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1,25-Dihydroxyvitamin D_3 Enhances the Transcription and Expression of the Inositol Trisphosphate Receptor Gene in HL-60 Cells

PETER G. BRADFORD, YOUYU JIN, and PEI HUI

Department of Pharmacology and Therapeutics, State University of New York at Buffalo, Buffalo, New York 14214 (P.G.B., P.H.), and Department of Pharmacology, Capital Institute of Medicine, Beijing, 100054, People's Republic of China (Y.J.)

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SUMMARY

Human leukemic HL-60 cells were treated with 1,25-dihydroxyvitamin D_3 ($VitD_3$) to induce monocytic cell differentiation. Concomitant with differentiation there was increased inositol-1,4,5-trisphosphate ($InsP_3$) receptor expression, as assessed by both [^{3}H]$InsP_3$ binding site density and maximal $InsP_3$-mediated Ca^{2+} mobilization from intracellular stores. Within 8 hr after $VitD_3$ treatment the steady state level of a 10-kilobase $InsP_3$ receptor mRNA was specifically elevated, and it continued to rise for 1–2 days. Nuclear run-off assays indicated a higher transcription rate of the $InsP_3$ receptor gene in response to $VitD_3$. The increased rate of transcription was sufficient to account for the increased steady state level of $InsP_3$ receptor mRNA in $VitD_3$-treated cells. $VitD_3$ had no effect on the decay of $InsP_3$ receptor mRNA in transcriptionally arrested cells; however, $InsP_3$ receptor mRNA decay was dependent upon continued protein synthesis. Moreover, in cycloheximide-treated cells $VitD_3$ was still able to induce an increase in the steady state level of $InsP_3$ receptor mRNA, indicating that protein synthesis was not required for the enhanced transcriptional response. The results suggest that $VitD_3$ directly enhances the transcription of the $InsP_3$ receptor gene in HL-60 cells.

The $InsP_3$ receptor regulates intracellular Ca^{2+} homeostasis in many eukaryotic cells including human peripheral blood monocytes and differentiated human monocytic cell lines (1–3). $InsP_3$ is formed rapidly within cells stimulated with Ca^{2+}-mobilizing agonists, and when $InsP_3$ is added to permeabilized cells Ca^{2+} is released from intracellular stores, supporting the second messenger function of $InsP_3$. $InsP_3$ receptors have been identified within cells and reconstitution experiments as well as transient expression systems have demonstrated that the $InsP_3$ receptor is a Ca^{2+} channel intrinsic to a specialized component of the endoplasmic reticulum (4–6). Stimulation of human monocytic cell lines and blood monocytes with formyl peptide chemoattractants or with thrombin elicits the rapid formation of $InsP_3$, with subsequent Ca^{2+} mobilization from intracellular stores and cellular activation (2, 3). Evidence also suggests that the release of tumor necrosis factor, interleukin-1, and interleukin-6 from human monocytes stimulated by platelet-activating factor and by the neuropeptides substance K and substance P proceeds in part through this $InsP_3$-dependent pathway (7–9). These observations suggest a critical role for the Ca^{2+}-mobilizing $InsP_3$ receptor in mediating several monocytic responses.

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HL-60 cells are derived from human promyelocytic leukemic cells and have been widely used in hematological research, in part because of their capacity to undergo monocytic or granulocytic differentiation under defined culture conditions (10). Treatment of cultured HL-60 cells with such diverse agents as γ-interferon, lymphocyte-conditioned medium, sodium butyrate, and $VitD_3$ induces the differentiation of HL-60 cells into cells with distinct monocytic characteristics (11–14). Among these acquired characteristics are the expression of monocyte-specific cell surface markers and an increase in the levels of signal transduction proteins, including the formyl peptide receptor and protein kinase C (15, 16). There is also a concomitant increase in the functional capacities for phagocytosis and cytotoxicity (17–19). Because the phosphoinositide pathway may be critical for some of these acquired functions, we examined the status of the $InsP_3$ receptor in untreated HL-60 cells and in HL-60 cells treated with concentrations of $VitD_3$ that induce monocytic differentiation. The results indicate that there is regulation of functional $InsP_3$ receptor expression in $VitD_3$-treated HL-60 cells and that the basis of this regulation is at the level of $InsP_3$ receptor gene transcription.

Experimental Procedures

Cell culture. Human leukemia HL-60 cells, obtained from the American Type Culture Collection (Rockville, MD), were cultured in

ABBREVIATIONS: $InsP_3$, inositol-1,4,5-trisphosphate; $VitD_3$, 1,25-dihydroxyvitamin D_3; CHX, cycloheximide.

supplemented RPMI 1640 medium (Life Technologies, Grand Island, NY) as described (20). $VitD_3$ (10 nM; Biomol Corp.) was added to cultures and incubations were continued for 1–3 days, after which time cells were harvested by centrifugation, washed in phosphate-buffered saline, and analyzed for various activities. The concentration and purity of $VitD_3$ were assessed by UV spectroscopy, with an absorbance maximum at 265 nm (ϵ_{265} = 18,200), minimum at 229 nm, and maximum/minimum ratio of 1.80. Monocytic differentiation of $VitD_3$-treated HL-60 cells was monitored by measuring the nonspecific esterase activity of cytocentrifuged cell preparations, using α-naphthyl acetate as substrate (Sigma Chemical Co.).

Fura-2 studies. Measurements of $InsP_3$-sensitive and ionomycin-releasable intracellular Ca^{2+} stores were carried out using digitonin-permeabilized cells essentially as described (21). Two milliliters of cells at 4×10^6/ml in an intracellular salt solution were placed in a quartz cuvette housed in a Shimadzu RF-5000 spectrofluorometer and were maintained at 22°, with stirring. Digitonin (25 μM) and the fluorescent Ca^{2+} indicator fura-2 (2 μM; Calbiochem) were added to the cell suspension. Oligomycin (10 μg/ml) and antimycin A (10 μM) were included to inhibit mitochondrial function. ATP (5 mM) was subsequently added to initiate uptake of ambient Ca^{2+} into vesicular stores. After 2 min, $InsP_3$ (3 μM; Boehringer Mannheim) or ionomycin (1 μM, free acid; Calbiochem) was added to stimulate release of stored Ca^{2+}. Excitation wavelengths were 340 nm and 380 nm and fluorescence emission was monitored at 500 nm. Ca^{2+} concentrations were calculated from 340/380 fluorescence ratios after subtraction of autofluorescence (22). Recoveries (>90%) and protein contents of permeabilized cells were comparable in untreated and $VitD_3$-treated cells.

Preparation of membranes. Untreated and $VitD_3$-treated HL-60 cells were harvested by centrifugation, washed in phosphate-buffered saline, and lysed by N_2 cavitation. Membrane-enriched fractions were prepared by differential centrifugation as described (20). Marker enzyme activities of the plasma membrane (5′-nucleotidase) and microsomes (glucose 6-phosphatase) in membranes from native and $VitD_3$-treated HL-60 cells were measured and shown to be similarly enriched 2–3-fold, compared with the respective total cell cavitates.

[^{3}H]$InsP_3$ binding assays. [^{3}H]$InsP_3$ binding to cell membrane preparations was measured as described (20). Membranes (0.2–0.5 mg of protein in 50 μl) from control or $VitD_3$-treated HL-60 cells were added to 50 mM Tris, pH 8.2, 1 mM EDTA buffer containing 3–60 nM [^{3}H]$InsP_3$ (17 Ci/mmol; NEN DuPont). Total [^{3}H]$InsP_3$ binding was determined by vacuum filtration. Nonspecific binding was assessed in the presence of 10 μM $InsP_3$ and was routinely <10% of total binding except at 60 nM radiolabeled ligand, when it amounted to approximately 15% of the total. Binding data were analyzed by the ELF/EDBA LIGAND computer program.

Poly(A)$^+$ RNA isolation and analysis. Poly(A)$^+$ RNA was isolated from cells by guanidine thiocyanate extraction, CsCl ultracentrifugation, and oligo(dT)-cellulose chromatography, according to established methods (21, 23). For Northern blot analyses, 2 μg of poly(A)$^+$ RNA were electrophoresed through formaldehyde-agarose gels and blotted onto Gene Screen Plus nylon membranes (NEN DuPont). For dot blot analyses, serial dilutions of poly(A)$^+$ RNA were applied to Gene Screen Plus membranes using a Bio-Rad apparatus and protocols. Membranes were prehybridized at 65° for 6–14 hr in a solution of 0.9 M NaCl/90 mM sodium citrate (6× standard saline citrate), 2% sodium dodecyl sulfate, 10% dextran sulfate, and 100 μg/ml denatured salmon sperm DNA and were then hybridized at 65° for 12–18 hr in the aforementioned solution containing $0.1–0.4 \times 10^6$ cpm/ml (approximately 10^9 cpm/μg) of a random hexamer-primed, [^{32}P]dCTP-labeled (3000 Ci/mmol; NEN DuPont), PCD6 cDNA. The PCD6 cDNA was sequenced and confirmed to encode part (nucleotides 7076–9050) of the murine $InsP_3$ receptor (24). This cDNA was generously provided by Dr. Harry T. Orr of the University of Minnesota. In some cases, membranes were hybridized with a probe derived by reverse transcription and polymerase chain reaction amplification of HL-60 cell poly(A)$^+$ RNA. The amplified product was confirmed by DNA sequencing to contain a 482-base pair segment (base pairs 489–970) of the human $InsP_3$ receptor cDNA. Results using either $InsP_3$ receptor probe were similar. For detection of β-actin mRNA, a 42-mer oligonucleotide antisense probe synthesized according to the murine β-actin cDNA sequence (25) was end-labeled using [γ-^{32}P]ATP (6000 Ci/mmol; NEN DuPont) and T4 polynucleotide kinase, as described by Sambrook *et al.* (26).

Nuclear run-off transcription assay. Assays were carried out essentially as described by McKnight and Palmiter (27), with minor variation. Control and $VitD_3$-treated HL-60 cells were washed and nuclei were isolated by lysis in reticulocyte standard buffer (10 mM Tris·HCl, pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$) containing 0.5% Nonidet P-40. The washed nuclei were resuspended at a DNA concentration of 1 mg/ml in a solution of 40% glycerol, 50 mM Tris·HCl, pH 8.3, 5 mM $MgCl_2$, 0.1 mM EDTA. Reaction volumes of 0.2 ml contained nuclei (0.2 mg of DNA), 30% glycerol, 2.5 mM dithiothreitol, 1 mM $MgCl_2$, 70 mM KCl, 0.25 mM GTP and CTP, 0.5 mM ATP, and 100 μCi of [α-^{32}P] UTP (800 Ci/mmol; NEN DuPont). After 30 min at 26°, RNA was extracted and isolated by ultracentrifugation as described above. Radiolabeled nuclear RNA ($3–5 \times 10^5$ dpm/ml) was allowed to hybridize to Gene Screen Plus nylon membranes containing heat-denatured target plasmids (50–150 ng). After hybridization for 36 hr at 65° the membranes were washed and exposed for autoradiography. Target plasmids included pUC118/PCD6 (for $InsP_3$ receptor RNA detection) and pBR322/β-actin (containing the complete coding sequence of β-actin) (28).

Results and Discussion

$VitD_3$ induction of monocytic differentiation of HL-60 cells. $VitD_3$ at nanomolar concentrations induces the monocytic differentiation of human promyelocytic leukemia cells and human bone marrow cells (10, 17–19). Accompanying this differentiation, there is increased expression of monocytic markers and functions including cell surface C3 receptors, enhanced phagocytic and antibody-dependent cytotoxicity activities, and the induction of α-naphthyl acetate esterase activity, a monocyte- and macrophage-specific plasma membrane marker. When treated with 10 nM $VitD_3$ for 3 days, the HL-60 cells used in the experiments reported here acquired α-naphthyl acetate esterase activity (data not shown). In the cytocentrifuged cell preparations that were examined, untreated HL-60 cells had virtually no detectable α-naphthyl acetate esterase activity, whereas 35–55% (three experiments) of $VitD_3$-treated cells exhibited activity, as measured by the deposition of black granulated esterase products on cytocentrifuged cells.

$InsP_3$ receptor expression and function. Because the acquired phagocytic activity and formyl peptide responsiveness in monocytes and in $VitD_3$-treated HL-60 cells involve changes in intracellular Ca^{2+}, it was of interest to us to examine the expression and function of the $InsP_3$ receptor in these cells. Untreated HL-60 cells express an $InsP_3$ receptor but only at a relatively low level (20). The results of [^{3}H]$InsP_3$ binding experiments using membranes prepared from native HL-60 cells and HL-60 cells that had been treated for 3 days with 10 nM $VitD_3$ are summarized in Table 1. Scatchard analyses showed linear plots, indicating that both untreated and $VitD_3$-treated cells express a single, high affinity $InsP_3$ receptor with a K_d of approximately 17–18 nM. The similarity of the $InsP_3$ binding dissociation constants in the two cell types suggests that native and $VitD_3$-treated HL-60 cells express the same $InsP_3$ receptor. These K_d values are also similar to the value of 21.7 nM observed for $InsP_3$ binding to the clonally expressed murine $InsP_3$ receptor (29). Despite the similarity of K_d values,

TABLE 1

[^{3}H]$InsP_3$ binding parameters in membranes from HL-60 cells and $VitD_3$-treated HL-60 cells

Steady state binding of [^{3}H]$InsP_3$ to membranes derived from untreated HL-60 cells or HL-60 cells that had been treated for 3 days with 10 nM $VitD_3$ was measured according to the described procedures. Values are means ± standard errors of three determinations, each from independent passages of cells.

	K_d	B_{max}
	nM	pmol/mg
Untreated	17 ± 2	0.24 ± 0.03
$VitD_3$-treated	18 ± 2	0.66 ± 0.05

the maximum $InsP_3$ binding site density (B_{max}) was increased in membranes prepared from $VitD_3$-treated HL-60 cells, compared with untreated cells. The density of $InsP_3$ receptors increased approximately 3-fold, from 0.24 pmol/mg of protein (untreated HL-60 cells) to 0.66 pmol/mg of protein ($VitD_3$-treated HL-60 cells), after 3 days of treatment with 10 nM $VitD_3$. Recoveries of protein and marker enzyme activities (5′-nucleotidase and glucose-6-phosphatase) were similar in both membrane preparations, suggesting that the increase in $InsP_3$ receptor density is specific.

Accompanying this apparent induction of $InsP_3$ receptor protein, there was also an increase in $InsP_3$-stimulated Ca^{2+} mobilization in $VitD_3$-treated cells, compared with untreated HL-60 cells (Fig. 1; Table 2). To assess the Ca^{2+}-mobilizing function of $InsP_3$ receptors, cells were permeabilized with digitonin and incubated with fura-2, the fluorescent Ca^{2+} indicator dye. In the presence of mitochondrial inhibitors, Ca^{2+} is taken up by an ATP-dependent mechanism into nonmitochondrial stores, including the $InsP_3$-sensitive Ca^{2+} pool. If the capacities of these pools are the same in control and $VitD_3$-treated HL-60 cells, then the amount of Ca^{2+} released by a saturating concentration of $InsP_3$ should reflect the density of functional $InsP_3$ receptors within the respective cells.

In response to ATP and digitonin permeabilization, HL-60 cells and HL-60 cells that had been treated with 10 nM $VitD_3$ for 3 days lowered the ambient Ca^{2+} concentration from 0.9–1 μM to 345 ± 21 nM (eight experiments, mean ± standard error) and 333 ± 24 nM (eight experiments), respectively (Table 2). This Ca^{2+} was sequestered into vesicular pools, because 1 μM ionomycin was able to release the entire ATP-dependent store (Fig. 1B). These results suggest that control and $VitD_3$-treated cells have similar abilities to sequester Ca^{2+} into nonmitochondrial vesicular stores and that the capacities of these stores are comparable in the two cell types.

A portion of the stored Ca^{2+} is releasable in response to $InsP_3$. Upon addition of 3 μM $InsP_3$, Ca^{2+} was rapidly released from the vesicular store. However, significantly more Ca^{2+} was released from stores of $VitD_3$-treated cells than from stores of untreated HL-60 cells (Fig. 1A; Table 2). In the experiment shown in Fig. 1A, $InsP_3$ stimulated Ca^{2+} mobilization in untreated HL-60 cells, raising the Ca^{2+} concentration from 400 nM to 427 nM, whereas in $VitD_3$-treated cells $InsP_3$ raised the Ca^{2+} concentration from 400 nM to 520 nM. Results from all experiments are summarized in Table 2. On average, after 3 days of treatment with $VitD_3$ the Ca^{2+} release response of treated cells was 2.5-fold greater than that of control cells (net increase of 199 ± 14 nM in $VitD_3$-treated cells versus 80 ± 18 nM in untreated cells). The amount of Ca^{2+} released by 10 μM $InsP_3$ in either cell type was not significantly different from that released by 3 μM $InsP_3$, indicating that a saturating concentration of $InsP_3$ was used in these experiments. Because steady state extravesicular Ca^{2+} levels are dictated by the opposing rates of uptake and release and because Ca^{2+} uptake was similar in both cell types, the results from the experiments described above suggest that the rate of $InsP_3$-dependent Ca^{2+} release is greater in $VitD_3$-treated HL-60 cells than in untreated cells. This suggestion is consistent with the contention that

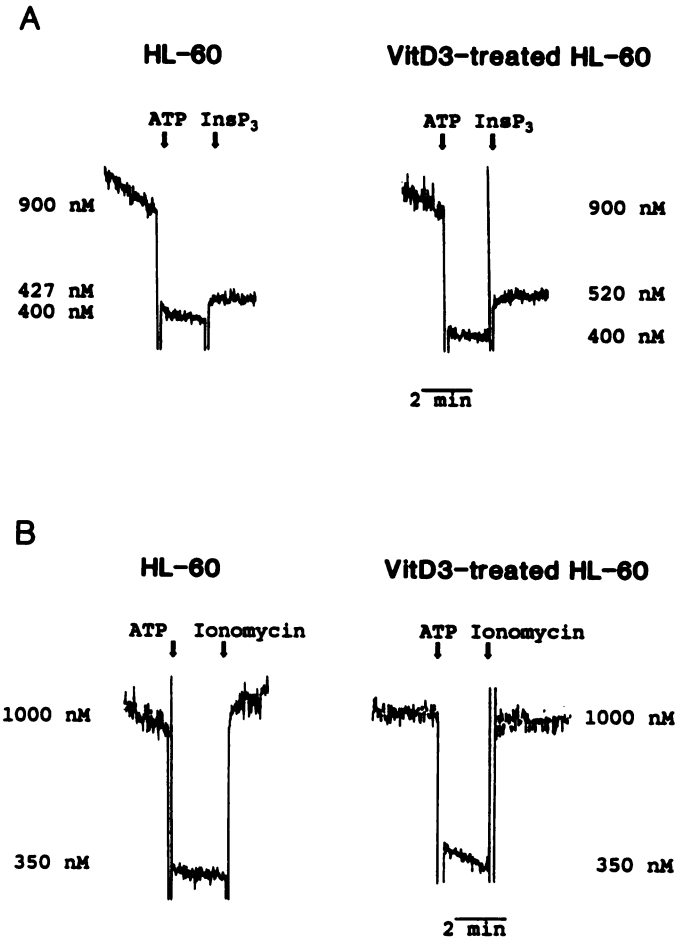

Fig. 1. Measurements of Ca^{2+} mobilization stimulated by $InsP_3$ and ionomycin. Untreated HL-60 cells or HL-60 cells that had been treated for 3 days with 10 nM $VitD_3$ were permeabilized with digitonin and incubated in an intracellular salt solution containing mitochondrial inhibitors and the fluorescent Ca^{2+} indicator dye fura-2. ATP (3 mM) and then either $InsP_3$ (3 μM) (A) or ionomycin (1 μM) (B) were added as indicated. Changes in Ca^{2+} concentrations were monitored as fluorescence emissions from fura-2, as described in Materials and Methods. *Margin values*, calculated Ca^{2+} concentrations.

TABLE 2

$InsP_3$-stimulated Ca^{2+} release from permeabilized HL-60 cells and $VitD_3$-treated HL-60 cells

Untreated HL-60 cells or HL-60 cells that had been treated for 3 days with 10 nM $VitD_3$ were permeabilized with digitonin, and Ca^{2+} concentrations were monitored using the indicator dye fura-2. Values (means ± standard errors, eight experiments) are calculated Ca^{2+} concentrations after 3 mM ATP addition and after subsequent 3 μM $InsP_3$ addition. Δ, Net increases from basal Ca^{2+} concentration after $InsP_3$ addition.

	Ca^{2+} concentration		Δ
	+ATP	+$InsP_3$	
	nM		nM
Untreated	345 ± 21	426 ± 22	80 ± 18
$VitD_3$-treated	333 ± 24	532 ± 46	199 ± 14[a]

[a] $p < 0.05$, $VitD_3$-treated versus untreated HL-60 cells, paired t test.

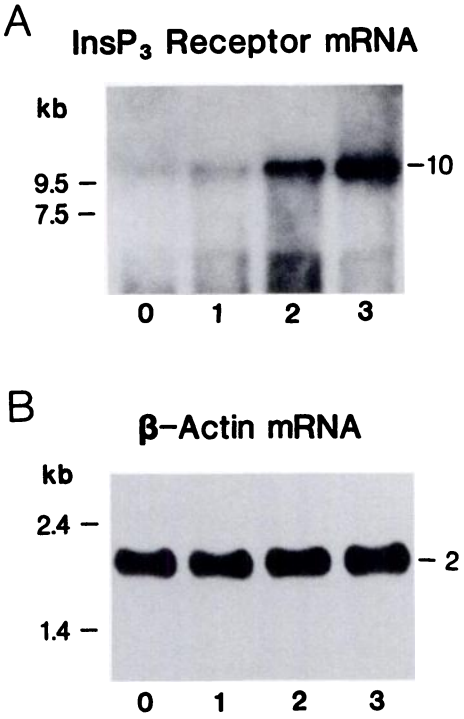

Fig. 2. Northern blot analyses of InsP$_3$ receptor and β-actin mRNA. Size-separated poly(A)$^+$ RNAs from untreated HL-60 cells (*lane 0*) and from HL-60 cells that had been treated with 10 nM VitD$_3$ for 8 hr (*lane 1*), 24 hr (*lane 2*), or 48 hr (*lane 3*) were hybridized with radiolabeled probes for InsP$_3$ receptor mRNA (A) or β-actin mRNA (B). Migration of RNA molecular size standards is indicated to the *left* and the estimated sizes of hybridizing species are shown to the *right* of each blot.

there is a higher density of Ca^{2+}-mobilizing InsP$_3$ receptors in VitD$_3$-treated cells and is supported by the increased [^{3}H]InsP$_3$ binding site density observed after VitD$_3$ treatment.

VitD$_3$-dependent increase in InsP$_3$ receptor mRNA levels. Northern blot analyses were performed to examine the possibility that the observed VitD$_3$-dependent increase in InsP$_3$ receptor expression and function is the result of higher levels of InsP$_3$ receptor mRNA in these cells. PCD6 cDNA is a partial clone (2 kilobases) of the murine InsP$_3$ receptor (24, 30) and was used to prepare radiolabeled probes for the detection of InsP$_3$ receptor mRNA in HL-60 cells. The results of Northern blots (Fig. 2) showed that this probe hybridizes to a single poly(A)$^+$ RNA species of approximately 10 kilobases. Similarly sized InsP$_3$ receptor mRNA transcripts have been observed in mouse and rat brain, mouse placenta, and cytokine-treated human K562 myeloblast cells (21, 29–32). Full length InsP$_3$ receptor cDNA clones from rat and mouse have been reported to be approximately 9900 base pairs (29, 31). The Northern blot analyses of HL-60 cell poly(A)$^+$ RNA showed that untreated HL-60 cells have low but detectable InsP$_3$ receptor mRNA levels (Fig. 2A, *lane 0*). However, after 8-hr treatment of cells with 10 nM VitD$_3$, a small but reproducible increase in InsP$_3$ receptor mRNA levels was observed (Fig. 2A, *lane 1*), and after 24- and 48-hr treatment the InsP$_3$ receptor mRNA level was substantially increased (Fig. 2A, *lanes 2* and *3*). The level of β-actin mRNA in HL-60 cells was not appreciably altered by VitD$_3$ over this time course (Fig. 2B).

InsP$_3$ receptor mRNA levels in control and VitD$_3$-treated HL-60 cells were quantitatively compared by poly(A)$^+$ RNA dot blot dilution analyses (data not shown). After 8-hr treatment, InsP$_3$ receptor mRNA levels had more than doubled, compared with untreated cells. After 24 hr the InsP$_3$ receptor mRNA level had more than quadrupled, and it thereafter remained elevated above controls for 2–3 days. β-Actin mRNA levels were indistinguishable in untreated and VitD$_3$-treated HL-60 cells. These experiments indicate that treatment of HL-60 cells with 10 nM VitD$_3$ resulted in a rapid and selective elevation of InsP$_3$ receptor mRNA.

VitD$_3$ stimulation of InsP$_3$ receptor gene transcription. Steady state mRNA levels are dictated by the relative rates of mRNA transcription and degradation. Specific mRNAs in HL-60 cells, for example those encoding the α and β subunits (CD11b/CD18) of the Mac-1 adhesion molecule, have been shown to be regulated both by enhanced transcription and by decreased degradation (33, 34). To determine the potential contribution of either of these mechanisms to the observed VitD$_3$-dependent increase in steady state InsP$_3$ receptor mRNA level, nuclear run-off experiments were performed. Nuclear run-off assays allow an estimation of the relative rates of specific RNA synthesis, reflecting transcriptional start rates at specific genes (27). Nascent RNA transcripts in isolated nuclei were labeled with [^{32}P]UTP and then hybridized to specific cDNAs encoding either the InsP$_3$ receptor or β-actin. Representative results from experiments with nuclei from untreated and 2-day VitD$_3$-treated HL-60 cells are shown in Fig. 3. Quantitation by scanning densitometry in two independent experiments showed that nuclei from VitD$_3$-treated cells contained approximately 3–4-fold more nascent [^{32}P]UTP-labeled InsP$_3$ receptor RNA transcripts than did nuclei from untreated cells. In the same preparations of nuclei, the levels of newly synthesized β-actin RNA transcripts were unchanged by VitD$_3$ treatment. These results suggest that, in response to treatment of HL-60 cells with VitD$_3$, the InsP$_3$ receptor gene is specifically transcribed at a rate 3–4-fold greater than observed in untreated cells. This increased rate of transcription is sufficient to account for the observed increase in the steady state level of mRNA.

Effects of CHX on InsP$_3$ receptor mRNA induction. VitD$_3$, through its intracellular receptor, may act directly on the InsP$_3$ receptor gene to enhance transcription or, alternatively, it may act indirectly by affecting transcription of other regulatory genes, whose products in turn regulate InsP$_3$ receptor gene transcription. To address these possibilities, analyses of mRNAs were carried out in the presence and absence of CHX, an inhibitor of protein synthesis. In control studies, HL-60

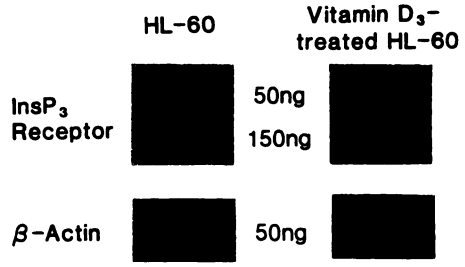

Fig. 3. Nuclear run-off transcription assays. Transcription assays were performed using nuclei from untreated HL-60 cells and from HL-60 cells that had been treated for 2 days with 10 nM VitD$_3$. Nascent RNA transcripts were elongated in nuclei labeled with [α-^{32}P]UTP, and then equal amounts of isolated [^{32}P]RNA were hybridized with denatured recombinant plasmids containing DNA for the InsP$_3$ receptor (pUC118/PCD6, 50 and 150 ng) or β-actin (pBR322/β-actin, 50 ng). Parental plasmids (pUC118 and pBR322) showed no reactivity (data not shown).

cells treated for 16 hr with 10 nM $VitD_3$ showed an approximately 2-fold increase in $InsP_3$ receptor mRNA levels (Fig. 4A). However, when CHX (3 μg/ml) was included in these experiments there was a surprising increase in $InsP_3$ receptor mRNA levels in both untreated and $VitD_3$-treated cells. These results suggest that protein synthesis may be required to degrade $InsP_3$ receptor mRNA and/or to repress gene transcription. These results are specific for $InsP_3$ receptor mRNA, because no such enhancement was observed with β-actin mRNA (Fig. 4B). In fact, CHX caused a slight decrease in β-actin mRNA levels.

Significantly, the observed induction of $InsP_3$ receptor mRNA by $VitD_3$ was still seen in the presence of 3 μg/ml CHX (Fig. 4A). Despite the mRNA levels being elevated by CHX, the amount of $InsP_3$ receptor mRNA was still approximately 2-fold greater if $VitD_3$ was included. Similar results were seen with 10 μg/ml CHX. These results suggest that protein synthesis is not required for the increase in $InsP_3$ receptor mRNA stimulated by $VitD_3$. Furthermore, these results are consistent with the hypothesis that $VitD_3$, via its receptor, may directly regulate $InsP_3$ receptor gene transcription.

Conclusions

Cytosolic receptors for $VitD_3$ have been observed in $VitD_3$-responsive cells, including HL-60 cells (16, 35–37). The specificities of $VitD_3$ and its analogs for the expression of biological activity in Ca^{2+} homeostasis and for binding with high affinity to the $VitD_3$ receptor have been well correlated with their specificities for inducing HL-60 cell monocytic differentiation (18). This evidence suggests that the phenotypic changes associated with monocytic differentiation in $VitD_3$-treated HL-60 cells are mediated by specific intracellular $VitD_3$ receptors. It is of interest for the understanding of hematopoietic growth and differentiation to determine the relevant genes that are regulated both directly and indirectly by $VitD_3$ receptors.

$VitD_3$-differentiated HL-60 cells but not native HL-60 cells have been shown to be capable of chemoattractant-mediated Ca^{2+} mobilization and of Ca^{2+}-mediated functional responses (38). Because these responses are dependent upon functional Ca^{2+}-mobilizing $InsP_3$ receptors, it was important to determine the status of $InsP_3$ receptor expression after $VitD_3$ treatment. $InsP_3$ receptor expression is only one factor among many that enable differentiated HL-60 cells to be functionally competent; however, the analysis of this one protein and its encoding gene may yield insights into the actions of $VitD_3$ and into the differentiation process. The results of the experiments reported here suggest that, as part of the $VitD_3$-driven differentiation process, functional $InsP_3$ receptor expression is increased. Northern blot and nuclear run-off analyses showed that $InsP_3$ receptor gene transcription is specifically and directly stimulated in $VitD_3$-treated cells and that $InsP_3$ receptor mRNA accumulates by a process not requiring protein synthesis.

Study of the direct transcription-enhancing activity of $VitD_3$ for the human osteocalcin gene has led to the identification of a $VitD_3$ response element in the osteocalcin gene promoter (39, 40). This response element is necessary for $VitD_3$ receptor-dependent enhancement of osteocalcin gene transcription. The characteristics of the enhanced transcription of the $InsP_3$ receptor gene reported in the present study suggest that a similar $VitD_3$-responsive regulatory region may exist in the $InsP_3$ receptor gene.

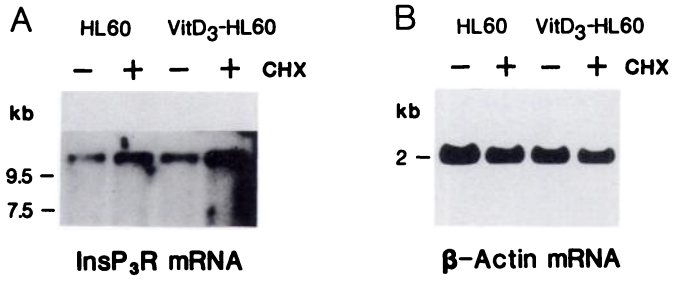

Fig. 4. Effect of CHX on $InsP_3$ receptor mRNA. Poly(A)$^+$ RNAs were prepared from HL-60 cells that had been treated for 16 hr as indicated, with or without 10 nM $VitD_3$ and in the presence (+) or absence (–) of 3 μg/ml CHX. Northern blots of poly(A)$^+$ RNA (2 μg) were sequentially hybridized with radiolabeled probes for the $InsP_3$ receptor (A) and β-actin (B). Migration of RNA molecular size standards is shown to the *left* of each blot.

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Send reprint requests to: Peter G. Bradford, Department of Pharmacology and Therapeutics, 102 Farber Hall, State University of New York at Buffalo, Buffalo, NY 14214–3000.