statistical Analyses

Values represent the mean ± SEM of 4–6 observations from separate experiments. Probability of difference for phospholipid changes was determined by the paired *t*-test. Differences among several groups were assessed by Duncan's test for multiple comparisons.

RESULTS

$1\alpha,25\text{-}(OH)_2D_3$, 20 nM, significantly increased ^{3}H-inositol triphosphate (^{3}H-IP_3) levels in the nuclear envelope after 5 min (65 ± 14 vs. 77 ± 15 cpm/5×10^6 nuclei, $P < 0.01$) while no effect was seen after 15 s of treatment (43 ± 7 vs. 38 ± 10 cpm/5×10^6 nuclei) (Fig. 2). This effect was relatively specific since the vitamin D epimer, $1\beta,25\text{-}(OH)_2D_3$, 20 nM, did not increase ^{3}H-IP_3 levels in the nuclei (57 ± 11 vs. 48 ± 8 cpm/5×10^6 nuclei). Similarly, ATP, 1 mM, significantly increased ^{3}H-IP_3 levels in the nuclear envelope after 5 min (70 ± 12 vs. 86 ± 6 cpm/5×10^6 nuclei, $P < 0.05$) but not after 15 s of treatment (74 ± 3 vs. 73 ± 1 cpm/5×10^6 nuclei) (Fig. 2).

$1\alpha,25\text{-}(OH)_2D_3$, 20 nM, gradually increased calcium levels in the isolated nuclei, with increased fluorescence noted after 3 min (Fig. 3). After 5 min of treatment, the hormone significantly increased nuclear calcium levels (123 ± 7 vs. 219 ± 28 nM, $P < 0.01$). $1\alpha,25\text{-}(OH)_2D_3$ increased calcium levels in isolated nuclei only in the presence of extranuclear calcium. Chelation of calcium with EGTA prevented the hormone-induced increase in nuclear calcium (140 ± 6 vs. 130 ± 4 nM). ATP, 1 mM, also gradually increased fluorescence intensity in the isolated nuclei with changes apparent after 2 min (Fig. 4). By 5 min, both ATP, 1 mM, and IP_3, 1 mM, significantly increased nuclear calcium (control = 168 ± 8 nM; ATP = 223 ± 16 nM*; IP_3 = 247 ± 18 nM*; $P < 0.05$ compared to control). The effects of $1\alpha,25\text{-}(OH)_2D_3$, 20 nM, and ATP, 1 mM, on nuclear calcium were not additive (Table I). The hormone increased nuclear calcium levels by 78% while the nucleotide increased levels by 83%. The combination of $1\alpha,25\text{-}(OH)_2D_3$ and ATP increased nuclear calcium levels by 59%.

DISCUSSION

The results of this study demonstrate that $1\alpha,25\text{-}(OH)_2D_3$ and ATP rapidly alter phospholipid metabolism in isolated nuclei. $1\alpha,25\text{-}$

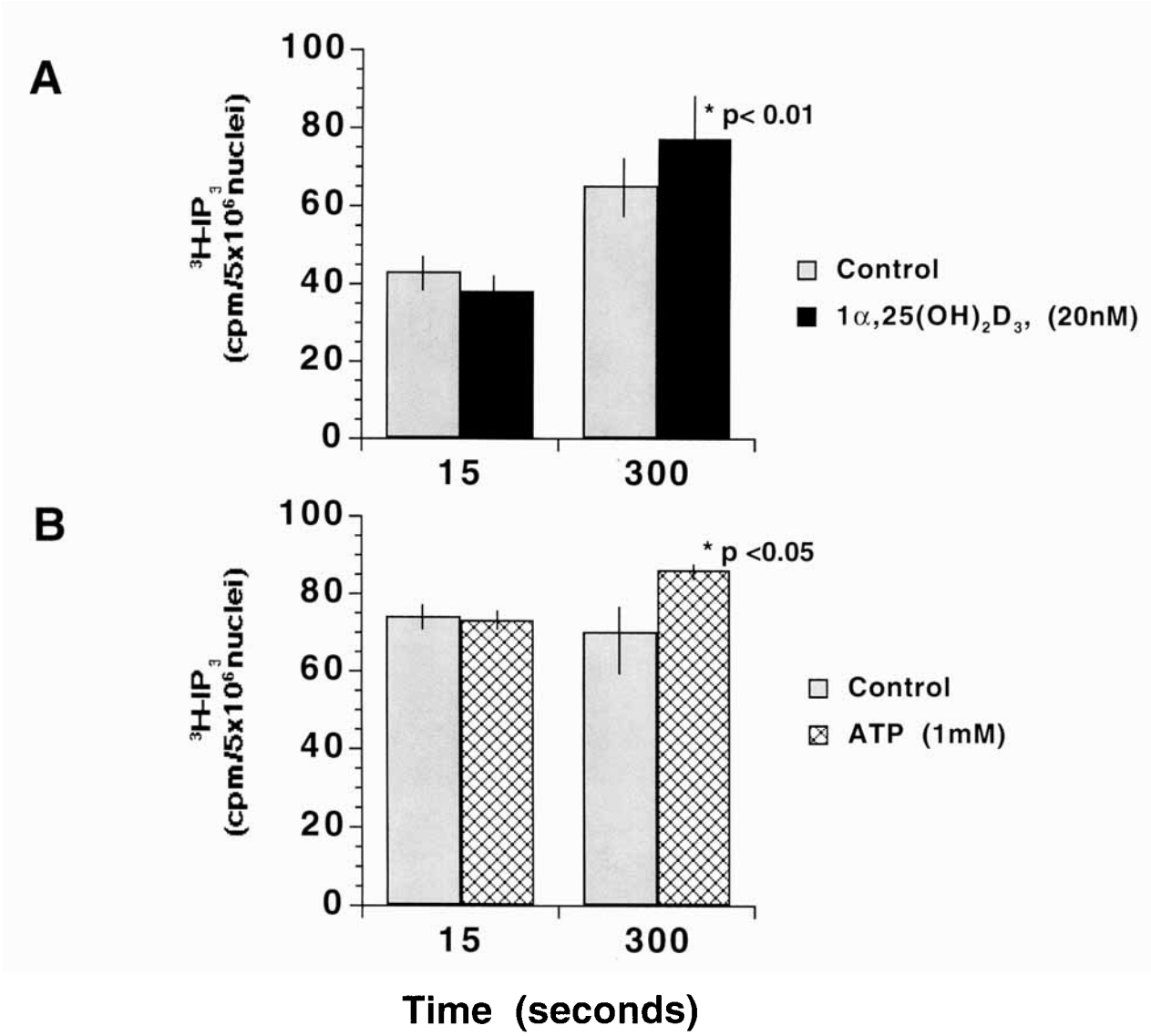

Fig. 2. Nuclear ^{3}H-inositol-triphosphate levels as affected by (A) 1α,25-dihydroxyvitamin D_3 (20 nM) at 15 and 300 secs; (B) ATP (1 mM) at 15 and 300 secs.

TABLE I. Effect of 1α, 25-Dihydroxyvitamin D_3, ATP, and the Combination on Nuclear Calcium Levels

	Calcium (nM)	% Increase
Control	123 ± 6	—
1α,25-dihydroxyvitamin D_3 (20 nM)	219 ± 25*	78
ATP (1 mM)	225 ± 29*	83
1α,25-dihydroxyvitamin D_3 + ATP	196 ± 21*	59

*$P < 0.05$ compared to control by Duncan's test for multiple comparisons.

$(OH)_2D_3$ has previously been reported to increase the production of ^{3}H-IP$_3$ in ROS 17/2.8 cells, presumably by increasing phospholipase C activity in the plasma membrane [Civitelli et al., 1990]. This effect occurred within 10 s of hormone addition. More recently, 1α,25-$(OH)_2D_3$, 10 pM to 1 nM, has also been shown to increase ^{3}H-IP$_3$ accumulation in freshly isolated mouse osteoblasts by 12–50% [Grosse et al., 1993]. Similar degrees of accumulation were noted after stimulation with 24,25-$(OH)_2D_3$ and 25-$(OH)_2D_3$, suggesting a direct interaction of vitamin D metabolites with membrane secosteroid recognition sites [Grosse et al., 1993]. 1α,25-

$(OH)_2D_3$ also activates phospholipase C and increases ^{3}H-IP_3 production in rat enterocytes [Lieberherr et al., 1989], rat colonic epithelium [Wali et al., 1990, 1992], human colonic carcinoma cells [Tien et al., 1993], murine keratinocytes [Tang et al., 1987], and avian myoblasts [Morelli et al., 1993]. In contrast, the hormone appears to activate phospholipase A_2 in hepatocytes [Baran et al., 1988, 1990; Baran and Kelly, 1988] and their nuclei [Baran et al., 1989], and chondrocytes [Schwartz et al., 1988]. Thus, various doses of the secosteroid have been shown to alter phospholipid metabolism in a variety of cell types by activation of either phospholipase C or phospholipase A_2.

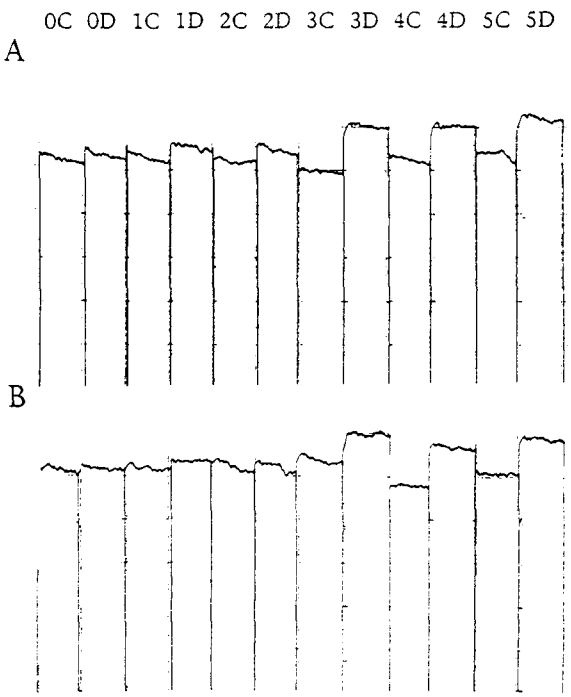

Fig. 3. Time course of the effect of 1α,25-dihydroxyvitamin D_3, 20 nM, on fluorescence measured at 340 nm in Fura 2 loaded intact nuclei. Calcium values listed in the text are calculated by using a ratio of the 340/380 fluorescence measurements. **A** and **B** refer to 2 experiments performed on separate days. The numbers above each bar refer to time in minutes, while the letters designate control (C) or 1α,25-Dihydroxyvitamin D_3 (D) treated nuclei.

ATP has also been previously shown to increase phospholipid turnover in a variety of cell types. The nucleotide increases ^{3}H-IP_3 in UMR-106 cells [Kumagai et al., 1991], Ehrlich ascites tumor cells [Dulyak, 1986], and human amnion cells [Kooy et al., 1989] within 30 s.

In the present report, we show that 1α,25-$(OH)_2D_3$ increases ^{3}H-IP_3 levels by 20% in isolated nuclei of rat-osteoblast-like cells. In contrast to intact osteoblasts, where an effect of 1α,25-$(OH)_2D_3$ on ^{3}H-IP_3 levels was observed within 30 s [Civitelli et al., 1990; Grosse et al., 1993], we saw no increase in ^{3}H-IP_3 in the nuclei at that time but observed the increment after 5 min (Fig. 2). The effect of 1α,25-$(OH)_2D_3$ is relatively specific since 1β,25-$(OH)_2D_3$ had no effect on ^{3}H-IP_3 levels. Similarly, ATP increases ^{3}H-IP_3 levels in the nuclei of the ROS 17/2.8 cells after 5 min. Like the 1α,25-$(OH)_2D_3$ effect on phospholipid turnover, the nucleotide action is observed only after 5 min of treatment (Fig. 2). This contrasts with the more rapid (30 s) effect of ATP on ^{3}H-IP_3 production in intact osteoblasts [Kumagai et al., 1991]. These differences are not methodologic in nature, since we also observe increases in ^{3}H-IP_3 levels in intact ROS 17/2.8 cells treated with 1α,25-$(OH)_2D_3$ within 15 s (data not shown). Thus, variations in response time may reflect differences in the

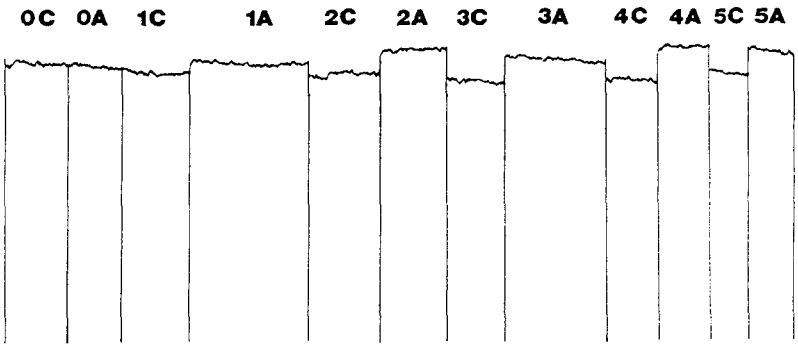

Fig. 4. Time course of the effect of ATP, 1 mM, fluorescence measured at 340 nm in Fura 2 loaded intact nuclei in a single representative experiment. The numbers above each bar refer to time in minutes, while the letters designate control (C) or ATP (A) treated nuclei.

recognition sites on the plasma membrane and nuclei, differences in phospholipid turnover at the two sites, or alterations in turnover induced during the process of nuclei isolation. Although the most likely source of IP_3 in these studies is the nuclear envelope, the results do not rule out possible generation of IP_3 from intranuclear sites.

The phospholipid environment has been postulated to be important in the regulation of nuclear function [Cocco et al., 1989; Divecha et al., 1991]. The inositol phospholipids in particular have been shown to modulate RAS gene activity during mitogenic stimulation [Berezney et al., 1979; Tsai et al., 1989] and to stimulate the initiation of DNA synthesis in rat liver cells [Hill et al., 1989]. Nuclear specific phosphatidylinositol lipid breakdown has been shown to play a direct role in DNA synthesis in HeLa cells [York and Majerus, 1994]. Phospholipid phosphorylation takes place in isolated rat liver nuclei [Capitani et al., 1989, 1990]. This may be related to signals to the genetic apparatus [Capitani et al., 1989] and/or interaction with the genetic apparatus in the nuclear matrix [Cocco et al., 1980; Payrastre et al., 1992].

The nuclear membrane has a calcium pump that is similar to that of the endoplasmic reticulum [Layini et al., 1992]. In addition, nuclei bind IP_3 and posses an IP_3 sensitive calcium pool [Nicotera et al., 1990; Malriya et al., 1990]. Our results indicate that IP_3 increases nuclear calcium levels in Fura 2 loaded isolated nuclei. The observation that the increase in nuclear calcium after treatment with both $1\alpha,25\text{-}(OH)_2D_3$ and ATP is similar to that noted after treatment with either agent alone, and after treatment with IP_3, suggests that inositol triphosphates mediate the hormone and nucleotide-induced increments in nuclear calcium. It has recently been reported that inositol tetrakisphosphate also binds to rat liver nuclei and plays an important role in nuclear calcium uptake [Koppler et al., 1993]. Thus, is appears that the inositol phosphates play a role in the regulation of nuclear calcium uptake and that the phospholipids themselves may be partly regulated by the hormonal and nucleotide environment.

In conclusion, it is apparent that $1\alpha,25\text{-}(OH)_2D_3$ exerts rapid effects at both the cellular and subcellular level. The hormone-induces changes in nuclear phospholipid metabolism and nuclear calcium within 5 min. Phospholipid metabolism has been linked to the regulation of gene expression. Likewise, the rapid nongenomic actions of $1\alpha,25\text{-}(OH)_2D_3$ modulate the hormone-induced increments in gene transcription. The $1\alpha,25\text{-}(OH)_2D_3$-mediated changes in nuclear IP_3 and calcium movement may play a role in modulating the hormone's control of gene expression. These studies suggest that both $1\alpha,25\text{-}(OH)_2D_3$ and ATP increase nuclear calcium by increasing nuclear IP_3 levels. Further studies evaluating the time course of these effects and employing inhibitors of phospholipase C may assist in proving this relationship.

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REFERENCES

Baran DT, Sorensen AM, Honeyman TW (1988): Rapid actions of 1,25-dihydroxyvitamin D_3 on hepatocyte phospholipids. J Bone Miner Res 3:593–600.

Baran DT, Kelly AM (1988): Lysophosphatidylinositol: A potential mediator of 1,25-dihydroxyvitamin D_3-induced increments in hepatocyte cytosolic calcium. Endocrinology 122:930–934.

Baran DT, Sorensen AM, Honeyman TW, Ray R, Holick MF (1989): Rapid actions of $1\alpha,25$-dihydroxyvitamin D_3 on Ca^{+2} and phospholipids in isolated rat liver nuclei. FEBS Lett 259:205–208.

Baran DT, Sorensen AM, Honeyman TW, Ray R, Holick MF (1990): $1\alpha,25$-Dihydroxyvitamin D_3-induced increments in hepatocyte cytosolic calcium and lysophosphatidylinositol: inhibition by pertussis toxin and $1\beta,25$-dihydroxyvitamin D_3. J Bone Miner Res 5:517–524.

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

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

Berezney R, Basler J, Kaplan SC, Hughes BB (1979): The nuclear matrix of slowly and rapidly proliferating liver cells. Eur J Cell Biol 20:139–142.

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.

Capitani S, Helms B, Mazzoni M, Previati M, Bertagnolo V, Wirtz KWA, Manzoli FA (1990): Uptake and phosphorylation of phosphlatidylinositol by rat liver nuclei: role of phosphatidylinositol transfer protein. Biochem Biophys Acta 1044:193–200.

Civitelli R, Kim YS, Gunsten SL, Fujimori 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.

Cocco L, Maraldi NM, Manzoli FA (1980): Phospholipid interactions in rat liver matrix. Biochem Biophys Res Commun 96:890–898.

Cocco L, Martelli AM, Gilmour RS, Ognibene A, Manzoli F, Irvine RF (1989): Changes in nuclear phospholipids induced in intact cells by insulin-like growth factor I. Biochem Biophys Res Commun 159:720–725.

Divecha N, Banfic H, Irvine RF (1991): The polyphosphoinositide cycle exists in the nuclei of Swiss 3T3 cells under the control of a receptor (for IGF-1) in the plasma membrane, and stimulation of the cycle increases nuclear diacylglycerol and apparently induces translocation of protein kinase C to the nucleus. EMBO J 10:3207–3214.

Dulyak GR (1986): Extracellular ATP actives polyphosphoinositide breakdown and Ca^{+2} mobilization in Ehrlich ascites tumor cells. Arch Biochem Biophys 245:84–95.

Farach-Carson MC, Sergeev I, Norman HW (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-trisphosphate and diacylglycerol induced by vitamin D metabolites in confluent mouse osteoblasts. J Bone Miner Res 8:1059–1069.

Hill TD, Zwiller J, Boynton AL (1989): Inositol 1,3,4,5 tetrakisphosphate stimulates the initiation of DNA synthesis in Ca^{+2} deprived rat liver cells. J Cell Physiol 140:403–407.

Jenis L, Lian J, Stein G, Baran D (1993): 1α,25-dihydroxyvitamin D_3-induced changes in intracellular pH in osteoblast-like cells modulate gene expression. J Cell Biochem 53:234–239.

Kooy DV, Dulyak GR, Moore RM, Moore JJ (1989): Adenosine triphosphate actives the phospholipase-C cascade system in human amnion cells without increasing prostaglandin production. Endocrinology 124:2005–2012.

Koppler P, Matter N, Malvirya AN (1993): Evidence for stereospecific inositol 1,3,4,5,-^{3}H-tetrakisphosphate binding sites on rat liver nuclei: delineating inositol 1,3,4,5-tetrakisphosphate interaction in nuclear calcium signalling process. J Biol Chem 268:26248–26252.

Kumagai H, Sakamoto H, Guggino S, Filburn CR, Sacktor B (1989): Neurotransmitter regulation of cytosolic calcium in osteoblast-like bone cells. Calcif Tissue Int 45:251–254.

Kumagai H, Sacktor B, Filburn CR (1991): Purinergic regulation of cytosolic calcium and phosphoinositide metabolism in rat osteoblast-like osteosarcoma cells. J Bone Miner Res 6:697–708.

Layini L, Bachs O, Carafoli E (1992): The calcium pump of the liver nuclear membrane is identical to that of enoplasmic reticulum. J Biol Chem 267:11548–11552.

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

Lieberherr M, Grosse B, Duchambon P, Drueke T (1989): A functional cell surface type receptor is required for the early action of 1,25-dihydroxyvitamin D_3 on the phosphoinositide metabolism in rat enterocytes. J Biochem 264: 20403–20406.

Malriya AN, Rogue P, Vincendon G (1990): Stereo-specific inositol 1,4,5-[^{32}P] trisphosphate binding to isolated rat liver nuclei: Evidence for inositol trisphosphate receptor mediated calcium release from the nucleus. Proc Natl Acad Sci U S A 87:9270–9274.

Morelli S, deBoland AR, Boland RL (1993): Generation of inositol phosphates, diacylglycerol and calcium fluxes in myoblasts treated with 1,25-dihydroxyvitamin D_3. Biochem J 289:675–679.

Nicotera P, McConkey DJ, Jones DP, Orrenins S (1989): ATP stimulates Ca^{+2} concentration in isolated rat liver nuclei. Proc Natl Acad Sci U S A 86:453–457.

Nicotera P, Orrenius S, Nilsson T, Berggren PO (1990): An inositol 1,4,5-triphosphate sensitive Ca^{+2} pool in liver nuclei. Proc Natl Acad Sci U S A 87:6858–6862.

Payrastre B, Nievers M, Boonstra J, Breton M, Verkleij AJ, Van Bergenen, Henegonwen MP (1992): A differential location of phosphoinositide kinase S, diacylglycerol kinase, and phospholipase C in the nuclear matrix. J Biol Chem 267:5078–5084.

Reimer WJ, Dixon SJ (1992): Extracellular nucleotides elevate $[Ca^{+2}]i$ in rat osteoblastic cells by interaction with two receptor subtypes. Am J Physiol 263:C1040–C1048.

Schwartz Z, Schlader DL, Swain LD, Boyan BD (1988): Direct effects of 1,25-dihydroxyvitamin D_3 and 24,25-dihydroxyvitamin D_3 on growth zone and resting zone chondrocyte membrane alkaline phosphatase and phospholipase-A_2 specific activities. Endocrinology 123:2878–2884.

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

Tang W, Ziboh VA, Isseroff RR, Martinez D (1987): Novel regulatory actions of 1α,25-dihydroxyvitamin D_3 on the metabolism of polyphoinositides in murine epidermal keratinocytes. J Cell Physiol 132:131–136.

Tien X-Y, Brasitus TA, Qasawa BM, Normal AW, Sitirn MD (1993): Effect of $1,25(OH)_2D_3$ and its analogues on membrane phosphoinositide turnover and $[Ca^{+2}]i$ in Caco-2 cells. Am J Physiol 265:G143–G148.

Tsai MH, Yu CL, Wei FS, Stacey DW (1989): The effect of GTPase activating protein yoon RAS is inhibited by mitogenically responsive lipids. Science 243:522–526.

Wali RK, Baum CL, Sitrin MD, Brasitus TA (1990): $1,25(OH)_2$ vitamin D_3 stimulates membrane phosphoinositide turnover, activates protein kinase C, and increases cytosolic calcium in rat colonic epithelium. J Clin Invest 85:1296–1303.

Wali RK, Bolt MJG, Tien X-Y, Brasitus TA, Sitrin MD (1992): Different effect of 1,25-dihydroxycholecalciferol on phosphoinositide turnover in the antipodal plasma membranes of colonic epithelial cells. Biochem Biophys Res Commun 187:1128–1134.

Waybil MM, Yelamarty RV, Zhang Y, Scarderle RC Jr, LaNoul KF, Hsu C, Smith C, Tillotson DL, Ya FTS, Cheun JY (1991): Nuclear calcium gradients in cultured rat hepatocytes. Am J Physiol 261:E49–57.

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